Controlling antibiotic resistance in the ICU

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Controlling antibiotic resistance in the ICU

Het bestrijden van antibiotische resistentie op de intensive care

(met een samenvatting in het Nederlands)

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Colonization and infection in ICU patients

Patients admitted to intensive care units (ICUs) are frequently colonized with (antibiotic-resistant) bacteria. Some will be colonized when admitted, and some will acquire carriage during ICU-stay. Acquisition of colonization during ICU admission may occur through newly developed resistance in previously susceptible bacteria (i.e., *de novo* resistance), selection of resistant bacteria induced by antibiotic use (i.e., endogenous selection) or through patient-to-patient transmission of bacteria (i.e., cross-transmission).[1] The transfer of pathogens from patient to patient mostly occurs through temporarily contaminated hands of health-care workers (HCW).[2] This transmission route requires five sequential steps, as described in the World Health Organization (WHO) "Guidelines on Hand Hygiene in Health Care" in 2009: "(i) organisms are present on the patient's skin, or have been shed onto inanimate objects immediately surrounding the patient; (ii) organisms must be transferred to the hands of HCWs; (iii) organisms must be capable of surviving for at least several minutes on HCWs' hands; (iv) hand washing or hand antisepsis by the HCW must be inadequate or entirely omitted, or the agent used for hand hygiene inappropriate; and (v) the contaminated hand or hands of the caregiver must come into direct contact with another patient or with an inanimate object that will come into direct contact with the patient."[2] Preventing colonization (and subsequently infection) through breaking this chain of transmission is challenging.

Colonization may lead to healthcare associated infections (HCAI).[3] Prevalence rates of ICU acquired infections vary between 10% and 32% in Europe,[4] with crude mortality rates ranging from 12% to 80%.[5] Main risk factors for ICU acquired infections are underlying immunodeficiency, co-morbidities, use of invasive devices (e.g. mechanical ventilation, central venous catheter), and the intensity of patient care.[6] These factors, combined with extensive use of antibiotics, facilitate colonization and subsequent infection with antimicrobial-resistant bacteria (AMRB) in this vulnerable patient group.

The burden of HCAI is difficult to quantify because of the use of multiple - sometimes different - diagnostic criteria and methods varying between countries and studies. In surveillance studies, HCAI diagnosis usually relies on a combination of laboratory and microbiological criteria. In developed countries, HCAI is estimated to affect up to 15% of hospitalized patients and 9 to 37% of patients admitted to ICUs.[2,5] According to data from the Hospital in Europe Link for Infection Control through Surveillance (HELICS),[7] approximately 5 million HCAIs occur annually in acute

care hospitals in Europe. These HCAIs represent around 25 million excess days of hospital stay each year, and a corresponding economic burden of 13 to 24 billion euros. Mortality caused by HCAI in Europe is estimated to be about 50,000 deaths per year, but HCAI contribute to the death of at least 135,000 patients per year.[2]

Antimicrobial-resistant bacteria

Colonization and infection with AMRB, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE) and highly-resistant *Enterobacteriaceae* (HRE) has been increasing in hospitals worldwide over the last decade.[8-10] Recent data suggest that infections with resistant clones add to infections caused by susceptible bacteria, rather than replacing them, thus contributing to the burden of disease.[9,11]

General risk factors for the emergence of infections caused by AMRB in the ICU include the use of antimicrobial agents, the use of invasive devices, failing infection control policies and prolonged length of hospital stay.[12] For ventilator-associated pneumonia caused by AMRB, seven or more days of mechanical ventilation, previous antibiotic use, and previous use of broad-spectrum antibiotics were identified as most important risk factors.[13] The rise in infections caused by AMRB outside the hospital, such as long-term care facilities, could also increase the introduction rates of these pathogens in ICUs.[12]

Though the epidemiology of MRSA is changing, and new strains have occurred in the last decades in Europe and the United States, the majority of HCAI are still caused by traditional health-care associated MRSA genotypes.[9,14,15] For these genotypes risk factors include older age, prior hospitalization (especially in the ICU) or surgery, prior or prolonged antibiotic treatment, the presence of (surgical) wounds, co-morbidities and the use of invasive devices.[10,16,17]

Vancomycin resistance among Enterococci was described for the first time in Europe in 1988.[18] The risk factors for acquiring VRE are generally similar to those for MRSA and other antimicrobial-resistant bacteria. Patients colonized with VRE often contaminate their environment; and VRE has the ability to persist on environmental surfaces for months.[19]

Infections caused by HRE, such as extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, mostly *Escherichia coli* and *Klebsiella pneumoniae*, are rapidly increasing worldwide.[20] ESBLs are plasmid-mediated, which allows efficient inter-species and cross-species horizontal gene transfer.[21] This complicates infection control, and increases the risk of spreading resistance and outbreaks, especially in health-care facilities. More recently, carbapenemase-producing Gram-negatives have emerged in different parts of the world, and have imposed even bigger problems in controlling antibiotic resistance.[22-24]

Risk factors for acquisition of HRE colonization include age above 75 years, male gender, ICU admission, the use of invasive devices, increased disease severity, emergency intra-abdominal surgery, prior use of antibiotics (especially third generation cephalosporins) and residence in a nursing home.[20,25,26]

Infections caused by AMRB are increasing; and are associated with delayed initiation of appropriate therapy, failure of therapy, prolonged length of hospital stay, and increased morbidity and mortality.[27-30] In a recent study by De Kraker,[31] results from the Burden of Resistance and Disease in European Nations (BURDEN) project were combined with prevalence data from the European Antibiotic Resistance Surveillance Network (EARSSNet).[32-34] This allowed estimation of the burden of disease of AMRB bacteremias (measured as MRSA and third-generation cephalosporin-resistant *Escherichia coli*) in Europe. The authors concluded that excess mortality associated with AMRB bacteremia is high; and estimated the incidence to rise to 3.3 associated deaths per 100,000 inhabitants by 2015. The authors predict, based on current trends, that the observed increase in resistant Gram-negative bacteremias will become an even more pressing health care problem in the near future. This global increase in AMRB necessitates more effective control measures, especially in intensive care units.[27]

Current strategies to combat antibiotic resistance

Current strategies to detect and control antibiotic resistance include hand hygiene programs, the use of chlorhexidine body-washing or other decontaminating agents, such as mupirocin nasal ointment; and screening of patients on admission with contact precautions of identified carriers. Improving hand hygiene is considered a cornerstone of infection prevention. There is a firm body of evidence that low hand hygiene compliance is associated with higher bacterial transmission rates as well as increased incidence of HCAI.[2] However, this evidence mainly stems from observational studies and there is hardly any data that demonstrates that improving adherence - in an experimental setting - reduces transmission and infection rates. Hand hygiene compliance is generally low, especially in ICUs.[35] Though many studies have shown that improving hand hygiene is feasible, the variation in the content of these programs is large, and the optimal strategy unknown.[2] Poor adherence to hand hygiene has been associated with HCW category (physicians adhering less than nurses), type of activity performed (compliance being lower before patient contact than after patient contact), increased workload (measured as frequency of hand hygiene opportunities per hour), lower nurse-to-patient staffing ratio (NTPSR), and several other personal and institutional factors.[2] It is currently unknown if and how these factors can be influenced by a comprehensive hand hygiene improvement strategy to control colonization, and thus infection, of ICU patients. Also, the association between workload and compliance, as described by the Geneva group, has never been confirmed in the era of hand rubbing.[35,36] Pittet only assessed hand washing, the most applied method to perform hand hygiene at that time.[35]

In Hugonnet's study, an association was found between workload and hand washing, but not hand rubbing.[36] In the latter study, an association between workload and recourse to hand rubbing was found, as it was less time-consuming. Thereafter, hand rubbing, not washing, has become standard practice.

In 2009, the World Health Organization (WHO) published its "Guidelines on Hand Hygiene in Healthcare". This extensive review included a multimodal hand hygiene improvement strategy ("My 5 Moments for Hand Hygiene"), suitable for training, observation, and performance reporting across different health-care settings worldwide. It includes an extensive toolkit for implementation in hospitals or other healthcare facilities. The strategy identifies five indications for hand hygiene for trainers, observers and health-care workers; and aims to facilitate education, minimize inter-individual variation in observations and to increase adherence to hand hygiene centred around patient contact. Though based on a thorough review of the available scientific evidence, and an extensive testing and evaluation phase of about 3 years, the feasibility of this program, the effectiveness in reducing colonization and/or infection has not been evaluated clinically. Recently, combining the WHO "5 moments" method with a feedback intervention designed using behavioural theory, was effective in improving hand hygiene compliance, suggesting that audit and feedback is essential for the success of such programs.[37]

The use of chlorhexidine body washing has been associated with lower infection rates, especially in ICUs.[38-45] AMRB frequently colonize the skin of ICU patients, and decontamination of these body surfaces may not only prevent development of infections but also reduce the potential for cross-transmission. These conclusions are drawn in the absence of large, randomized clinical trials, and the evidence is weakened by inter-study differences in the protocol for chlorhexidine use, co-interventions, and patient case mix.

Another intervention associated with reduced transmission is treatment of AMRB carriers with contact precautions or in single-patient rooms.[46,47] Recently, new and faster diagnostic methods have come to the market, which might improve the effectiveness of this strategy.[48-51] However, reported effects of rapid screening for AMRB carriage followed by contact precautions are conflicting, and mostly including just MRSA, precluding evidence-based recommendations.[52] Two previous studies failed to demonstrate beneficial incremental effects of screening on ICU admission and contact precautions for identified carriers of AMRB.[53,54] However, these studies addressed Gram-positive bacteria only, and were criticized for not evaluating interventions based on rapid screening, as average turn-around times of cultures were 3 days[53] and 5.2 days,[54] respectively, implying that many screening results were not available before patient discharge. Furthermore, failure of isolation was accredited to a low 21% hand hygiene compliance in one study.[53]

Future of infection control strategies

Controlling resistance in the ICU is challenging. Recently, doubt has been raised as to whether, in this day and age, hand hygiene interventions can still prevent colonization and infection of patients.[55] Recent studies on chlorhexidine body-washing provide promising results, though its effectiveness against (resistant) Gram-negatives has not been demonstrated. If, in a background of optimal hygiene, there is incremental value of screening and isolation of identified carriers, is currently unknown. Additionally, the value of rapid diagnostic tests, that are usually much more costly compared to traditional or chromogenic culture methods, remains uncertain. In summary, the evidence base of these interventions is mainly based on small, quasi-experimental studies, mostly testing the effect of a single intervention for a single pathogen.

Objectives of this thesis

We assessed effects of interventions to reduce antibiotic resistance in ICUs in a multi-center cluster randomized trial. Additionally, we aimed to investigate the molecular epidemiology, risk factors and duration of colonization with MRSA, VRE and HRE in European ICUs.

Outline of this thesis

The first part of this thesis, including this introduction in **chapter 1**, focuses on current strategies to control antibiotic resistance. In **chapter 2** we conduct a systematic review of the use of chlorhexidine body washing to control antimicrobial-resistant bacteria in intensive care units.

In the second part of this thesis the results of a multi-center cluster-randomized trial are described. The objective of the trial was to quantify the incremental effects on acquisition of AMRB carriage by ICU patients of 1) unit-wide implementation of chlorhexidine body-washing, combined with a hand hygiene improvement program; and 2) rapid diagnostic testing followed by contact precautions for identified carriers, using either molecular-based testing for MRSA and VRE together with chromogenic-based screening for HRE, or chromogenic-based screening for MRSA and VRE only. The main results of the trial are described in **chapter 3**. In **chapter 4**, we describe in detail the results of the implementation of the hand hygiene improvement strategy, based on the WHO “5 moments” method, in 13 European intensive care units.

In the third part of this thesis the molecular epidemiology and risk factors of AMRB in Europe are addressed. In **chapter 5**, we describe MRSA epidemiology based on genotypic analyses of all first isolates of colonized patients, collected within the cluster-randomized trial described in part two of this thesis. In **chapter 6**, HRE epidemiology is assessed based on a similar collection of strains. In **chapter 7** we describe the clinical impact and risk factors for colonization with HRE, based on data from a French intensive care unit.

This part of the thesis includes assessment of the duration of colonization with antimicrobial-resistant bacteria after ICU discharge in **chapter 8**. A maximum likelihood analysis approach was used to calculate the survival function, taking censoring into account.
Finally, in **chapter 9**, the results of the different studies are summarized, and the implications for currently applied strategies to control AMRB, as well as remaining challenges, are discussed.

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Chlorhexidine body washing to control antimicrobial-resistant bacteria in intensive care units:

a systematic review

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Chlorhexidine body washing to control antimicrobial-resistant bacteria in intensive care units: a systematic review

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Abstract

Purpose: Infections caused by antimicrobial-resistant bacteria (AMRB) are increasing worldwide, especially in intensive care units (ICUs). Chlorhexidine body washing (CHG-BW) has been proposed as a measure to limit the spread of AMRB. We have systematically assessed the evidence on the effectiveness of CHG-BW in reducing colonization and infection with AMRB in adult ICU patients.

Methods: PubMed, Embase, CINAHL, and OpenSigle databases were searched using synonyms for "intensive care unit", "hospital", and "chlorhexidine". All potentially relevant articles were examined by two independent reviewers. Inclusion was limited to studies with ICU patients as domain, providing outcomes related to colonization or infection with AMRB. Data from 16 studies were extracted; 9 were excluded because of assessed high risk of bias or inadequate analyses. The remaining studies differed markedly in (co-)interventions and case mix, which precluded pooling of data in a formal meta-analysis.

Results: Incidences of MRSA acquisition were reduced significantly in three studies in which this was the primary endpoint. Significant reduction in MRSA infection rates was observed in only one of five studies. Carriage and bacteremia rates of VRE were assessed in one study, and both significantly declined. There were hardly any data on the effects of CHG-BW on antibiotic-resistant Gram-negative bacteria (ARGNB).

Conclusions: CHG-BW may be effective in preventing carriage, and possibly bloodstream infections, with MRSA and VRE in different ICU settings. As CHG-BW protocols, co-interventions and case mix varied widely, attribution of these effects to CHG-BW alone should be done with care. Evidence that CHG-BW reduces carriage of or infections with ARGNB is lacking.

Keywords ICU | Chlorhexidine body washing | Systematic review | AMRB

Introduction

The failing control of antimicrobial-resistant bacteria (AMRB) is an important and continuously growing threat to the delivery of adequate medical care in hospitals and the community.[1] Infections caused by AMRB usually require longer and more complex treatments than those caused by susceptible bacteria.[2, 3] Nosocomial infections with AMRB are associated with delayed initiation of appropriate therapy, failure of therapy, prolonged length of hospital stay, and increased mortality. Patients admitted to the intensive care (ICU) are extremely prone to infections, including those caused by AMRB. Main contributing factors are underlying immunodeficiency, co-morbidities, use of invasive devices, and the intensity of patient care. These factors, combined with extensive use of antibiotics, facilitate patient-to-patient transfer of AMRB.[4]

Chlorhexidine gluconate (CHG) is a cationic bisbiguanide developed in the UK around 1950. Recently, there has been a renewed interest in this antiseptic as a measure to prevent infections with, and transmission of, AMRB in ICU patients. Cross-transmission of AMRB is extremely important in the dynamics of these bacteria, and temporarily contaminated hands of health care workers are considered the most important vectors for spread.[5] AMRB frequently colonize the skin of ICU patients, and decontamination of these body surfaces may not only prevent development of infections but also reduce the potential for cross-transmission.

We aimed to evaluate the evidence for the effectiveness of chlorhexidine body washings (CHG-BW) in reducing colonization and infection with AMRB in adult ICU patients, measured as colonization or infection with methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and/or antibiotic-resistant Gram-negative bacteria (ARGNB). We assessed the effect, when possible, on different AMRB separately, as the effect might differ between bacterial species. We focused on CHG-BW, and not on the use of CHG for oral decontamination and pre-surgical skin preparation, which have been systematically reviewed recently.[6, 7]

Materials and methods

Search strategy

Methods and inclusion criteria of the review were specified in advance and documented in a protocol (see "Appendix"). All studies in PubMed, Embase, CINAHL, and OpenSigle from their inception to 1 April 2011 were considered. Databases were searched using "intensive care unit" and "hospital" (all variants and abbreviations) with Boolean "OR" to describe the setting and "chlorhexidine" OR "body wash" to describe the intervention. In OpenSigle, only "chlorhexidine" was used as search term to include all possibly relevant studies. We included all studies of adult ICU patients that investigated CHG-BW as an intervention to control AMRB and had colonization, clearance of colonization, or infection as an outcome. Non-English language papers were accepted if they fulfilled the above-mentioned criteria. A related article and reference search was performed.

Data collection and selection of studies

Duplicates were removed, and the title and abstract of all identified articles were screened for relevance, without blinding to journal and authors, by two independent reviewers (LD and MD). In case of discordant results consensus was reached by discussion with a third reviewer (MB). Reviews were included if there was any reason to assume that original data were present. Letters to scientific journals were not automatically excluded, as they could contain original data. Outbreak reports (an outbreak was defined as an increase in incidence lasting < 6 months) were excluded, as success in outbreak situations cannot be generalized to non-outbreak situations. Chart reviews were also excluded. Studies were eligible if the setting was the ICU, or hospital without explicit absence of an ICU, and if outcomes were related to colonization or infection with AMRB.

All potentially relevant articles were obtained, and the full text was examined. Because of the high proportion of studies with low quality design, a high possibility for bias or the absence of a control group, we decided at this point to limit inclusion to randomized controlled trials (RCTs) and interrupted time series (ITS) design with three or more time-points. In before-after design studies, proper ITS analyses and at least three time points before and after initiation of the intervention are recommended.[8, 9] Even then, there is a possibility that the internal validity of ITS is compromised by trends that are already present before the start of intervention, outcomes that are measured differently over time, and differential dropout in the intervention group. Therefore, we only selected ITS adhering to the recommendations of the Cochrane "Effective Practice and Organization of Care" Group (EPOC)[8] in order to limit these threats to internal validity. None of the studies including hospital patients included results for ICU patients separately, nor could these results be calculated from the data presented. Therefore, we excluded these studies. The data collection flowchart is shown in Figure 1.

Data extraction and management

For each of the 16 included studies, the following characteristics were extracted: design, setting, domain, co-interventions, outcome(s), possible sources of bias, missing data, and the statistical analyses used to evaluate the outcome(s). Only effect measures related to colonization and/or infection were included.

Only one study (one out of two RCTs) used the ICU rather than the individual patient as unit of analysis.[10] Because of this sparseness of RCTs using unit-based analyses, we did not exclude any studies based on this issue. As many studies did not mention missing data, nor the way missing data were handled, this was only assessed when present. Because of heterogeneity in designs, no meta-analyses to obtain pooled results could be performed.

Figure 1: Study flow diagram

Results

Our search yielded 2,477 abstracts; two extra records were retrieved by related article and reference search.[11,12] In both articles, neither "chlorhexidine" nor any of the other search terms were mentioned in the title or abstract. Seven studies were included in the final review (Table 1).[10,13-18] One of these studies investigated two interventions in a 2 x 2 factorial design. Patients (n = 515) were randomized to receive topical antibiotics (polymyxin and tobramycin; applied in the oropharynx and through the nasogastric tube) or placebo, and also to receive mupirocin ointment in the nose and CHG-BW or placebos. For the current review we only used the data from the patient groups that did not receive topical antibiotics, but CHG-BW with mupirocin (n = 130) or placebos (n = 126). Of nine excluded studies, seven were excluded based on inadequate analyses of ITS studies (following EPOC criteria).[19-25] In one RCT and one ITS, insufficient data were present to calculate the effectiveness of CHG-BW (Table 2).[26, 27]

Quality and completeness of the evidence

Of seven included studies three determined acquisition rates of MRSA carriage;[13,15,16] one determined acquisition rates of VRE carriage.[15] Five quantified MRSA[14-18] and one quantified VRE infection rates.[15] Four studies reported (limited) results on infections with ARGNB.[10,14,16,17] Co-interventions were used in four studies,[13,14,16,18] and CHG-BW protocols as well as patient case mix differed extensively between studies (Table 1).

Compliance with CHG-BW protocol was measured in one study only.[15] In this study, actual use of CHG was compared to predicted use, and coordinators urged better compliance if needed. However, compliance data were not presented. Hand hygiene compliance was not systematically assessed in any of the studies.

Risk of bias in included studies

There was no perceived risk of selection bias in the selected studies. In one study, 3,928 of 4,444 admitted ICU patients were excluded, but only 4.6% of these patients were excluded for other reasons than those stated in the exclusion criteria (mainly because of logistic issues on weekends (3.7%)).[14] A double-blind design was used in one[14] and partial blinding in another RCT in which one of three investigators categorizing bloodstream infections (BSIs) and the category adjudicator were blinded to the study arm.[10] Naturally, blinding was not used in the studies with ITS design.

Possibilities for detection bias were considered present in three studies.[13,15,16] In one study the method of screening changed during the trial;[13] in another one compliance with obtaining surveillance cultures increased during the study,[15] and in the third study screening cultures were used during intervention, but not during the baseline period.[16] In the latter two studies, though, detection bias may have underestimated the effectiveness of the intervention, as detection of the primary endpoint improved after the intervention was implemented.

Table 1: Characteristics of included studies

Study	Design	Patients included (n)	Duration (months)	Setting	Domain [a]	CHG intervention	Co-interventions or Control group	Primary outcome	Secondary outcome(s)
Batra [13]	ITS	4,570	51	Single center, mixed ICU	Patients colonized or infected with MRSA	1% CHG in nostrils/ mouth/tracheotomy site QID	Educational campaign (reinforcing HH and barrier nursing, covert HH and barrier nursing audit and monthly MRSA infection rate feedback)	Transmission of MRSA colonization	-
						1% CHA in groin/axillae/ skin folds daily	MRSA colonized nursed in side rooms or pairs		
						4% CHG body wash daily			
Bleasdale [10]	RCT	836	12	Single center, medical ICU	All patients	2% CHG body wash daily with impregnated cloths	Daily bathing with soap and water	All-cause primary BSIs	All-cause UTI, VAP, and secondary BSIs
Camus [14]	RCT	256 [b]	30	Multi-center, medical ICUs	Patients with expected duration of ventilation > 48 h	15 ml of 4% CHG body wash every 12 h for 5 days with or without SDD	SDD plus "body wash placebo" or placebo only	All-cause infections acquired until 48 h after termination of study treatments	All-cause total and device-related infections
Climo [15]	ITS	5,043	12	Multi-center, medical, surgical, cardiac surgery; and coronary/medical ICUs	All patients	4% CHG body wash daily	Daily bathing with non-medicated soap and water	Acquisition of MRSA and VRE colonization and BSIs	-
Gould [16]	ITS	2,653	48	Single center, mixed ICU	All patients	4% CHG body and hair wash daily	Nasal ointment QID (a) 2% fusidic acid; (b) 3% oxytetracycline (only available first 6 months) or (c) 0.5% neomycin sulphate w 0.1% chlorhexidine hydrochloride	Acquisition of MRSA colonization and infection	*S. aureus* bacteremia
Popovich [17]	ITS	3,048 [c]	24	Single center, surgical ICU	All patients	2% CHG body wash daily	Daily bathing with bar soap, warm water and cotton washcloths	Acquisition of all-cause CLABSIs	Acquisition of other nosocomial infections
Raineri [18]	ITS	3,978	120	Single center, mixed ICU	All patients	4% CHG body wash daily for 5 days CHG shampoo on day 1 and 5	Post-intervention education session for new HCWs, monthly infection control meetings, strict isolation, and cohorting	Acquisition of MRSA colonization and infection	-

BSI bloodstream infection, *CHA* chlorhexidine acetate, *CHG* chlorhexidine gluconate, *CLABSI* catheter-related bloodstream infections, *ICU* intensive care unit, *ITS* interrupted time series, *MRSA* methicillin-resistant *Staphylococcus aureus*, *RCT* randomized controlled trial, *S. aureus Staphylococcus aureus*, *SDD* selective digestive decontamination, *UTI* urinary tract infection, *VAP* ventilator-associated pneumonia, *VRE* vancomycin-resistant Enterococci

[a] The domain for the CHG intervention is stated. The domain for co-interventions can differ

[b] Only taking into account the “neither” and the “CHG” regimen patients

[c] Calculated from mean monthly admission rate of 138 during 12 months at baseline and 116 during 12 months at intervention phase

Attrition bias was not considered relevant in any of the included studies. In one study 3 of 391 patients in the CHG-BW arm did not receive bathing because of skin rashes (eventually considered as not related to CHG-BW), and these patients were included in the intention-to-treat analysis.[10] In another study 1 of 126 patients in the placebo group was withdrawn from the analysis because of premature unblinding.[14]

Though no formal meta-analysis was performed, the presence of studies with negative results demonstrates that publication bias was not complete.

Selective outcome reporting may have been present, but was difficult to assess as study protocols for studies using an ITS design were not available. Protocols were available for the two RCTs. For one RCT the protocol, as accessed through www.clinicaltrials.gov, stated that microbiological data were collected, but only data related to BSIs were reported.[10] The authors stated that these data will be published separately. The protocol of the other RCT was kindly provided by the authors, and no risk of selective outcome reporting was detected.[14] For the ITS studies, we compared information in the "methods" sections to the "results" sections, and evidence of selective outcome reporting was not detected. In one of these studies, it was stated that the intervention was not part of a pre-planned study protocol.[17]

Table 2: Characteristics of excluded studies

Study	Design	Reason for exclusion
Dixon [19]	ITS	Does not use time-series analysis Source and method of data collection not mentioned
Dryden [26]	RCT	CHG-BW was used in both groups
Evans [20]	ITS	Does not use time-series analysis
Fraser [21]	ITS	Does not use time-series analysis
		ITS with only one data point per period
Holder [22]	ITS	No formal analysis, only descriptive data. High risk of bias (regression to the mean)
Munoz-Price [23]	ITS	Does not use time-series analysis. Possible regression to the mean. Risk of reporting bias (the "unblinded" preventionist reported the number of infections). Substantial non-compliance, not quantified in intervention period
Popovich [24]	ITS	Does not use time-series analysis
Ridenour [25]	ITS	Does not use time-series analysis
Robicsek [27]	ITS	Not suitable to assess effectiveness of CHG-BW (focus on different types of surveillance)

CHG-BW chlorhexidine gluconate body washing, *ITS* interrupted time series, *RCT* randomized controlled trial

Effects of interventions

Incidences of acquisition of MRSA carriage were reduced significantly in the three studies in which this was the primary endpoint (Table 3).[13,15,16]

MRSA infection rates were a primary outcome in three studies,[15,16,18] and two studies presented limited data on MRSA infection rates.[14,17] A statistically significant incidence reduction was observed in one.[18] Two studies failed to demonstrate statistically significant effects on MRSA bacteremia, although MRSA-carriage rates decreased in both studies.[15,16] Absolute numbers of MRSA bacteremia, though, were only 40 (29 before and 11 after intervention) and 13 (8 before and 5 after intervention) in these studies. MRSA infections were even lower in the studies in which this was not a primary outcome. In one study there were two and five MRSA infections in the CHG-BW and placebo group,[14] and in the other study there were five and six clinical cultures yielding MRSA at baseline and during the intervention, respectively (incidence rate of 0.68 vs. 1.03 per 1,000 patient-days; p = .49).[17] Carriage and bacteremia rates due to VRE were analyzed in one study; these were reduced by 45 and 78%, respectively.[15] Reported results of CHG-BW on preventing all-cause infections were more heterogeneous. There was a statistically significant 61% decline in the incidence of all-cause primary BSIs in one study,[10] whereas no significant reductions in central line-associated BSIs (CLABSIs) were reported in two other studies.[14, 17]

Although incidences of colonization and/or infections with ARGNB were not primary outcomes in any of the studies, some results were provided. In one study, 1 out of 27 and 2 out of 11 primary BSIs were caused by Gram-negative bacteria before and after the introduction of CHG-BW, respectively.[10] In another study, 5 out of 13 and 1 out of 12 clinical cultures grew imipenem-resistant *A. baumannii* before and during the use of CHG-BW, respectively, although overall more CLABSIs were due to Gram-negative bacteria (and yeasts) during CHG-BW.[17] In a third study, the number of patients acquiring infections with Gram-negative bacteria was 50 of 126 randomized to placebo and 44 of 130 randomized to CHG-BW plus nasal mupirocin, without further information on antibiotic susceptibilities.[14] In a fourth study, carriage and bacteremia rates with ARGNB were 1% or lower in both study periods.[16] Therefore, there was hardly any evidence on the effects of CHG-BW on carriage with ARGNB.

Discussion

The results of this systematic review demonstrate that CHG-BW may be effective in preventing bloodstream infections and carriage with MRSA and VRE in different ICU settings. This conclusion is based on seven studies with good methodological quality and low risk of bias, but marked differences in interventions, co-interventions and patient case mix, which precluded pooling of data in a formal meta-analysis.

Table 3: Summary of findings

Study	Patients included (*n*)	Duration (months)	Infection	Colonization
Batra [13]	4,570	51		70% reduction in acquisition of endemic MRSA strains (rate ratio 0.3), but increased acquisition (rate ratio 3.85) with an outbreak MRSA strain
Bleasdale [10]	836	12	61% incidence reduction in all-cause primary BSIs; rate difference 6.3/1,000 ptdays 16.8 versus 6.4 BSIs per 1,000 central line-days (p = .01) No significant reduction in all-cause UTI, VAP, and secondary BSIs	
Camus [14]	256	30	No significant reduction in all-cause ICU-acquired infections (p = .919) [a] No significant reduction in all-cause total infections [a] No significant reduction in all-cause device-related infections [b]	
Climo [15]	5,043	12	No reduction in MRSA bacteremia [c]	25% reduction in acquisition of MRSA colonization (-0.66 per 1,000 ptdays) [c]
			78% reduction in ICU acquired VRE bacteremias (-2.64 per 1,000 ptdays) [c]	45% reduction in acquisition of VRE colonization (-1.51 per 1,000 ptdays) [c]
Gould [16]	2,653	48	No significant reduction in MRSA or MSSA bacteremia	11.4% decrease (p = .005) in proportion of patients with MRSA (colonization or infection)
Popovich [17]	3,048	24	No significant reduction in ICU-acquired all-cause CLABSIs (p = .57) Significant decrease in incidence rate of MRSA clinical cultures (0.68 versus 1.03 per 1,000 ptdays, p = .49) No significant reduction in ICU-acquired other infections (all p values > 0.18)	
Raineri [18]	3,978	120	Decrease of MRSA infection rate from 3.5 to 1.7 per 1,000 ptdays (p = .0023) No significant difference in MRSA-VAP [d] Decrease in MRSA-BSI incidence rate from 1.65 to 0.29 cases per 1,000 ptdays (p = .02)	

BSI bloodstream infection, *CHG* chlorhexidine gluconate, *CHG-BW* chlorhexidine gluconate body washing, *CLABSI* central line-associated bloodstream infection, *MRSA* methicillin-resistant *Staphylococcus aureus*, *PO* primary outcome, *Ptdays* patient-days, *SO* secondary outcome, *TW-MRSA* sequence type 239 MRSA outbreak strain, *UTI* urinary tract infection, *VAP* ventilator-associated pneumonia, *Vent days* ventilator days, *VRE* vancomycin-resistant Enterococci

[a] There was a significant effect for the polymyxin/tobramycin plus CHG/mupirocin group when compared to each regimen alone and neither regimen

[b] There was also no significant difference for the polymyxin/tobramycin plus CHG/mupirocin group when compared to each regimen alone and neither regimen

[c] Only the results of the time-series analysis are presented

[d] For period 1 compared to period 2. For the whole trial (period 1 to period 3), there was a significant decrease (p = .006 for trend)

Though much can be learned from less robust studies, like outbreaks, we chose methodological selection criteria to select only the best available evidence. Before-after studies not fulfilling these criteria have a high chance of inappropriately attributing the found effect to the intervention, as they do not correct for baseline trends.[8]

In four studies, co-interventions were present, such as the use of mupirocin intranasally, active surveillance cultures, isolation or other barrier precautions, and education programs.[13,14,16,18] Therefore, attribution of the beneficial effects on infections and carriage with MRSA and VRE to CHG-BW alone should be made with care.

There was no evidence (nor lack of evidence) that CHG-BW reduces acquisition of carriage or infections with ARGNB. CHG works by attachment to and disruption of cytoplasmic membranes of bacteria, and should, therefore, be effective against gram-positive and -negative bacteria.[5] In vitro, though, CHG has slightly better activity against gram-positive bacteria.[5]

Possible adverse events and the emergence of resistance against CHG are important issues that were not systematically assessed. However, no severe allergic skin reactions were reported in the included studies. In one study slightly higher median minimally inhibitory concentrations to CHG were observed among blood culture isolates during CHG-BW, compared to soap-and-water bathing, but this difference was attributed to isolation of fewer (very susceptible) gram-positive bacteria during CHG-BW rather than to an increase in the absolute number of bacteria with elevated minimally inhibitory concentrations for CHG.[10]

Since decontamination of body surfaces may not only prevent development of infections but also reduce the potential for cross-transmission, CHG-BW may influence the risk of non-treated patients to acquire bacterial carriage (i.e., colonization pressure).[28] The effects of CHG-BW are, therefore, best evaluated when applied to all patients in a unit simultaneously, and individual patient randomization may not be the most appropriate study design. There was only one RCT in which the effectiveness of CHG-BW was evaluated on the unit level.[10]

The most practical approach for unit-based interventions is a before–after study. Unfortunately, from a methodological perspective, this is a weak study design because of intrinsic risks of bias.[9] Moreover, not incorporating patient dependency in the statistical analysis may lead to wrong inferences.[29] Therefore, seven studies employing ITS design, but not complying with EPOC guidelines were excluded from our analyses.[19-27]

In a recent meta-analysis of 12 studies investigating the effects of CHG-BW on the incidence of BSIs, no methodological criteria were applied for study selection.[30] Five of those studies were also included in our study,[10,14-17] and reductions in BSIs were apparent in three.[10,15,16] However, our study adds important nuances to the conclusions of O'Horo et al. In one of the abovementioned three studies, only a reduction in primary, but not in secondary all-cause BSIs was apparent;[10] in one study a significant reduction in ICU-acquired VRE bacteremia, but not of MRSA bacteremia was demonstrated,[15] and in the remaining study there were no reductions in MRSA and MSSA bacteremia in the original manuscript.[16] The reduction as demonstrated

in the pooled estimate of O'Horo's meta-analysis was caused by a decrease in BSI caused by coagulase-negative staphylococci only.[30] Two studies included in our review were excluded in O'Horo's meta-analysis, one study because the outcome was colonization instead of infection.[13] The reason for exclusion of the second study investigating BSIs, albeit with MRSA only, is unknown.[18] Five studies, excluded for methodological reasons in our study were included in O'Horo's study. In 4 of these (from a total of 12 studies) statistically significant effects were obtained. Though the authors touch upon the subject of heterogeneity in their discussion, they do not comment on their reasons for pooling data. In summary, both the present study and O'Horo's meta-analysis suggest an effect of CHG-BW on BSIs. Our study adds that the benefit for preventing BSI is limited to gram-positives (VRE and possibly MRSA) and that evidence for Gram-negatives is lacking. Moreover, our findings also suggest that colonization with gram-positives is reduced by CHG-BW.

Conclusions

Based on this systematic review we conclude that there is evidence that CHG-BW is effective in preventing carriage, and possibly BSI, with MRSA and VRE in ICU patients, although this evidence is weakened by inter-study differences in intervention, co-interventions, and patient case mix. Overall, the quality of the studies was good, with low to medium risk of bias. There was no evidence (or lack of evidence) that CHG-BW reduces acquisition of carriage or infections with ARGNB. Future studies should address the effects of CHG-BW on acquisition of carriage and infections with ARGNB, preferably by investigating the effects of CHG-BW with the ICU as level of inference to account for colonization pressure, for instance by applying an ITS design with sufficient data points or a cluster-randomized trial design.

Conflicts of interest: None.

Appendix: Research protocol

Objective: To evaluate the evidence for the effectiveness of the use of chlorhexidine body washings in reducing colonization and infection with AMRB in adult ICU patients.

Databases to be searched: PubMed, Embase, CINAHL, and OpenSigle from their inception until 1 April 2011. The search was last performed on 15 September 2011.

Population: Adult ICU patients.

Intervention: Chlorhexidine body washings.

Outcomes: All outcomes related to colonization, infection, and/or bacteremia with AMRB.

Study design: All.

Free text search terms: chlorhexidine, chlorhexidine gluconate, critical care, icu, intensive care, critical* ill, critical* illness, intensive treatment unit*, hospital, hospitals, inpatient*, hospitalis*, hospitaliz*.

MeSH terms: Chlorhexidine ("Chlorhexidine"[Mesh]), Chlorhexidine gluconate ("Chlorhexidine gluconate"[Substance Name]).

Study selection (after removing duplicates): Screen title and abstract of all identified articles for relevance, without blinding to journal and authors, by two independent reviewers (LD and MD). In case of discordant results consensus by discussion with a third reviewer (MB).

(A) Inclusion

Body washing with CHG as an intervention to control AMRB.

Colonization or infection with MRSA or VRE or ARGNB or any combination of those micro-organisms or clearance of colonization with these micro-organisms as an outcome.

Setting: ICU or hospital without explicit absence of an ICU.

Patient population: Adults.

Non-English language papers and non-published papers all accepted.

Reviews included.

Letters to scientific journals included.

(B) Exclusion

Outbreak reports (defined as an increase in incidence that lasted < 6 months) excluded as having weak evidence.

Chart reviews excluded.

Studies specifically on the subject of oral or topical decontamination, and studies on hand hygiene only excluded.

PubMed search strategy: [(critical care unit* OR ccu NOT coronary care unit*) OR critical care OR cc OR intensive care unit* OR icu OR intensive care OR ic OR (critical* AND ill) OR (critical* AND illness) OR intensive treatment* OR (intensive treatment* AND unit)* OR itu OR hospital OR hospitals OR inpatient* OR hospitaliz* OR hospitalis*] AND (chlorhexidine gluconate* OR chlorhexidine* OR body wash*).

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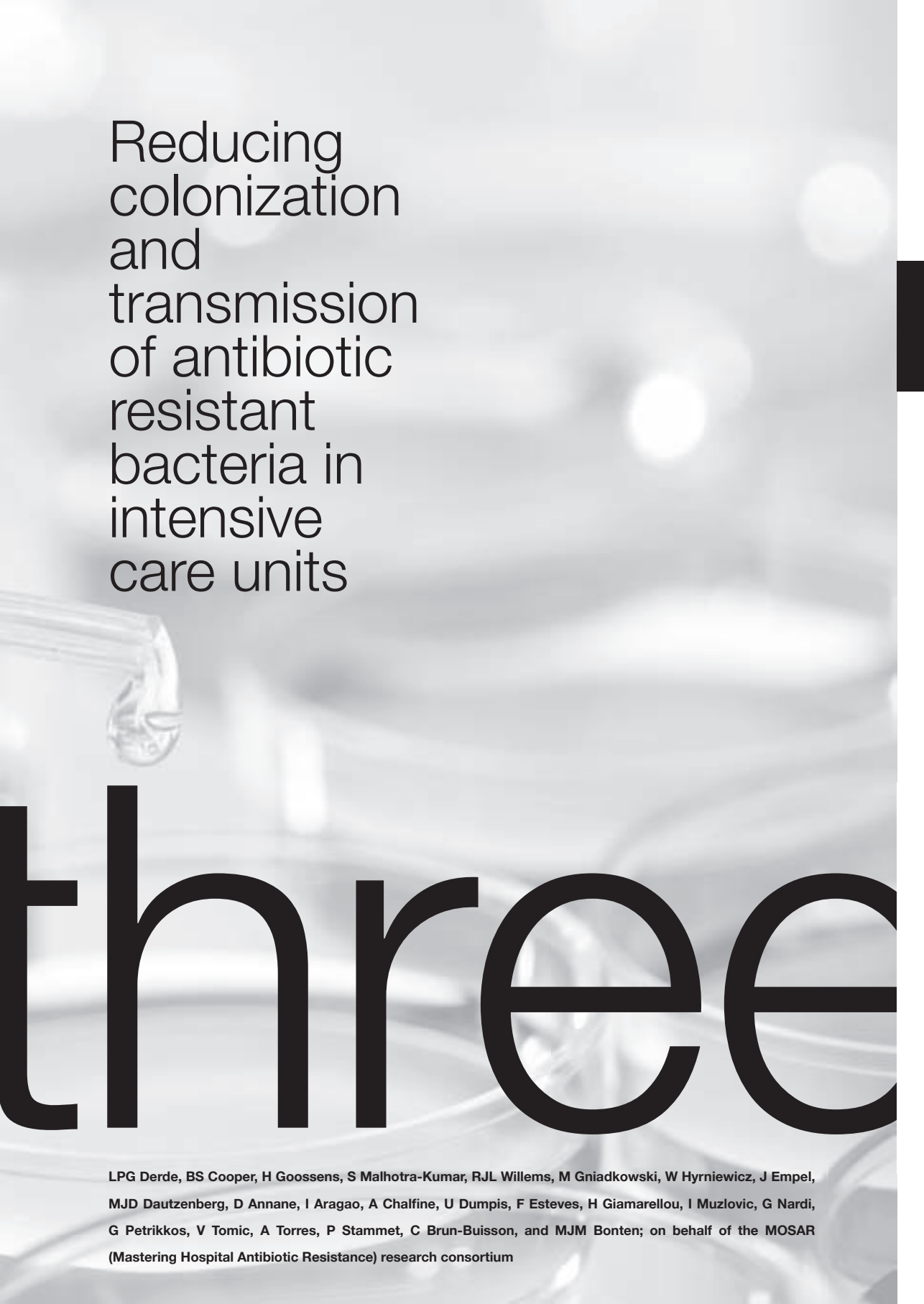
Reducing colonization and transmission of antibiotic resistant bacteria in intensive care units



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Submitted

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Reducing colonization and transmission of antibiotic resistant bacteria in intensive care units

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Abstract

Background: Intensive Care Units (ICUs) are high-risk areas for transmission of antimicrobial-resistant bacteria (AMRB).

Methods: We conducted an interrupted time-series and cluster-randomized trial in 13 ICUs consisting of a 6-month baseline period (P1), followed by universal chlorhexidine body-washing combined with hand hygiene improvement during 6 months (P2). Subsequently (P3; 12 to 15 months), ICUs were randomized to rapid screening (7 ICUs), including PCR testing for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) and chromagar screening for highly-resistant *Enterobacteriaceae* (HRE), or to chromogenic screening for MRSA and VRE (6 ICUs); with contact precautions for identified carriers. Acquisition of AMRB carriage was determined by microbiological surveillance and analyzed using multilevel Poisson segmented regression.

Results: Hand hygiene compliance improved from 52% (P1) to 69% (P2) and 77% (P3). Median proportions of patients receiving chlorhexidine body-washing increased by 86.6% at the start of P2. Weekly AMRB acquisition tended to increase in P1 (weekly incidence rate ratio [IRR] 1.4% [95% CI -0.4%, 3.1%]), decreased in P2 (0.98 [0.95, 1.00]) with no stepwise change in incidence, and remained unchanged in P3 compared to P2, without significant differences between rapid and chromagar screening (p = .06). The fall in AMRB was largely due to changes in the rate of MRSA acquisition, which was initially increasing, but which had a clear decreasing trend in P2 (weekly IRR 7.5% [3.9%, 11.0%]).

Conclusions: Improved hand hygiene plus unit-wide chlorhexidine body-washing reduced AMRB acquisition and MRSA in particular. Adding isolation of identified carriers had no incremental effects.

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Introduction

The global increase of antimicrobial-resistant bacteria (AMRB), such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE) and highly-resistant *Enterobacteriaceae* (HRE), necessitates more effective control measures, especially in intensive care units (ICUs).[1] Improving hand hygiene is considered a cornerstone of infection prevention.[2] Treatment of AMRB carriers with contact precautions or in single-patient rooms has been associated with reduced transmission,[3, 4] and this might be more effective if carriage is rapidly detected after admission. Chlorhexidine body-washing has been associated with lower infection rates and lower bacterial loads of MRSA and VRE on skin surfaces, which also may reduce cross-transmission.[5-10] However, the evidence base of these interventions is mainly based on small, quasi-experimental studies, mostly testing the effect of a single intervention for a single pathogen. Moreover, reported effects of rapid screening for AMRB carriage followed by contact precautions are conflicting, precluding evidence-based recommendations.[11] We, therefore, set out to quantify the incremental effects on acquisition of MRSA, VRE or HRE carriage by ICU patients of 1) unit-wide implementation of chlorhexidine body-washing, combined with a hand hygiene improvement program; and 2) rapid diagnostic testing followed by contact precautions for identified carriers, using either molecular-based testing for MRSA and VRE together with chromogenic-based screening for HRE, or chromogenic-based screening for MRSA and VRE only.

Methods

Study design:

We combined an interrupted time-series and cluster-randomized study design in 13 European ICUs between May 2008 and April 2011. Adult ICUs with at least 8 beds were eligible, provided that proportions of MRSA, VRE or extended-spectrum beta-lactamases (ESBLs) among ICU-acquired bacteremias recorded in 2006 or 2007 were >10%, >5% and >10% among *S. aureus*, enterococcal or *Enterobacteriaceae* bacteremias, respectively.

Written approval of the study protocol was obtained from each institution's review board or national ethics committee as appropriate. As the study was considered to involve no more than minimal risk of harm to patients, a waiver for informed consent was granted for all participants. The study was registered on www.clinicaltrials.gov under number NCT00976638.

Microbiology:

Surveillance cultures of perineum, nose and wounds (if present) were obtained within 2 days of admission and twice weekly thereafter, from all patients admitted for 3 days or longer. After 21 days, culture frequency was reduced to once weekly. All surveillance swabs were analyzed locally, according to a standardized protocol (Appendix A), using chromogenic media (BBL CHROMagar MRSA II for MRSA detection or Enterococcosel Agar with vancomycin 8 µg/mL for VRE

detection (Becton, Dickinson and Company, Franklin Lakes, NJ USA) and Brilliance ESBL 2 (Oxoid Limited, Cambridge, UK) for HRE detection).[12-14] In ICUs randomized to rapid screening, Xpert MRSA and Xpert VanA/VanB (Cepheid, Sunnyvale, CA USA) were used following protocols.[15,16]

All microbiology laboratories were required to complete proficiency panels.[17-19] All MRSA, VRE and HRE first isolates from surveillance cultures or blood were shipped to a central laboratory for species confirmation, susceptibility testing and genotyping. To assess chlorhexidine resistance the presence of qacA/B genes was investigated by PCR.[20]

Interventions:

After a baseline period of six months (P1), both a hand hygiene improvement program and universal daily body-washing with 0.16 grams/Liter chlorhexidine gluconate were implemented for six months (P2; Appendix B) in all ICUs. The hand hygiene improvement program was derived from the WHO "My 5 Moments" concept.[2] In phase 3 (P3; 12-15 months) these interventions continued; additionally, ICUs were randomly assigned by an independent data manager to either chromogenic agar-based screening for MRSA and VRE (conventional arm, CA) or to PCR-based screening for MRSA and VRE combined with chromogenic agar-based screening for HRE (rapid arm, RA). Contact precautions for identified carriers of targeted AMRB were implemented in this phase (Appendix C). During P1 and P2, barrier precautions were used based on pre-study local isolation protocols and not as part of study interventions. During P1 and P2, surveillance cultures were stored and processed with a two-month delay to maintain blinding of ICU personnel to the colonization status of patients. In P3, PCR and chromagar results were immediately disclosed to the ICU staff. In P3 times from obtaining swabs until start of processing (time-to-test) and until reporting of results (time-to-result) was determined for all surveillance cultures. The sum of both times was the turn-around time of tests.

Measurements:

Patient demographics, reason for ICU admission, length of stay, disposition at discharge and 28-day mortality, as well as occurrence of bacteremia with *S. aureus*, Enterococci, and *Enterobacteriaceae* were collected for all patients. Weekly point prevalence data were collected on bed occupancy, staffing ratios, numbers of patients ventilated, use of invasive devices, and isolation details (Appendix D).

Two research nurses per ICU were centrally trained to perform hand hygiene observations according to the WHO "My 5 Moments" concept.[2] Fifteen 15-30 minute observation sessions were performed per month throughout the study in each ICU, randomly scheduled within three 4-hour time intervals (08:00-12:00 hrs, 12:00-16:00 hrs and 16:00-20:00 hrs).

Data were collected through an online data entry system, consisting of an independently managed secure data processing center, including plausibility checks for data entry.

A 10% sample of all data collected in each ICU was monitored during the trial, and procedures were checked for consistency with the study protocol at all sites.

Definitions and outcomes:

Colonization was defined as growth on chromogenic plates in all phases and arms. Colonization and bacteremia were considered ICU-acquired if detected on or after the third day of ICU-admission, following a negative swab. The primary endpoint was the number of acquisitions of MRSA, VRE or HRE carriage, based on local laboratory testing, per 100 patient-days at risk in ICU. Secondary outcomes included incidence density of ICU-acquired colonization and bacteremia for MRSA, VRE, and HRE individually, hand hygiene compliance, length of ICU-stay, length of hospital-stay and 28-day mortality.

Patients staying in ICU for less than three days were not considered at risk for ICU-acquired colonization and bacteremia, and were excluded from the analyses of ICU-acquired endpoints. For calculations of secondary outcomes, patients were considered at risk for acquisition of an individual pathogen if not colonized or infected on admission with that specific pathogen (e.g., patients colonized with VRE were still at risk for acquiring MRSA colonization).

Statistical analyses:

A pre-study sample size calculation showed that to demonstrate a 10% absolute difference (from 15% to 5%) in the probability of acquiring colonization with a target organism between the two phase 3 arms, with a two-sided test, type I and II error rates of 0.05 and 0.2, and an intra-cluster correlation coefficient of 0.05, 960 patients per ICU would be needed. Pre-specified multilevel Poisson segmented regression analysis was used to determine the effect of each intervention on incidence density of acquisitions with AMRB (primary endpoint) and on secondary outcomes, comparing ICUs implementing conventional methods and ICUs implementing rapid tests, allowing for random between-ICU variation in baseline levels and trends. Potential confounding factors were fitted as covariates. In unplanned exploratory analyses time-dependent Cox regression was used to determine effects of colonization pressure on acquisition rates. Analysis was performed in STATA version 11 (StataCorp), and SPSS (PASW statistics) version 17.

Results

Characteristics of ICUs and patients:

In all 14,390 patients were assessed for eligibility, of whom 8,976 were admitted for at least three days and 8,519 were analyzed (Figure 1). ICU and patient characteristics are listed in Table 1 and proportions of patients colonized on admission in Table 2.

Figure 1: MOSAR-ICU trial flowchart

* = Those patients that were admitted for at least 3 days, of whom admission and discharge date were available and of whom at least one nasal, rectal or wound swab was obtained during ICU admission. Patients at risk for acquiring colonization with AMRB excludes all patients having colonization on admission with any of MRSA, VRE, HRE, or in whom a first (admission) swab was taken after the first 2 days of ICU stay and was found positive

Table 1: Baseline characteristics of patients and ICUs per study phase, presented by phase 3 randomization status (conventional and rapid arm)

	Phase 1	Phase 2	Phase 3	
			CA	RA
	(n = 2043)	(n = 2072)	(n = 2348)	(n = 2513)
	Mean% [a]	Mean% [a]	Mean% [a]	Mean% [a]
ICU characteristics:				
Beds occupied	84.7	87.9	84.2	86.6
Nurse to patient staffing ratio	0.55	0.53	0.55	0.55
Location before ICU admission:				
Home or private residence	38.2	35.6	38.2	37.2
Health care facility	59.1	60.3	58.6	58.6
Unknown or other	2.7	4.2	3.2	4.3
Risk factors for colonization before admission:				
Admitted to a hospital for > 24 hours in the last year	52.6	47.6	56.6	41.4
Any type of surgery in the last year	20.3	22.2	20.9	18.9
Urgent or emergency surgery prior to ICU-admission	17.4	15.3	14.8	17.4
Patient history:				
Solid tumor	14.2	12.0	13.5	15.8
Hematologic malignancy	3.9	4.1	4.2	4.1
Hematopoietic stem cell or bone marrow transplant	0.7	0.8	0.8	0.8
Solid organ transplant	2.2	1.6	1.8	1.5
HIV/ AIDS	1.9	1.4	1.4	1.6
Patient demographics:				
Age (median, years)	65	64	64	65
Male gender	61.0	60.0	60.9	59.3
Non-surgical ICU admission reason	76.5	79.8	81.4	73.4
APACHE-II score (median) [b]	16	16	15	15
SAPS-II (median) [c]	40	38	37	35
Invasive devices (during first 3 days):				
Endotracheal tube	60.4	60.4	59.4	52.1
Tracheostomy tube	4.2	4.3	3.2	5.8
Central venous catheter	69.8	68.3	61.5	73.5
Arterial intravascular catheter	64.2	63.4	59.2	69.0

CA = conventional arm; RA = rapid arm; ICU = intensive care unit; HIV= human immunodeficiency virus; AIDS = acquired immune-deficiency syndrome; APACHE = Acute Physiology and Chronic Health Evaluation; SAPS = Simplified Acute Physiology Score

Data were taken from all patients admitted for at least 3 days

[a] = Unless otherwise specified

[b] = APACHE-II score available for 709 patients in P1, 780 in P2 and 1724 in P3

[c] = SAPS-II score available for 1334 patients in P1, 1292 in P2 and 3134 (3 scores missing) in P3

Table 2: On admission carriage rates with antimicrobial-resistant bacteria per study phase and according to screening strategy uses in phase 3

ICUs using conventional screening	Phase 1 (n = 979)	Phase 2 (n = 1020)	Phase 3 (n = 2280)	ICUs using rapid screening	Phase 1 (n = 983)	Phase 2 (n = 906)	Phase 3 (n = 2351)
AMRB (%)	13.5	12.5	10.3	AMRB (%)	12.3	11.0	14.1
MRSA (%)	5.4	3.7	4.1	MRSA (%)	3.3	4.6	3.3
VRE (%)	3.3	2.9	1.1	VRE (%)	3.5	2.2	5.8
HRE total (%) [a]	6.8	7.0	6.0	HRE total [a] (%)	7.0	5.7	7.7
E.coli (%)	4.0	2.8	3.7	E.coli (%)	2.7	2.2	3.8
PPM (%)	0.3	0.4	0.2	PPM (%)	0.3	0.0	0.2
KESC (%)	3.4	4.8	2.7	KESC (%)	4.8	3.6	4.2

CA = conventional arm; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis*; HRE = highly resistant *Enterobacteriaceae*; *E. coli* = *Escherichia coli* ; PPM = Proteus-, Providencia- and Morganella species ; KESC = Klebsiella-, Enterobacter-, Serratia- and Citrobacter species ; RA = rapid arm
Note that patients of whom both the admission swab was not obtained during the first 2 days of admission, and the first swab taken during ICU admission was positive, are not included in the on admission carriage rates. Consequently, the number of patients on whom the rates presented here are based, might differ slightly from the number of patients in the "patients analyzed" category in figure 1 of this manuscript

[a] = Number of patients admitted with HRE, irrespective of the species. HRE are divided into individual species (E Coli) or subgroup (PPM, KESC)

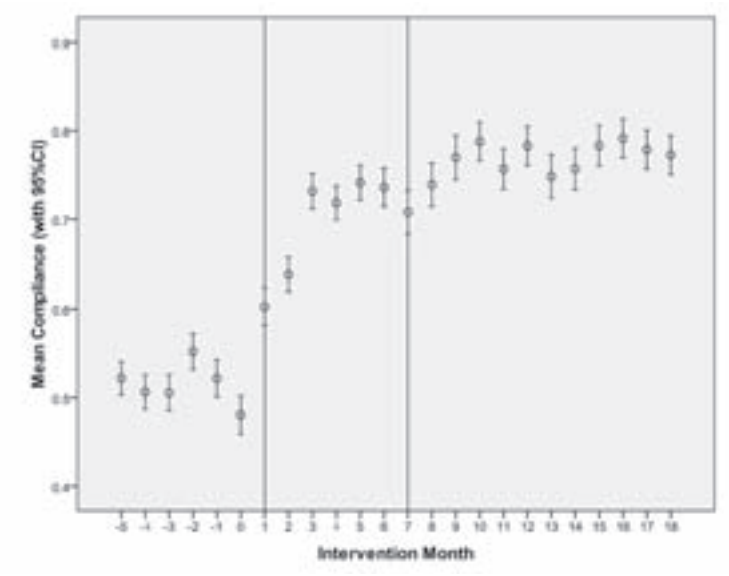
Interventions:

At least one nasal, perineal and wound swab was obtained from 8,517 (94.9%), 8,501 (94.7%) and 931 (10.4%) patients, respectively. Analysis of 41,558 hand hygiene opportunities showed that mean compliance was 52% in P1, 69% in P2 and to 77% in P3 (Figure 2; Appendix E, Table 1). Based on 1,188 point-prevalence measurements, the median percentages of patients receiving chlorhexidine body-washing were almost 100% in P2 and P3 (Appendix E, Figure 1), with an immediate 86.6% absolute increase after P2 started.

In ICUs randomized to rapid screening proportions of patients on contact precautions increased for all AMRB compared to P2. In ICUs randomized to conventional screening only MRSA and VRE related contact precautions increased, consistent with protocol (Appendix E, Table 2).

In P3 median turn-around times of chromagar and PCR tests were 48 and 26 hours, respectively (Appendix E, Table 3).

Figure 2: Mean hand hygiene compliance per month during the 3 phases of the study



Note: Months -5 to 0 are the 6-month baseline phase (P1) with no intervention; month 1 is first month of the hand hygiene improvement intervention

Acquisition of colonization:

Analyses of 64,997 swabs obtained from 8,976 patients revealed ICU-acquired colonization with MRSA, VRE or HRE in patients at risk (not colonized on admission) in 296 of 8184 (3.6%) patients, 384 of 8243 (4.7%) and 1014 of 7943 (12.8%) patients, respectively. Confirmation rates of first isolates were 92% for MRSA and 92% for ESBL-producers (of which 29% were also resistant to carbapenems). Eleven percent of HRE were AmpC producers. For VRE, 51% of the isolates were vanA or vanB containing *E. faecium* and *E. faecalis*, and 35% were *E. gallinarum* and *E. casseliflavus*.

There was large between-center variation in both baseline AMRB acquisition rates and baseline trends (Appendix E Figure 2-4) with an indication that the mean P1 trend was increasing by 1.4% (Table 3). During P2 there was a weekly 2% reduction in trend but no evidence of a step-wise change in incidence. No incremental effect on prevention of AMRB acquisition could be demonstrated during P3 (Table 3; Figure 3). The null hypothesis that conventional and rapid screening were equivalent could not be rejected (p = .06, likelihood ratio test).

The MRSA acquisition rate was increasing by 4.2% per week during P1 and this trend was reduced by 7.5% in P2, resulting in a mean 3.6% weekly decrease. MRSA levels plateaued in P3, with an increase in trend relative to P2. No significant difference between conventional and rapid screening was demonstrated (Table 3; Appendix E Table 4 and Figure 2). For HRE and VRE there was no evidence from the planned analysis that acquisition rates changed in either P2 or P3, and the null hypotheses that arms were equivalent could not be rejected (Table 3; Appendix E, Figure 3 and 4). Cox regression confirmed the results of the Poisson model and indicated that trends in acquisition rates could not be wholly explained by changes in colonization pressure. It also provided some evidence that the P2 intervention led to a trend for reduced VRE acquisition (Appendix E, Table 5 and Figure 5).

Table 3: Poisson segmented regression results [a] of weekly acquisition with antimicrobial-resistant bacteria, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci and highly-resistant *Enterobacteriaceae*

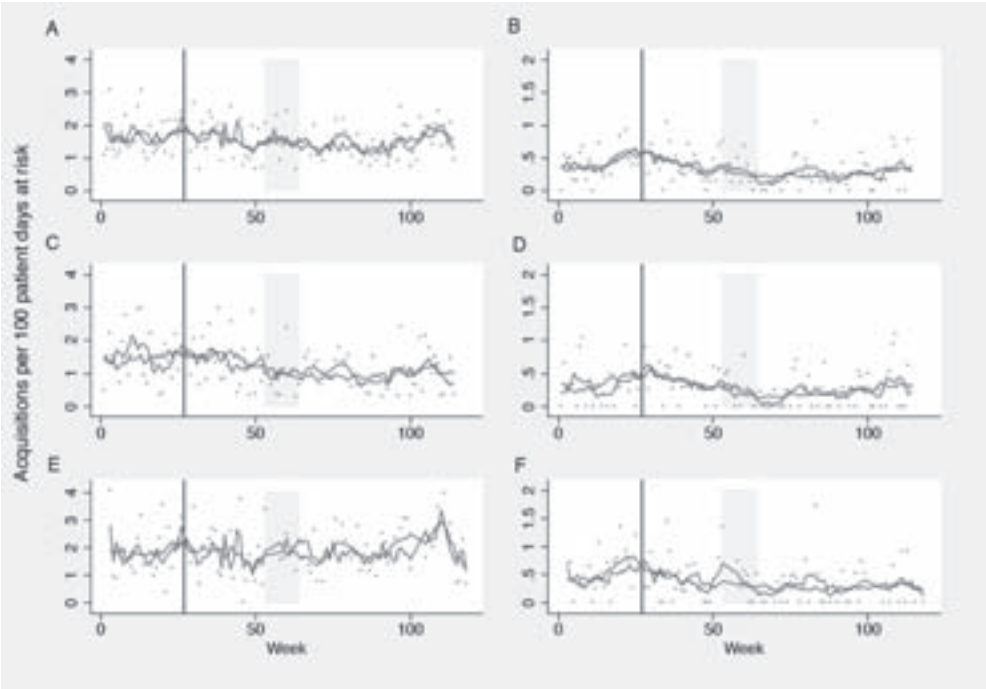
	AMRB	MRSA	VRE	HRE
	IRR (95%CI)	**IRR (95%CI)**	**IRR (95%CI)**	**IRR (95%CI)**
Trend P1	1.014 (0.996, 1.031)	1.042 (1.009, 1.075) **	1.000 (0.971, 1.030)	1.012 (0.992, 1.032)
Step P2	0.955 (0.676, 1.348)	1.159 (0.654, 2.053)	0.884 (0.481, 1.626)	0.831 (0.559, 1.235)
Trend P2	0.976 (0.954, 0.999) *	0.925 (0.890, 0.962) ***	0.982 (0.945, 1.020)	0.994 (0.968, 1.021)
Step P3, CA	0.634 (0.349, 1.153)	0.755 (0.252, 2.257)	0.651 (0.209, 2.031)	0.525 (0.263, 1.048)
Trend P3, CA	1.015 (0.998, 1.032)	1.057 (1.029, 1.086) ***	1.015 (0.984, 1.048)	0.991 (0.971, 1.011)
Step P3, RA	1.075 (0.625, 1.849)	1.309 (0.520, 3.292)	1.130 (0.447, 2.852)	0.888 (0.479, 1.646)
Trend P3, RA	1.010 (0.994, 1.027)	1.042 (1.014, 1.070) **	1.008 (0.981, 1.036)	0.991 (0.973, 1.009)
LRT CA vs RA	p = .06	p = .34	p = .47	p = .10

AMRB = antimicrobial resistant bacteria; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis* ; HRE = highly resistant *Enterobacteriaceae* ; IRR = incidence risk ratio; CA = conventional arm; RA = rapid arm; LRT = likelihood ratio test

IRR values smaller than one represent a decrease, while values above one represent an increase. Significance is indicated by * ($p < .05$), ** ($p < .01$) or *** ($p < .001$).

[a] = Cluster effects were accounted for in the analyses, and potential confounding factors (sex, age, month, invasive devices, nurse-to-patient staffing ratio, location before ICU admission, admission reason, APACHE/SAPS, hospital and number of days-at-risk for acquisition) were fitted as covariates

Figure 3: Acquisition of antimicrobial-resistant bacteria and methicillin-resistant *Staphylococcus aureus* per 100 patient-days at risk



A = all AMRB, both arms combined; B = MRSA, both arms combined; C = all AMRB, conventional arm (CA); D = MRSA, conventional arm (CA); E = all AMRB, rapid arm (RA); F = MRSA, rapid arm (RA).

Weeks are numbered from 26 weeks before phase 2. Shaded area corresponds to start of phase 3.

Green dots represent data, green line represents the 7 week moving average of these data. Red line represents the predicted value from the multilevel Poisson segmented regression model. Cluster effects were accounted for, and potential confounding factors (sex, age, month, invasive devices, nurse-to-patient staffing ratio, location before ICU admission, admission reason, APACHE/ SAPS, hospital and number of days-at-risk for acquisition) were fitted as covariates.

Secondary outcomes:

The total number of ICU-acquired bacteremias recorded during the trial was 83 for HRE, 28 for MRSA and 9 for VRE (*E. faecium* and *E. faecalis* only). There was no evidence of a change in overall rates of ICU-acquired AMRB bacteremia between phases or of differences between the intervention arms in P3 (Appendix E, Table 6). There was a trend for a decrease in ICU-acquired HRE bacteremia in P1, no evidence that this trend changed in P2 or P3, and no evidence for differences between the intervention arms. For MRSA and VRE, numbers were too low to perform statistical analyses.

The mean length of ICU stay (LOS) of patients at risk for acquisition of AMRB was 8 days in P1. In P2, LOS decreased by 1.2% per week (0.6%, 1.8%), to give a net reduction of 26% (16%, 48%) at the end of P2. At the end of P3, LOS had increased by 18% (1%, 39%) compared to P2 in ICUs using rapid screening, but by only 8% (-8%, 27%) in those using conventional screening. Rapid screening was also associated with a stepwise increase in LOS at the start of P3. There was no evidence that hospital length of stay and mortality at day 28 were affected by any of the interventions (Appendix E, Table 6).

Presence of qacA/B was evaluated in 223 MRSA isolates and detected in 14 of 110 isolates from P1 and in 16 of 113 isolates from P3 (p = .75; data not shown).

Discussion

In this trial in 13 European ICUs, optimizing hand hygiene combined with universal chlorhexidine body-washing (P2) was associated with a trend for reduced acquisition of AMRB, mainly by reducing MRSA acquisition. The same interventions did not reduce acquisition of HRE. There was no incremental effect of implementing contact precautions for carriers identified from screening on admission by either chromogenic-based or PCR-based screening. The P2 intervention was associated with a reduced length of ICU stay, while PCR-based screening in P3 was associated with increased length of stay. Reasons for these changes are unclear.

The selective efficacy of P2 interventions on MRSA and possibly VRE, but not HRE, might be partly explained by differences in bacterial epidemiology. Whereas HRE mainly colonize the digestive tract, MRSA and VRE are also known colonizers of the skin and environment. Though chlorhexidine body-washing eradicates MRSA and VRE, we found this intervention did not reduce intestinal HRE acquisition rates. This suggests that patient-to-patient transmission may not be the dominant acquisition route for HRE in these ICU patients, and other prevention methods will be needed to reduce colonization and infections rates with HRE, such as improved antibiotic stewardship programs or intestinal decolonization with non-absorbable antibiotics.[21-23] The ecological safety and effectiveness of the latter measure in ICUs with endemic levels of HRE remains to be determined.[24]

The gradual decrease in AMRB acquisition in P2 suggests that hand hygiene improvement was important, as compliance also improved gradually, whereas chlorhexidine body-washing increased immediately in P2 (Appendix E, Figure 1). Our study design, however, precludes determination of the relative importance of these two interventions.

Our study failed to demonstrate beneficial incremental effects of screening on ICU admission and contact precautions for identified carriers of AMRB. Although a previous controlled study reached a similar conclusion, this addressed Gram-positive bacteria only, did not use rapid screening, and had an average turn-around time for cultures of 5.2 days implying that many screening results were not available before patient discharge.[25] The present study, with a turn-around time of molecular tests for MRSA and VRE of 25 hours on average, does not suffer from this limitation

and we were able to demonstrate that the proportions of patients in isolation increased with implementation of PCR-based screening and good adherence to all aspects of the study protocol. Our study has some limitations. With 13 ICUs in eight countries there was considerable heterogeneity across ICUs in resistance rates and unit characteristics. However, in unplanned explanatory subgroup analyses, we found no evidence of differential intervention effects across wards according to higher or lower median MRSA, VRE or HRE admission prevalence, or that effectiveness of any interventions varied according to baseline trends or level (data not shown). Furthermore, confidence intervals for primary and secondary outcomes were generally small. As the participating ICUs represent typical ICU populations we feel that their heterogeneity and the consistent results for higher and lower prevalence wards add to the external validity of our results. The large changes in length of stay were not anticipated and could affect the number of AMRB acquisitions (and therefore the Poisson regression results). However, the very similar results obtained with the Cox regression analysis (which considers the acquisition rate) indicate that changes in acquisition rates cannot be explained by changes to the length of stay.

Processing surveillance cultures at participating sites may be associated with heterogeneity in microbiological procedures and results. We chose to use local laboratories instead of a central laboratory to implement contact precautions for identified carriers in a timely manner, and to adequately represent daily practice. All local microbiology laboratories used standardized operating procedures to process samples, and completed quality assessments for detection of MRSA, VRE and HRE;[17-19] All first isolates were confirmed at a central laboratory with high confirmation rates for MRSA and HRE. For VRE only 51% of the isolates contained *van*A or *van*B. However, analyses using only confirmed vanA or vanB data did not change results (data not shown). A further limitation is that the phase 2 intervention was not protected by randomization and is potentially vulnerable to maturation effects. However, the primary analysis adjusted for seasonal effects, and accounted for baseline ICU-specific levels and trends. Moreover, visual inspection of MRSA outcomes in the two study arms shows a remarkably similar patter of phase 2 decline. A final limitation is that contact precautions were not audited on a patient level, as research personnel determined weekly isolation rates per ICU.

Conclusion

In this multi-center ICU study optimization of hand hygiene together with universal chlorhexidine body-washing reduced acquisition of AMRB, especially of MRSA. Our findings do not support the use of universal screening for AMRB with contact precautions for identified carriers. PCR-based screening was associated with increased length of ICU stay.

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The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix

Appendix A
Sample collection, storage, microbiological procedures and transport

1. Sample collection:

Of all patients admitted for 3 days or longer and of a sample of patients admitted for less than 3 days, admission (within 48 hours) and twice weekly swabs were taken from the anterior nares (MRSA), perineum (VRE, HRE) and wounds (if present; MRSA, VRE and HRE). The frequency of culturing was reduced to once weekly if the patient had a LOS longer than 21 days.
All cultures were obtained using regular dry swabs by experienced ICU nurses or the appointed research nurses.

2. Storage of surveillance cultures:

Surveillance cultures were stored in cryopreservative fluid for a minimum of two months before analysis, to prevent feedback of results to clinicians. To prepare 100 ml of cryopreservative fluid, 3.7 grams of Brain Heart Infusion broth and 85 ml distilled water was added to 15 ml glycerol (or 15 grams glycerol by weight as it is very viscous). The fluid was boiled to dissolve the Brain Heart Infusion powder and obtain a homogeneous solution. In each 2 or 1.5 ml micro tube, 1 ml of cryopreservative fluid was dispensed. The micro tubes with cryopreservative fluid were autoclaved for 15 minutes at 121ºC and were cooled to room temperature.
In each micro tube, one screening swab was inserted. Each swab was vortexed in the tube for 30 seconds, then expressed gently but firmly to remove all medium from the swab. The swab was removed and discarded; and the cap was carefully screwed back on the tube.
Micro tubes were labeled clearly using non-removable ink (appropriate also for freezing at -80ºC) with the date and site of sampling and the MOSAR study ID. The micro tubes were placed directly into the -70/80ºC freezer, to be thawed and processed later.

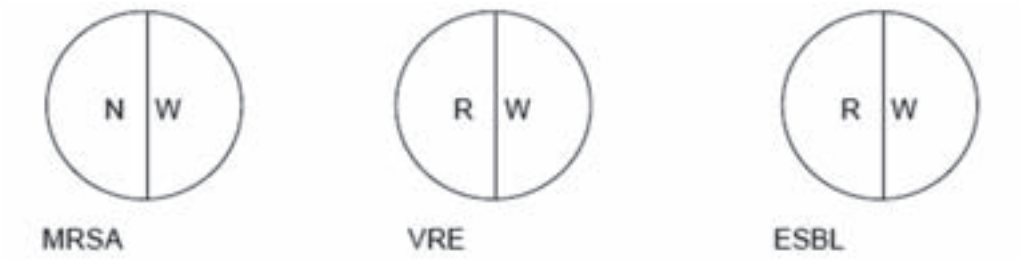
3. Microbiological procedures:

3.1 Use of chromogenic agar plates

For detection of MRSA, BBL CHROMagar MRSA II (CMRSA II) medium (Becton, Dickinson and Company, Franklin Lakes, NJ USA) was used; Enterococcosel agar (ECCV) with 8 µg/ml vancomycin (Becton, Dickinson and Company, Franklin Lakes, NJ USA) was used for detection of VRE and Brilliance ESBL 2 (Oxoid Limited, Cambridge, UK) medium was used to detect *Enterobacteriaceae* resistant to third- or fourth generation cephalosporins (HRE). Training and instructions on the use of the chromogenic media were provided by the manufacturers.
Each plate was divided into two, labeling each half as N (nose), R (rectal), or W (wound) as appropriate (see Supplementary Figure 1). A loopful (10 µl) of each sample was plated on the

appropriate location on the different chromogenic media. Incubation instructions for the relevant medium as provided by the manufacturer were followed. After 18 to 24 hours, chromogenic media were checked for characteristically colored colonies.

Figure 1



Type of sample	To be screened for	Media inoculated
Nose swab	MRSA	BBL CHROMagar MRSA II
Wound swab	MRSA	BBL CHROMagar MRSA II
	VRE	ECCV with 8 µg/ml vancomycin
	ESBL-harboring *Enterobacteriaceae*	Brilliance 2 ESBL
Rectal swab	VRE	ECCV with 8 µg/ml vancomycin
	ESBL-harboring *Enterobacteriaceae*	Brilliance 2 ESBL

For the Gram-negatives, only colonies from the groups of E coli (pink and blue on the Brilliance ESBL 2 medium); Klebsiella/ Enterobacter/ Serratia/ Citrobacter (KESC) group and the Proteus/ Providencia/ Morganella (PPM) group were picked. A double disk synergy test (DDST) using ceftazidime (30 µg), cefotaxim (30 µg), or cefepime (30 µg) discs placed 20 mm apart (center to center) from a co-amoxyclav disc (clavulanic acid 10 µg) was performed on all types of bacterial colonies taken from the Brilliance ESBL 2 medium. The test was considered positive when a decreased susceptibility to cefotaxim was combined with a clear-cut enhancement of the inhibition zone of cefotaxim in front of the clavulanate-containing disk, often resulting in a characteristic shape-zone referred to as 'champagne-cork' or 'keyhole'. DDST was performed on Mueller-Hinton agar according to CLSI guidelines for non-fastidious bacteria. Colonies that showed a positive DDST were considered to be HRE positive.

3.2 Use of PCR tests

For detection of MRSA and VRE on admission swabs, Xpert MRSA and Xpert VanA/VanB (Cepheid, Sunnyvale, CA USA) were used with the GeneXpert platform. Training and instructions on the use of the PCR were provided by the manufacturer at each site.

Double headed swabs were used to facilitate use of the surveillance swab for both PCR and chromogenic agar. Swabs were labeled appropriately with the information written in permanent/ non-removable ink. During P3 of the study, screening swab samples were directly processed and were not frozen and stored.

Double headed swab

Each test was started within 15 minutes of adding the reagents to the cartridge.
The cartridge and reagents were removed from the package. Swabs were removed from the transport container and then one swab was removed from the red cap. The swab was inserted into the tube containing the Elution Reagent (using sterile gauze to minimize risks of contamination). The swab was held by the stem near the rim of the tube, lifted a few millimeters from the bottom of the tube and the stem pushed against the edge of the tube to break it. The cap was closed tightly and vortexed at high speed for 10 seconds. The cartridge lid was opened. Using a sterile transfer pipette, the entire contents of the Elution Reagent were transferred to the "S" chamber of the GeneXpert® MRSA or Xpert vanA/vanB cartridge. Reagent 1 was added into cartridge chamber 1. The ampoule was squeezed until the entire contents were added to the cartridge. Reagent 2 was added into cartridge chamber 1. The ampoule was squeezed until the entire contents were added to the cartridge. Lastly, the cartridge lid was closed.

Figure 1. Xpert MRSA cartridge (top view)

Before starting the test, the Xpert MRSA assay or Xpert vanA/vanB assay definition was imported into the GeneXpert software. For detailed instructions, the GeneXpert Dx System Operator Manual was used.

The test procedure was carried out as listed below:

- Turn on the computer, and then turn on the GeneXpert® Dx instrument.
- On the Windows® desktop, double-click the GeneXpert® Dx shortcut icon.
- Log on to the GeneXpert® Dx System software using your user name and password.
- In the GeneXpert® Dx System window, click "Create Test". The Scan Cartridge Barcode dialog box appears.
- Scan the barcode on the Xpert MRSA or vanA/vanB cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

- In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.
- Click Start Test. In the dialog box that appears, type your password.
- Open the instrument module door with the blinking green light and load the cartridge.
- Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
- Wait until the system releases the door lock before opening the module door and removing the cartridge.
- Dispose of the used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

4. Transport of strains:

Two microbanks with beads and liquid medium were provided to the sites for storage of each strain (one microbank for the MOSAR central laboratory and one microbank as back-up; to be stored at the hospital site). For storage of the strains, the screw cap of the microbank was opened under aseptic conditions. A large disposable 10 µl loop or a swab was used to pick up as many colonies as possible and was dispensed in the microbank medium by rotating the loop several times to make sure the bacteria were completely dispersed in the fluid. The vial was closed tightly, inverted 4-5 times and vortexed for 20-25 seconds to let more bacteria stick to the beads. The vial was opened again and excess fluid removed with a sterile plastic Pasteur pipette. The vial was then re-capped and screwed as tight as possible. Both microbanks from one strain were stored together preferably at -70°C, and if not available at -20°C in the freezer until shipment to the central laboratory.

From each patient, the first isolated strain of MRSA, VRE and each different HRE was shipped to the central laboratory for confirmation and molecular typing. Shipment was in one or more batches per center; 1 microbank per strain was shipped on dry ice in small boxes (one box could hold ~100 microbanks).

Appendix B
Phase 2 procedures

I Chlorhexidine gluconate body washing protocol

Selection of patients

All patients that are present on the ICU on the first day of phase 2 of the trial, and all patients admitted to the ICU after that day will be bathed daily for the whole length of their stay with chlorhexidine gluconate, unless there is a contra-indication. If there is a contra-indication, please note this clearly at the patient's bedspace and use appropriate alternative bathing methods.

Contra-indications

- Burn patients.
- Patients with extensive skin damage, not allowing bathing with chlorhexidine gluconate.
- Patients known to be allergic to chlorhexidine gluconate.
- Patients known to be allergic to one of the components of the product used.

Product

Any product of 4% chlorhexidine (digluconate) that can be dissolved in warm water may be used.

Preparation of washing solution

Dissolve a 4 fluid ounce bottle of 4% chlorhexidine (digluconate) in 6 quarts of warm water (= one washing basin). In the metric system, the amounts equal:

- 120 mL of chlorhexidine in 6 liters of warm water.
- 20 mL of chlorhexidine in 1 liter of warm water.

Both add up to 0.16 grams chlorhexidine (digluconate) per liter.

Bathing the patient

- Wash the face and neck of the patient with warm water without chlorhexidine.
- Take care not to wash any mucous membranes or damaged skin with the prepared washing solution. Wash these parts with warm water without chlorhexidine.
- If the patient is heavily soiled (e.g.: diarrhea), use warm water without chlorhexidine first, to remove the soiling.
- Wash the patient's arms, chest, back, legs and bottom with the prepared washing solution.
- Use the order/ procedure of bathing the patients you always use in daily care.

Body lotion/ skin care

Because chlorhexidine is a cationic molecule, its activity can be reduced by natural soaps, various inorganic anions, non-ionic surfactants, and hand creams containing anionic emulsifying

agents. Use an approved lotion for skin care of the patient; make sure you check this in the package insert of the chlorhexidine product.

Adverse reaction

Chlorhexidine is a product that has been extensively investigated and used. It is registered for the indication of skin antisepsis and side effects have seldom been described. However, with any product adverse reaction can occur. If a patient without a history of allergy against the product develops a (possible) adverse reaction to the chlorhexidine body washing (e.g.: skin rash), do not continue daily bathing with chlorhexidine.

II Hand hygiene improvement program

Design of the intervention

Dedicated research nurses are trained in direct observation of hand hygiene, using the WHO method of observation. Training occurs through centralized full-day teaching sessions, distribution of training materials, and access to all online WHO resources (www.who.int/gpsc/en). The trained research nurses are responsible for observations, and for locally implementing the hand hygiene improvement program after P1, which includes educational sessions, visual reminders and direct feedback after observations. Monthly feedback of local compliance rates is provided to ICUs to guide the content of each local hand hygiene program. Newsletters and monthly conference calls will be initiated to share ideas and address questions related to the conduct of the study. In P3, monitoring and feedback will be continued; whist the hand hygiene improvement program is maintained as much as possible. The five different indications for hand hygiene in the WHO concept are separated into indications that occur before an action ("before patient" and "before aseptic"), and indications occurring after an action ("after patient", "after body fluid" and "after surroundings"). The "before" indications protect the patient, whereas the "after" indications protect health care workers and the patients' environment (and ultimately other patients).

Data collection and monitoring

The WHO form (with minor adaptations for this study, Figure 2) is used to register observed opportunities for HH, and data are entered within a week in an online case record form. During the trial, all sites are visited by the research coordinators. During these visits, methods of observation are reviewed and 10% of the monitoring forms cross-checked with data entered into the online case record form. Written scenarios as well as newsletters and conference calls are used throughout the trial to continuously validate observers' methods.

Informing ICU staff

Prior to the initiation of bedside monitoring, the ICU staff should be informed about the purpose and general procedures for bedside monitoring. ICU staff should be informed that:

- The purpose of these observations is to determine if differences in patient care practices occur:
  1) Between ICUs randomized to the two different strategies during the intervention period in order to assess the effect of both strategies.
  2) Between the baseline and intervention periods in all ICUs to assess the effect of the Hygiene Improvement Program.
- The purpose of these observations is not to evaluate and critique practices of individual healthcare workers or individual ICUs.
- Data will be recorded regarding the opportunities for hand hygiene and the type of action taken. Possible actions taken are handwashing, handrubbing, using gloves or missing the opportunity to perform hand hygiene. No unique identifying data regarding the patient or the healthcare worker will be recorded.
- Monitoring will occur on random dates at random times; bedspaces will be chosen at random.
- Monitors will be present in the room for approximately 30 minutes, or until the maximum amount of opportunities and/ or professionals have been observed. The monitoring form allows observation of a maximum of 9 opportunities for hand hygiene for a maximum of 4 professionals.
- Monitors will stand in a spot where they can observe patient care. Monitors may be asked to move, if necessary.
- Monitors cannot assist in patient care. If a healthcare worker is not present in the room, monitors will notify healthcare workers immediately about urgent patient care issues (e.g.: patient attempting to remove catheter or get out of bed unassisted). Routine requests or questions from patients and family will be referred to a healthcare worker when he or she returns to the room.
- Healthcare workers, patients, or family members may request that the bedside monitor not perform observations or stop observations at any time for any reason.
- The cooperation of healthcare workers with bedside monitoring is greatly appreciated.

Instructions for the bedside monitor

The worksheet/ observation form can be downloaded from the website (www.mosar-sic.org).

- Complete the top portions of the worksheet (date of the performed observation, start & stop time using a 24-hour clock, your initials and the bedspace number).

- Identify the location of the sink for handwashing and any bottles or dispensers of alcohol-based waterless hand rub, gel, or foam in the room or immediately outside the room. Identify where gloves and gowns for use in the room are kept and where they are disposed.
- Identify the first four people to enter the room and assign to them the columns "professional" 1, 2, 3 and 4.
- Observe closely for any opportunity for hand hygiene that occurs, and whenever one occurs, check the appropriate "moment" and corresponding action on the worksheet.
- After completing the observations from the 4-hour interval, the data collected on the worksheets must be entered into the electronic recording system. Data from all sessions, completed and uncompleted, should be entered into the online data capturing system.
- The monitor should save and store the worksheets in a separate binder/folder and file forms in reverse chronological order (i.e. every new worksheet is placed on top of the filed worksheets).

Schedule of monitoring dates and time intervals

- Each site will be supplied with a list of pre-selected dates and 4-hour intervals for monitoring. The following 4-hour time intervals will be used on the schedule: 08:00-12:00 hrs, 12:00-16:00 hrs, 16:00-20:00 hrs. There will be no compulsory monitoring on weekends or at nights.
- A session is defined as the time spent observing one bedside. For example, for the 4-hour time interval of 8 am-12 pm, the observation period could be from 10 am to 11 am and consist of two 30-minute sessions observing one bedspace each.
- If the monitor is unable to perform observations on a scheduled day during the scheduled time-interval, they should plan to "make-up" these observations during the same 4-hour time interval on a date as close as possible to the scheduled date. The make-up day may occur before or after the scheduled day but the time interval must be the same.
- The monitor may make-up no more than 1 observation period on any given day. A make-up observation period may occur on a day that is also a scheduled day but the time intervals for the make-up time interval and the scheduled time interval must remain as originally scheduled.

Selection of bedspaces

- For each 30-minute session, the monitor will identify a bedspace to monitor using a random number table provided at the start of the trial.

  At the start of the study, each bedspace number in the ICU should be associated with numbers 1-20. For example, if the ICU bedspace numbers are 350-365, bedspace 350 would be assigned 1, bedspace 351 would be assigned 2, etc. It is only necessary to do this once unless the bedspace numbers change during the study.

- The monitor will review the list of random numbers and identify the next unused random number on the list. The monitor will determine the ICU bedspace number associated with that number, and cross off the random number on the table so that it is no longer available. If the random number does not have an associated bedspace, that number will be crossed off and the next number will be chosen.
- If the bedspace is occupied by a patient, the monitor will observe the contact by health care workers in that bedspace. If there is no patient in the bedspace, the next random number will be chosen and the associated bedspace will be monitored if it is occupied by a patient.
- If the bedspace is occupied by a patient who is on airborne precautions, that bedspace will not be observed. The next random number will be chosen and the associated bedspace will be monitored if it is occupied by a patient.

Completed sessions

If an observation period ends before 30 minutes, and before the maximum amount of opportunities has been observed, the reason the session was ended early should be recorded on the paper worksheet. If the period was 15 minutes or longer, it qualifies as a completed session. If it was shorter than 15 minutes, the session does not qualify as a completed session. Data from all sessions, completed and uncompleted, should be entered into the electronic record system.

Materials

All materials used, including the "WHO Guidelines on Hand Hygiene in Health Care (revised Aug 2009)" and the "Guide to implementation of the WHO multimodal hand hygiene improvement strategy (revised Aug 2009)", can be found at www.who.int/gpsc/5may/en.

Statistical analysis: To account for the hierarchical structure of the data, hand hygiene compliance is analyzed using a mixed effects logistic regression analysis to determine the effect of the hand hygiene improvement program. A model with random intercept for hospital and random effect per phase per hospital is used to account for between-hospital differences and differing effects of the intervention per hospital. Intervention month and the interaction between intervention month and phase are added to the model to examine trends over time. Measures of association are summarized by odds ratios, displayed with their 95% confidence intervals. Models are compared, and the best model selected, on the basis of likelihood ratio tests. All tests are 2-tailed unless otherwise specified, and $p < .05$ is considered statistically significant. Correlation between compliance and opportunities for HH per hour is assessed by Spearman's correlation coefficient (one-tailed).

Figure 2: Hand hygiene observation form

mosar

Hand Hygiene Observation Form

Date (dd/mm/yyyy): ___ / ___ / ____
Start time (hh :mm): ___ : ___
End time (hh :mm): ___ : ___

Observer (initials): _____
Bedspace _____

Professional 1	Professional 2	Professional 3	Professional 4
☒ Medical doctor ☒ Nurse ☒ Auxillary ☒ Other HCW	☒ Medical doctor ☒ Nurse ☒ Auxillary ☒ Other HCW	☒ Medical doctor ☒ Nurse ☒ Auxillary ☒ Other HCW	☒ Medical doctor ☒ Nurse ☒ Auxillary ☒ Other HCW

Opp	Indication	Action
1	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
2	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
3	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
4	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
5	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
6	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
7	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
8	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves
9	☒ Bef-pat ☒ Bef-asept ☒ Aft-bfluid ☒ Aft-pat ☒ Aft-surr	☒ Rub ☒ Wash ☒ missed ☒ gloves

This form was adapted from the Observation Form, Annex 34 of the Guide to Implementation (WHO/EIP/SPO/QPS/07.2, World Health Organization 2007)

Appendix C
Phase 3 procedures

Indication for contact precautions

I Chromogenic arm

1. Take admission swabs of nose, perineum and wounds (if present).
2. After 24 hrs, preliminary chromagar results are available for MRSA and VRE.
   - If any chromagar shows growth > contact precautions for this patient, according to protocol.
   - If none of the chromagar show growth > no contact precautions, go to point 4.
3. After about 48 hrs, confirmation test results are available.
   - If no MRSA or VRE was confirmed > remove contact precautions.
   - If MRSA or VRE was confirmed > keep contact precautions.
4. On the next Monday or Thursday, new swabs will be taken.
   - If the patient is not on contact precautions when taking these swabs, start this protocol from point 2.
   - If the patient is on contact precautions when taking these swabs.
   - Only remove contact precautions if all cultures and confirmation tests for MRSA and VRE are negative on 2 consecutive days that swabs are obtained according to the surveillance protocol.
   - After removing contact precautions, start this protocol from point 4.

II Molecular arm

1. Take admission swabs of nose, perineum and wounds (if present).
2. After performing MRSA and VRE PCR the result is directly available.
   - If either MRSA or VRE PCR is positive > contact precautions for this patient, according to protocol.
3. After 24 hrs, preliminary Gram-negative chromagar results and “backup” MRSA and VRE chromagar results are available.
   - If any chromagar shows growth > contact precautions for this patient, according to protocol.
     Note: also isolate if chromagar is positive but PCR results were negative.
   - If none of the chromagar show growth and PCR was positive > keep contact precautions for this patient, go to point 5.
   - If none of the chromagar show growth and PCR was negative or invalid > no contact precautions for this patient, go to point 5.

4. After about 48 hrs, confirmation test results are available.
    - If no MRSA or VRE or ESBL was confirmed by chromagar and PCR was positive > keep contact precautions for this patient, go to point 5.
    - If no MRSA or VRE or ESBL was confirmed by chromagar and PCR was negative > remove contact precautions for this patient, go to point 5.
    - If MRSA or VRE or ESBL was confirmed by chromagar and PCR was negative > keep contact precautions for this patient.
5. On the next Monday or Thursday, new swabs will be taken.
    - If the patient is not on contact precautions when taking these swabs, start this protocol from point 3.
    - If the patient is isolated when taking these swabs.
    - Only remove contact precautions if *all* cultures and confirmation tests are negative on 2 consecutive days that swabs are obtained according to the protocol.
    - After removing contact precautions, start this protocol from point 5.

Contact precautions protocol in phase 3

If a patient has an indication for contact precautions the following procedure will be implemented:

- On the bed of the patient a notice/ sign will be clearly visible, stating the micro-organism the patient is colonized with (e.g.: “MRSA positive”).
  Note: Please distinguish signs for “suspected colonization” and “proven colonization”
- The patient will be put in a single room, and gloves and gowns will be used for all procedures involving patient contact. The “contact precautions” rules below should be followed. Supplies for proper hand hygiene should be available at the bedside.
- If you do not have a single room available for the colonized patient, then nurse cohorting should be used in the care of this patient. This means that preferably one nurse should care for all patients colonized with the same micro-organism. Also, gloves and gowns will be used for all procedures involving patient contact. The “contact precautions” rules below should be followed. Supplies for proper hand hygiene should be available at the bedside. Avoid unnecessary contact with healthcare workers.
- If you do not have a single room available for the colonized patient, patients carrying the same micro-organism should be cohorted in double- or multi-patient rooms. Also, gloves and gowns will be used for all procedures involving patient contact. The “contact precautions” rules below should be followed. Supplies for proper hand hygiene should be available at the bedside.
- In multi-patient rooms, 3 or more feet spatial separation of beds is advised.

Contact precautions rules

- Health care workers caring for patients on contact precautions wear a gown and gloves for all interactions that may involve contact with the patient or potentially contaminated areas in the patient's environment. The patient's environment includes the bed, bed linen, equipment, and items and surfaces in the patient's room or bedspace (if the patient is in a multi-patient room).
- Use of gloves and a gown are additional precautions to reduce hand and clothing contamination during care of a patient requiring contact precautions. Use of gloves and a gown is not a replacement for hand hygiene or other elements of standard precautions (i.e., use of mouth, nose, eye protection, as indicated).
- Clean gloves and a clean gown should be put on when entering the patient's room or bedspace and be worn during all contact with the patient and with the patient's environment (all items and surfaces in the patient's room or bedspace).
- Gloves should be changed and hand hygiene performed after direct contact with the patient (before touching items and surfaces in the environment), after contact with blood, body fluids, secretions, excretions, mucous membranes, or non-intact skin.
- If care of the patient will be continued, a new clean pair of gloves should be put on.
- The gown should be changed if it becomes visibly soiled or is likely to have become contaminated (i.e., the healthcare worker has direct, close physical body contact with the patient, such as might occur during transfer from the bed to a chair or commode, or contact with the patient's bed linen, such as might occur during a change of the patient's bed linen).
- If care of the patient will be continued, a clean gown should be put on.
- Gloves and the gown should be removed and hand hygiene performed when leaving the room.
- Contact precautions can be combined with other transmission-based isolation precautions as clinically indicated.

Contact precautions in phase 1 and 2

Use of contact precautions during phase 1 and 2 is according to local protocols, and may include contact precautions for patients colonized or infected with MRSA, VRE or HRE based on clinical culture results, or on surveillance culture results obtained as part of local standard practice. Existing local screening protocols are allowed if based on conventional culture methods, and if decolonization therapies (e.g.: mupirocin, chlorhexidine gluconate) are not used.

Appendix D
Measurement schedule

Measurement	Collection schedule	Collection source
ICU level data:		
Beds available	Weekly	Point prevalence survey
Beds occupied	Weekly	Point prevalence survey
Beds available for mechanical ventilation	Weekly	Point prevalence survey
Nurse to patient staffing ratio	Weekly	Point prevalence survey
Use of invasive devices and TPN	Weekly	Point prevalence survey
Chlorhexidine use	Weekly	Point prevalence survey
Reason for isolation	Weekly	Point prevalence survey
Hand hygiene compliance	+/- 5 Randomly scheduled observations per week	Hand hygiene observation form
Patient level data:		
Long stay (LS) versus short stay (SS) patient	Day 3 of ICU admission	Patient medical record
Demographics	At ICU admission	Patient medical record
Main reason for admission	At ICU admission	Patient medical record
APACHE II or SAPS II	At ICU admission	Patient medical record
Location prior to ICU admission, 28-day mortality, length of stay	At ICU admission and ICU discharge	Patient medical record
Length of hospital stay	At ICU admission and hospital discharge	Patient medical record
History of colonization with MRSA, VRE or HRE	At ICU admission	Patient medical record
MRSA risk factors	At ICU admission	Patient medical record
Surveillance cultures for MRSA, VRE and HRE during ICU stay (date, site and result)	At ICU admission (within 48 hrs) and twice weekly. Frequency reduced to once weekly after 21 days of ICU stay	Cultures collected by experienced ICU staff or research staff
Bacteremia with *S aureus*, Enterococci or *Enterobacteriaceae*	Throughout trial	Patient medical record

TPN = total parenteral nutrition; Long Stay (LS) = patients admitted for 3 days or longer; Short Stay (SS) = patients admitted for less than 3 days; ICU = intensive care unit; APACHE = Acute Physiology and Chronic Health Evaluation; SAPS = Simplified Acute Physiology Score; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus faecium or Enterococcus faecalis* ; HRE = highly resistant *Enterobacteriaceae*

Appendix E
Online tables and figures

Table 1: Number of hand hygiene opportunities observed and mean hand hygiene compliance

	Phase 1		Phase 2		Phase 3	
Hospital	**Opportunities (count)**	**HHC (mean%)**	**Opportunities (count)**	**HHC (mean%)**	**Opportunities (count)**	**HHC (mean%)**
1	648	43.7	1156	61.4	701	71.2
2	939	12.6	884	35.9	589	60.8
3	591	51.1	721	66.0	846	66.5
4	661	73.2	626	77.3	897	73.4
5	2192	49.9	1799	77.6	3445	84.2
6	618	31.2	584	72.4	592	79.1
7	746	89.8	665	82.0	716	81.6
8	1004	52.1	968	65.3	1065	74.2
9	1002	37.0	1360	63.5	1701	72.5
10	1123	6.1	890	42.7	1053	56.4
11	947	62.3	780	69.1	1051	73.4
12	375	45.6	440	63.4	452	81.2
13	3222	73.9	1821	94.5	1688	92.8
Total	**14068**	**51.5**	**12694**	**69.0**	**14796**	**76.7**

HHC = hand hygiene compliance

Table 2: Percentage of patients on contact precautions

	Phase 1	Phase 2	Phase 3, CA	Phase 3, RA
	Mean (%)	**Mean (%)**	**Mean (%)**	**Mean (%)**
Patients isolated for MRSA or VRE	3.9	3.7	4.6	7.8
Patients isolated for HRE	4.3	3.0	1.5	5.9
Patients isolated for any AMRB (total)	8.3	6.7	6.1	13.7
Patients isolated for non-AMRB reasons	5.3	10.6 [a]	11.3 [a]	10.6 [a]
Patients in single room	18.1	20.8	15.2	22.4

[a] = The increase relative to P1 was the result of the 2009 H1N1 Influenza A outbreak, and outbreaks of Clostridium difficile in some hospitals. No breach of protocol could be observed.

Table 3: Time-to-test (TTT) and time-to-result (TTR) for chromagar-based and PCR-based screening, per type of antimicrobial-resistant bacteria [a]

		Median TTT (IQR)	Median TTR (IQR)
PCR	MRSA	22:21 (20:11-25:50)	02:00 (01:50-03:32)
	VRE	22:51 (20:15-26:20)	01:55 (01:14-03:11)
chromagar	MRSA	24:00 (04:00-36:30)	24:00 (23:00-26:00)
	VRE	24:10 (05:38-48:00)	24:30 (23:00-26:00)
	HRE	24:00 (20:05-48:00)	24:00 (23:00-26:40)

PCR = polymerase chain reaction; AMRB = antimicrobial resistant bacteria; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis* ; HRE = highly resistant *Enterobacteriaceae*

[a] = based on P3 results, times in hours and minutes

Table 4: Full results of multilevel segmented Poisson regression models for acquisition of antimicrobial-resistant bacteria, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci and highly-resistant *Enterobacteriaceae*

	AMRB			MRSA			VRE			HRE		
Variable	**aIRR**	**95% CI**	**p value**	**aIRR**	**95%CI**	**p value**	**aIRR**	**95%CI**	**p value**	**aIRR**	**95%CI**	**p value**
P1 trend (days since P1 start)	1.01	(1.00,1.03)	0.12	1.04	(1.01,1.07)	0.01	1.00	(0.97,1.03)	0.99	1.01	(0.99,1.03)	0.25
P2 Step change	0.95	(0.68,1.35)	0.79	1.16	(0.65,2.05)	0.61	0.88	(0.48,1.63)	0.69	0.83	(0.56,1.23)	0.36
P2 Change in trend	0.98	(0.95,1.00)	0.04	0.93	(0.89,0.96)	<0.001	0.98	(0.95,1.02)	0.36	0.99	(0.97,1.02)	0.66
P3 CA Step change	0.63	(0.35,1.15)	0.14	0.75	(0.25,2.26)	0.61	0.65	(0.21,2.03)	0.46	0.53	(0.26,1.05)	0.07
P3 CA Change in trend	1.01	(1.00,1.03)	0.09	1.06	(1.03,1.09)	<0.001	1.02	(0.98,1.05)	0.34	0.99	(0.97,1.01)	0.35
P3 RA Step change	1.07	(0.63,1.85)	0.79	1.31	(0.52,3.29)	0.57	1.13	(0.45,2.85)	0.80	0.89	(0.48,1.64)	0.71
P3 RA Change in trend	1.01	(0.99,1.03)	0.21	1.04	(1.01,1.07)	0.003	1.01	(0.98,1.04)	0.55	0.99	(0.97,1.01)	0.31
Calendar month:												
January	1.00	-	-	1.00	-	-	1.00	-	-	1.00	-	-
February	1.07	(0.78,1.46)	0.68	0.92	(0.53,1.60)	0.77	1.64	(0.91,2.96)	0.10	1.14	(0.82,1.59)	0.44
March	1.12	(0.82,1.54)	0.47	0.75	(0.41,1.37)	0.35	1.48	(0.79,2.78)	0.22	1.13	(0.80,1.61)	0.49
April	1.03	(0.73,1.44)	0.88	0.82	(0.45,1.50)	0.53	1.73	(0.94,3.19)	0.08	0.91	(0.62,1.34)	0.63
May	1.18	(0.86,1.62)	0.31	1.04	(0.59,1.83)	0.90	0.74	(0.35,1.56)	0.43	1.31	(0.93,1.86)	0.13
June	1.13	(0.81,1.57)	0.48	0.81	(0.44,1.49)	0.50	1.59	(0.84,2.99)	0.15	1.15	(0.80,1.65)	0.45
July	1.47	(1.07,2.03)	0.02	1.15	(0.65,2.04)	0.63	2.16	(1.17,3.96)	0.01	1.19	(0.82,1.73)	0.37
August	1.13	(0.81,1.58)	0.47	1.03	(0.57,1.87)	0.91	1.64	(0.88,3.07)	0.12	1.02	(0.70,1.49)	0.91

Table 4: continued

	AMRB			MRSA			VRE			HRE		
Variable	**aIRR**	**95% CI**	**p value**	**aIRR**	**95%CI**	**p value**	**aIRR**	**95%CI**	**p value**	**aIRR**	**95%CI**	**p value**
September	1.37	(1.02,1.84)	0.04	1.24	(0.72,2.13)	0.45	2.34	(1.32,4.14)	0.00	1.26	(0.91,1.76)	0.17
October	1.43	(1.08,1.90)	0.01	1.07	(0.62,1.84)	0.82	2.74	(1.59,4.74)	0.00	1.22	(0.89,1.68)	0.22
November	0.93	(0.68,1.26)	0.63	0.79	(0.44,1.42)	0.43	1.36	(0.74,2.50)	0.32	0.87	(0.62,1.23)	0.43
December	1.01	(0.75,1.36)	0.93	1.09	(0.65,1.84)	0.73	1.42	(0.79,2.56)	0.24	0.94	(0.67,1.31)	0.71
Patient characteristics:												
Intracranial monitoring [1]	1.00	(0.98,1.02)	0.93	1.00	(0.97,1.03)	0.85	1.01	(0.97,1.04)	0.65	1.00	(0.98,1.03)	0.93
Tracheostomy [1]	1.00	(0.99,1.00)	0.19	0.99	(0.98,1.00)	0.17	1.00	(0.99,1.01)	0.84	1.00	(0.99,1.01)	0.95
Arterial line [1]	1.00	(0.99,1.01)	0.77	1.00	(0.99,1.01)	0.44	1.00	(0.99,1.01)	0.54	1.00	(1.00,1.01)	0.59
Central venous line [1]	1.00	(0.99,1.01)	0.87	1.00	(0.99,1.02)	0.40	1.01	(0.99,1.02)	0.33	1.00	(0.99,1.00)	0.60
Parenteral nutrition [1]	1.00	(0.99,1.00)	0.25	1.00	(0.99,1.01)	0.79	1.00	(0.99,1.01)	0.58	1.00	(0.99,1.00)	0.27
Surgical [1]	1.09	(0.63,1.87)	0.76	0.86	(0.34,2.19)	0.76	0.46	(0.18,1.17)	0.11	1.14	(0.62,2.08)	0.67
Proportion male	1.07	(0.64,1.78)	0.81	0.91	(0.36,2.33)	0.85	1.31	(0.52,3.30)	0.56	1.14	(0.63,2.07)	0.66
Median age (years)	1.01	(1.00,1.02)	0.22	1.00	(0.98,1.01)	0.64	1.02	(1.00,1.04)	0.05	1.00	(0.99,1.01)	0.72
Standardized severity [2]	0.97	(0.78,1.21)	0.79	1.19	(0.81,1.74)	0.37	0.92	(0.61,1.39)	0.70	1.08	(0.99,1.01)	0.53
Prior location [3]	1.29	(0.77,2.14)	0.33	1.10	(0.46,2.66)	0.83	2.41	(0.95,6.12)	0.07	1.34	(0.75,2.40)	0.33
Nurse to patient ratio	0.90	(0.50,1.64)	0.73	0.62	(0.24,1.61)	0.33	1.54	(0.53,4.43)	0.43	1.07	(0.48,2.39)	0.87
Random effects	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI				
Intercept	1.13	(0.73,1.74)		1.32	(0.79,2.19)		1.34	(0.74,2.44)		1.14	(0.74,1.77)	
P1 trend	0.01	(0.00,0.01)		0.01	(0.00,0.02)		0.01	(0.01,0.03)		0.01	(0.00,0.01)	

AMRB = antimicrobial resistant bacteria; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis* ; HRE = highly resistant *Enterobacteriaceae* ; CI = confidence interval; aIRR = adjusted incidence risk ratio; P1 = phase 1; P2 = phase 2; P3 = phase 3; CA = conventional arm; RA = rapid arm; LRT = likelihood ratio test.

Shown are model results from multilevel Poisson segmented regression, allowing for ICU-level random effects for the intercept and P1 trend, and with an unstructured covariance matrix. A log link function was used so trend terms are assumed to be exponential. All models included an offset term to account for the weekly exposure in each ICU (number of days at risk for acquiring each organism, where a patient was considered at risk on any day prior to the first positive isolate for the specified organism or, if never positive, prior to the last negative swab).

1 Expressed as a weekly percentage of patients in each ICU.

2 The ICU-specific standardized severity score for each week was calculated from either the mean weekly day 1 APACHE II score for ICUs where this was available or from the SAPS score on admission. For each ICU these mean weekly scores were standardized to have a mean of 0 and standard deviation of 1.

3 Prior location is expressed as the weekly proportion of patients in each ICU who were admitted from a healthcare facility.

Table 5: Full cox regression results of daily hazard of acquisition with methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci and highly-resistant *Enterobacteriaceae*

	MRSA			VRE			HRE		
Variable	**aHR**	**95% CI**	**p value**	**aHR**	**95% CI**	**p value**	**aHR**	**95% CI**	**p value**
P1 trend (days since P1 start)	1.00	(1.00, 1.01)	0.15	1.00	(1.00, 1.00)	0.65	1.00	(1.00, 1.00)	0.37
P2 Step change	1.25	(0.70, 2.23)	0.46	0.97	(0.54, 1.73)	0.92	1.11	(0.77, 1.60)	0.59
P2 Change in trend	0.99	(0.99, 1.00)	0.003	1.00	(0.99, 1.00)	0.06	1.00	(0.99, 1.00)	0.14
P3 CA Step change	1.17	(0.39, 3.49)	0.78	0.91	(0.30, 2.77)	0.87	0.94	(0.49, 1.81)	0.86
P3 CA Change in trend	1.01	(1.00, 1.01)	0.001	1.00	(1.00, 1.01)	0.08	1.00	(1.00, 1.00)	0.58
P3 RA Step change	1.78	(0.69, 4.61)	0.24	2.01	(0.84, 4.82)	0.12	1.45	(0.82, 2.58)	0.21
P3 RA Change in trend	1.01	(1.00, 1.01)	0.01	1.00	(1.00, 1.01)	0.02	1.00	(1.00, 1.00)	0.53
Calendar month:									
January	1.00	-	-	1.00	-	-	1.00	-	-
February	0.88	(0.51, 1.52)	0.65	1.56	(0.86, 2.81)	0.14	1.16	(0.83, 1.60)	0.39
March	0.70	(0.39, 1.28)	0.25	1.73	(0.96, 3.12)	0.07	1.05	(0.75, 1.46)	0.79
April	0.82	(0.45, 1.48)	0.50	2.03	(1.11, 3.71)	0.02	0.78	(0.53, 1.15)	0.20
May	0.90	(0.51, 1.60)	0.73	0.85	(0.42 1.71)	0.65	1.16	(0.83, 1.63)	0.38
June	0.91	(0.52, 1.61)	0.75	1.65	(0.89, 3.06)	0.12	1.20	(0.85, 1.69)	0.31
July	0.99	(0.55, 1.77)	0.96	2.81	(1.56, 5.07)	0.00	1.14	(0.79, 1.64)	0.48
August	1.16	(0.66, 2.03)	0.60	2.07	(1.13, 3.79)	0.02	1.14	(0.80, 1.63)	0.47
September	1.17	(0.68, 2.01)	0.56	2.91	(1.67, 5.06)	<0.001	1.28	(0.90, 1.67)	0.13
October	0.97	(0.57, 1.67)	0.92	2.80	(1.61, 4.87)	<0.001	1.22	(0.90, 1.67)	0.20
November	0.78	(0.44, 1.35)	0.37	1.83	(1.03, 3.27)	0.04	0.93	(0.67, 1.29)	0.65
December	1.00	(0.59, 1.68)	0.99	1.47	(0.82, 2.64)	0.20	0.90	(0.65, 1.26)	0.54
Patient characteristics:									
Male	1.54	(1.19, 1.98)	0.001	0.92	(0.74, 1.14)	0.43	0.90	(0.78, 1.04)	0.14
Age	1.00	(0.99, 1.00)	0.22	1.00	(1.0, 1.01)	0.40	1.00	(1.00, 1.01)	0.28
Standardized severity score [1]	1.23	(1.09, 1.37)	<0.001	1.26	(1.13, 1.40)	<0.001	1.16	(1.08, 1.24)	<0.001
Urgent surgery prior to ICU	0.96	(0.72, 1.28)	0.79	1.44	(1.14, 1.83)	0.002	1.22	(1.01, 1.44)	0.03
Colonization pressure: [2]									
1-3 MRSA/VRE/HRE patients	1.30	(0.95, 1.76)	0.10	1.04	(0.69, 1.55)	0.86	1.39	(1.03, 1.89)	0.03
4-6 MRSA/VRE/HRE patients	1.57	(0.97, 2.54)	0.07	0.91	(0.54, 1.52)	0.71	2.18	(1.53, 3.11)	<0.001
7+ MRSA/VRE/HRE patients	1.64	(0.81, 3.29)	0.17	0.90	(0.52, 1.54)	0.69	1.89	(1.27, 2.76)	0.002

MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis*; HRE = highly resistant *Enterobacteriaceae*; aHR = adjusted Hazard Ratio; P1 = phase 1; P2 = phase 2; P3 = phase 3; CA = conventional arm; RA = rapid arm.

Shown is survival analysis using a Cox regression model stratified by ICU, where failure events correspond to acquisition of MRSA, VRE or HRE and patients are censored at ICU discharge. Acquisition is assumed to occur two days before the first positive isolate. aHRs greater than one indicate that a variable is associated with an increased risk of the failure event in a small time interval; aHRs less than one correspond to decreased risk. Analysis using a frailty model with random effects showed a similar effect (data not shown).

[1] Severity calculated from either the mean weekly day 1 APACHE II score where this was available or from the SAPS score on admission. For each ICU these scores were standardized to have a mean of 0 and standard deviation of 1.

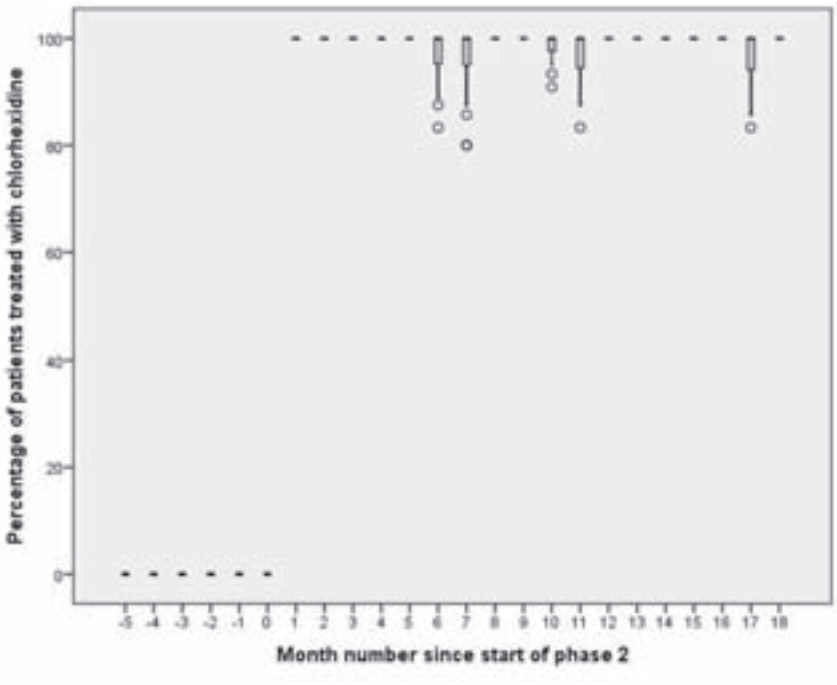
[2] Colonization pressure in the three models corresponds to the number of patients known to be colonized with MRSA, VRE or HRE respectively on each day.

Table 6: Secondary outcomes

	IRR (95% CI)	IRR (95% CI)	OR (95% CI)	IRR (95% CI)	IRR (95% CI)
	LOS in ICU	LOS in hospital	28-day mortality	AMRB bacteremia	HRE bacteremia
Trend P1	1.002	1.002	1.000	1.001	0.928
	(0.997, 1.007)	(0.996, 1.008)	(0.986, 1.015)	(0.972, 1.032)	(0.868, 0.992) *
Step P2	0.998	1.001	0.932	0.930	3.485
	(0.904, 1.102)	(0.879, 1.139)	(0.679, 1.280)	(0.492, 1.760)	(0.823, 14.755)
Trend P2	0.988	0.997	1.002	1.012	1.060
	(0.982, 0.994) ***	(0.989, 1.004)	(0.983, 1.022)	(0.974, 1.051)	(0.968, 1.160)
Step P3, CA	1.082	0.888	0.899	0.633	3.338
	(0.921, 1.270)	(0.720, 1.094)	(0.544, 1.484)	(0.226, 1.775)	(0.239, 46.689)
Trend P3, CA	1.015	1.004	0.995	0.990	1.010
	(1.010, 1.020) ***	(0.998, 1.010)	(0.981, 1.009)	(0.964, 1.016)	(0.939, 1.086)
Step P3, RA	1.180	1.060	1.189	0.610	7.206
	(1.006, 1.384) *	(0.866, 1.297)	(0.721, 1.961)	(0.229, 1.626)	(0.747, 69.541)
Trend P3, RA	1.005	0.998	0.994	0.978	0.983
	(1.000, 1.010) *	(0.992, 1.004)	(0.980, 1.009)	(0.951, 1.005)	(0.921, 1.049)
LRT CA vs RA	p < .001	p = .006	p = .06	p = .09	p = .57

IRR = incidence risk ratio; CI = confidence interval; OR = odds ratio; LOS = length of stay; AMRB = antimicrobial resistant bacteria; HRE = highly resistant *Enterobacteriaceae*; P1 = phase 1; P2 = phase 2; P3 = phase 3; CA = conventional arm; RA = rapid arm; LRT = likelihood ratio test. IRR values smaller than one represent a decrease, while values above one represent an increase. Significance is indicated by * (p < .05), ** (p < .01) or *** (p < .001).

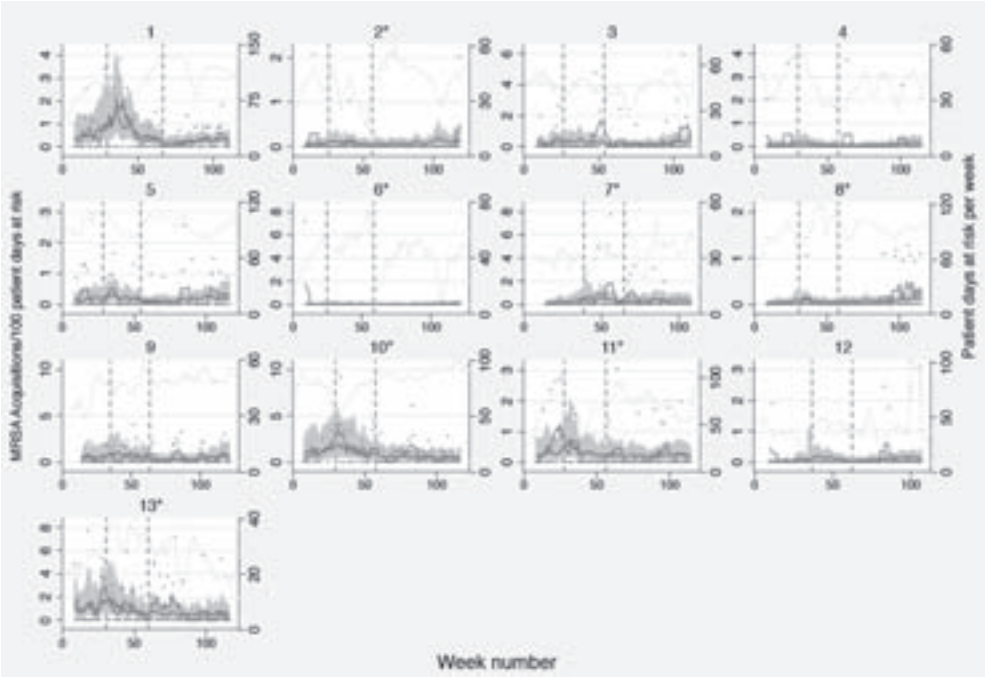
Figure 1: Use of chlorhexidine body washing



Median percentages based on 1,188 point prevalence surveys are shown with inter-quartile range (brown bars), range (black bars) and outliers smaller than or equal to the first quartile minus 1.5 times the inter-quartile range (circles).

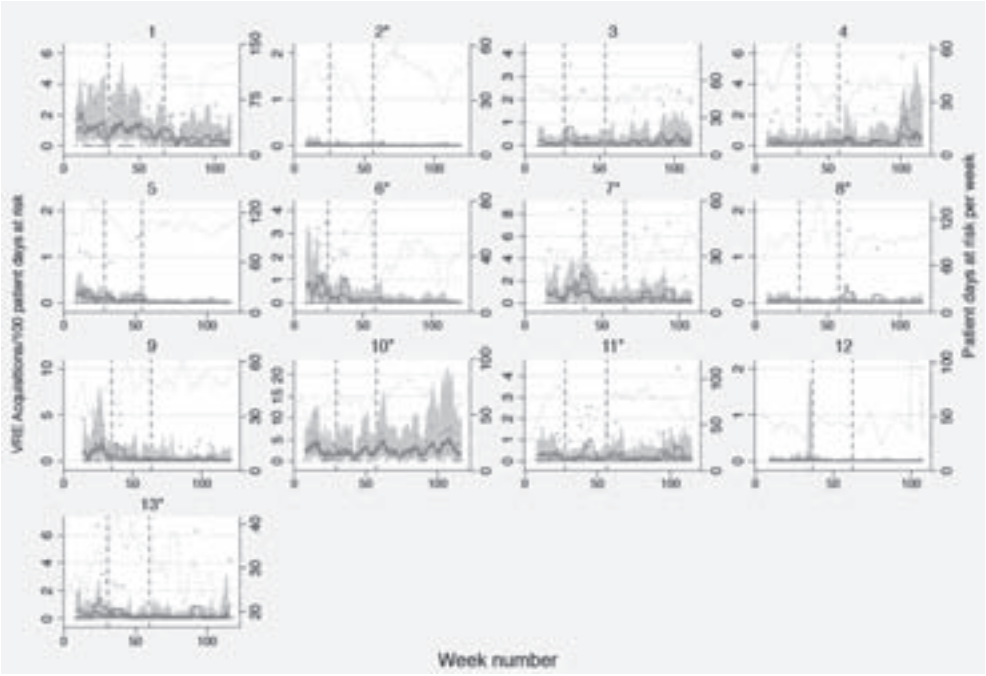
Note: month 1 is the first month of the daily chlorhexidine body washing intervention, which was applied throughout the remaining of the study to all patients in the participating ICUs.

Figure 2: Acquisition of MRSA per hospital



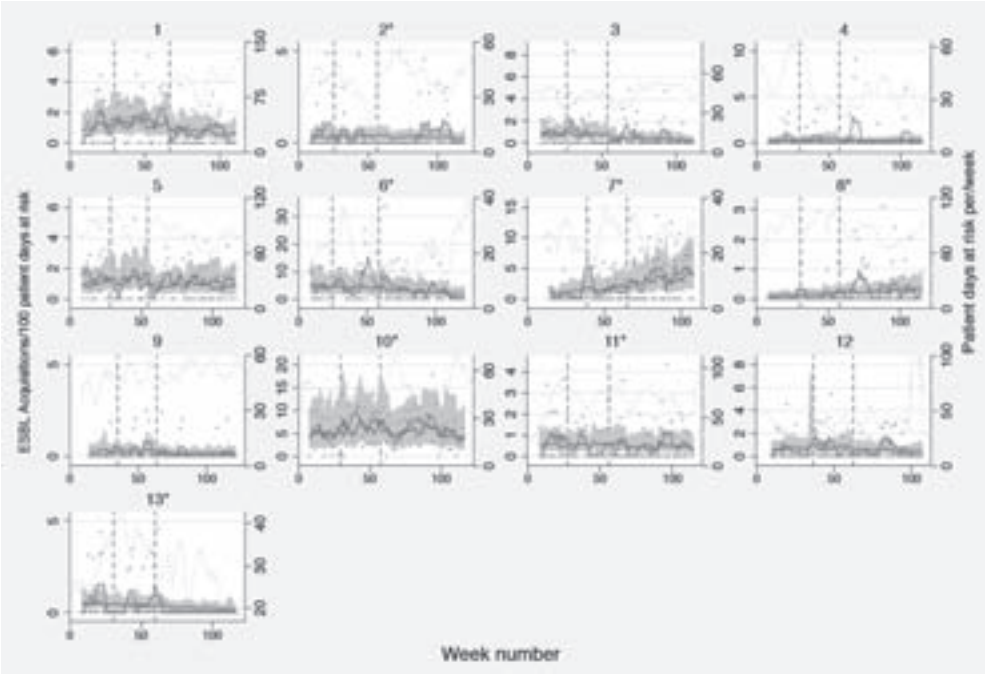
Green dots show weekly acquisitions. Green line represents 7 week moving average of weekly acquisitions. Dotted grey line shows 7 day moving average of number of patient days at risk per week. Red line is expected number of weekly acquisitions predicted by the full multilevel model, and shaded grey area represents 95% intervals for these predictions. Dashed vertical lines show timing of phase 2 and phase 3 interventions. Hospital numbers are shown above each graph; those followed by an asterisk were randomized to the molecular arm.

Figure 3: Acquisition of VRE per hospital



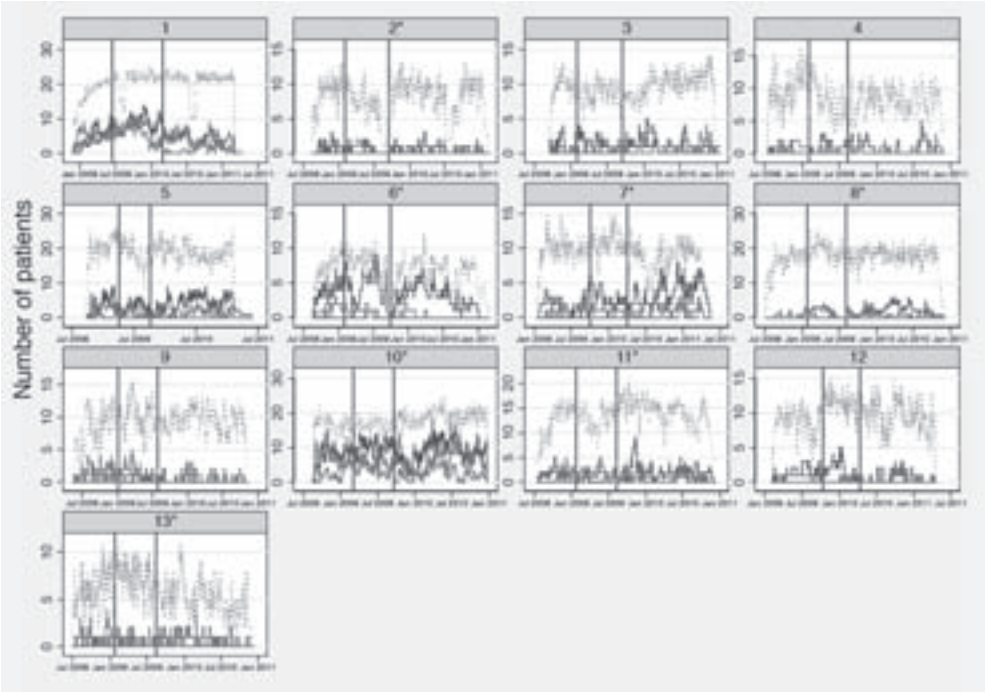
Green dots show weekly acquisitions. Green line represents 7 week moving average of weekly acquisitions. Dotted grey line shows 7 day moving average of number of patient days at risk per week. Red line is expected number of weekly acquisitions predicted by the full multilevel model, and shaded grey area represents 95% intervals for these predictions. Dashed vertical lines show timing of phase 2 and phase 3 interventions. Hospital numbers are shown above each graph; those followed by an asterisk were randomized to the molecular arm.

Figure 4: Acquisition of HRE per hospital



Green dots show weekly acquisitions. Green line represents 7 week moving average of weekly acquisitions. Dotted grey line shows 7 day moving average of number of patient days at risk per week. Red line is expected number of weekly acquisitions predicted by the full multilevel model, and shaded grey area represents 95% intervals for these predictions. Dashed vertical lines show timing of phase 2 and phase 3 interventions. Hospital numbers are shown above each graph; those followed by an asterisk were randomized to the rapid tests arm.

Figure 5: Prevalence of MRSA, VRE and HRE per hospital



Number of patients carrying MRSA is represented in green, VRE in red and HRE in blue. Total patient days of included patients are shown in grey. Hospital numbers are shown above each graph; those followed by an asterisk were randomized to the rapid tests arm.

Improving hand hygiene compliance in 13 European intensive care units:

An intervention study

four

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Submitted

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Improving hand hygiene compliance in 13 European intensive care units: An intervention study

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Abstract

Context: Intensive care patients are at high risk of developing nosocomial infections. Improved hand hygiene reduces the incidence of nosocomial infections, but is generally low in these wards.

Objective: To assess feasibility and effectiveness of the World Health Organization's "My 5 Moments for Hand Hygiene" program in intensive care units.

Design and setting: As part of a multi-center cluster-randomized trial, a hand hygiene program (interrupted time-series design) was implemented in 13 European intensive cares.

Interventions: A 6-month baseline, followed by a 6-month implementation phase of the "5 Moments" program and a 12-month continuation phase.

Main Outcome Measures: In all phases compliance was observed directly through trained monitors, at randomly selected time-intervals and bedspaces. Effects of the intervention on compliance were determined per unit, for different healthcare workers and types of activities.

Results: In all, 41558 hand hygiene opportunities were observed. At baseline, compliance varied from 6% to 90% (mean 52%). In phases 2 and 3 average compliance per unit was 69% and 77% (OR 2.68 and 3.78 for phase 2 and 3 respectively). There was a minor increase in compliance in phase 3 compared to phase 2 (OR 1.41). In all study phases, nurses' compliance was higher than physicians'. In baseline, compliance was better for "after" compared to "before" indications (OR 2.04), this difference increased in phase 2 (OR 5.34) and phase 3 (OR 6.96). In phase 1 there was a negative correlation between compliance and workload ($r = -.05$, $p = .03$), which reversed in phase 3 ($r = .09$, $p = .99$).

Conclusions: Implementation of the "My 5 Moments for Hand Hygiene" program in thirteen European intensive cares rapidly increased hand hygiene compliance from 52% to 69% and even higher during the ensuing twelve months.

Trial Registration: This study was registered on www.clinicaltrials.gov under number NCT00976638.

Introduction

Patients admitted to intensive care units (ICUs) are extremely vulnerable to infections.[1] The intensity of patient care in the ICU, necessitating multiple contacts between healthcare workers (HCW) and patients, facilitates transfer of pathogens from patient to patient through temporarily contaminated hands of HCW. Acquired colonization may subsequently lead to healthcare associated infections (HCAI).

Despite a firm body of evidence that improved hand hygiene compliance (HHC) is associated with lower bacterial transmission rates and reduced incidences of HCAI,[2] HHC is generally low, especially in ICUs.[3] Poor adherence has been associated with HCW category (physicians adhering less than nurses), type of activity performed, frequency of hand hygiene opportunities, nurse-to-patient staffing ratio (NTPSR), type of ward, education and several personal and institutional factors.[2]

In 2006 the World Health Organization (WHO) proposed a draft version, and in 2009 the final version, of a multimodal hand hygiene improvement strategy (the so-called "My 5 Moments for Hand Hygiene" concept), suitable for training, observation, and performance reporting across different health-care settings.[2] This concept summarizes the risk of transmission in five moments, which are considered opportunities for hand hygiene.

These moments are:

1) before touching a patient
2) before a clean/aseptic procedure
3) after body fluid exposure risk
4) after touching a patient
5) after touching patient surroundings.

Quantifying HHC, however, is difficult, and well-designed research is needed.[4] The most widely used method, direct observation of hand hygiene practices by unobtrusive monitors, may influence behavior of HCW and thereby increase compliance, especially when study periods are short. This type of observation bias, generally known as the Hawthorne effect, typically declines with time.[5, 6] Furthermore, validity of direct observation may be reduced because of inter-observer variability.

We performed a cluster-randomized study in thirteen European ICUs to assess the effectiveness of different interventions, including a program based on the WHO "My 5 Moments for Hand Hygiene" concept, to reduce acquisition of antimicrobial resistant bacteria (AMRB). Here we describe, based on more than 41,500 observations, the effectiveness of implementing this hand hygiene improvement program (HHIP) on HHC. The effectiveness of all interventions in reducing acquisition of AMRB will be reported elsewhere.

Materials and methods

Design and intervention: This interrupted time-series study was part of a multi-center

cluster-randomized study, with the ICU as unit of inference, to determine the incremental effects of different interventions in reducing acquisition with antimicrobial resistant bacteria, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci and highly resistant *Enterobacteriaceae*. After a baseline period of six months (P1, no interventions), the HHIP was implemented, together with daily chlorhexidine body washing, during a period of six months (P2, intensive-HHIP). Thereafter, surveillance screening for AMRB carriage at ICU-admission (followed by contact precautions for AMRB carriers) was added, and ICUs were randomized to PCR-based or chromogenic agar-based screening methods, whilst HHIP continued (P3, continued-HHIP). ICUs were eligible if they admitted adult patients only, had a medical, surgical or mixed patient population, and had at least 8 beds, all of which had possibility for mechanical ventilation. The main outcome measure was HHC, through either hand-washing or hand-rubbing, after each opportunity for hand hygiene. We did not assess quality of hand-washing or hand-rubbing.

Dedicated research nurses were trained in direct observation of hand hygiene (HH), using the WHO method of observation. Training occurred through centralized full-day teaching sessions, distribution of training materials, and access to all online WHO resources (www.who.int/gpsc/en). The trained research nurses were responsible for observations, and for locally implementing the HHIP after P1, which included educational sessions, visual reminders and direct feedback after observations. Monthly feedback of local compliance rates was provided to ICUs to guide the content of each local hand hygiene program. Newsletters and monthly conference calls were initiated to share ideas and address questions related to the conduct of the study. In continued-HHIP, monitoring and feedback were pursued; whilst the HHIP was maintained as much as possible. However, the implementation of the rapid processing and feedback of surveillance cultures-strategy obviously limited time spent on HHIP by research nurses. We therefore separate the observations in this study into baseline (6 months), intensive-HHIP (6 months) and continued-HHIP (12 months). The five different indications for hand hygiene in the WHO concept were separated into indications that occur before an action ("before patient" and "before aseptic"), and indications occurring after an action ("after patient", "after body fluid" and "after surroundings"). The "before" indications protect the patient, whereas the "after" indications protect HCW and the patients' environment (and ultimately other patients).

Observation schedule: Observations were performed according to a computer-generated list, with random dates, time slots and beds to be observed. Observers were provided with a list of pre-selected dates and 4-hour intervals for monitoring (08:00-12:00 hrs, 12:00-16:00 hrs and 16:00-20:00 hrs). HCW were not aware of the observation schedule. There were no scheduled observations in weekends or at night. Each site performed four to ten hours of observations each month (12-20 observation sessions of 15-30 minutes each).

Data collection and monitoring: The WHO form (with minor adaptations for this study, (eFigure 1) was used to register observed opportunities for HH, and data were entered within a week in an online case record form (CRF). During the trial, all sites were visited by the research coordinators. During these visits, methods of observation were reviewed and 10% of the monitoring forms were cross-checked with data entered into the online CRF. Written scenarios as well as newsletters and conference calls were used throughout the trial to continuously validate observers' methods.

The study protocol was approved by the institutional review board in each country and each participating ICU, and informed consent of patients and HCW was waived. This study was registered on www.clinicaltrials.gov under number NCT00976638. It was supported by the European Commission under the Life Science Health Priority of the 6th Framework Program (MOSAR network contract LSHP-CT-2007-037941).

Statistical analysis: To account for the hierarchical structure of the data, HHC was analyzed using a mixed effects logistic regression analysis to determine the effect of the HHIP-intervention.[7,8] A model with random intercept for hospital and random effect per phase per hospital was used to account for between-hospital differences and differing effects of the intervention per hospital. Intervention month and the interaction between intervention month and phase were added to the model to examine trends over time. Measures of association are summarized by odds ratios (ORs), displayed with their 95% confidence intervals (CIs). Models were compared, and the best model selected, on the basis of likelihood ratio tests. All tests were 2-tailed unless otherwise specified, and $p < .05$ was considered statistically significant. Correlation between compliance and opportunities for HH per hour was assessed by Spearman's correlation coefficient (one-tailed). Opportunities per hour were calculated by extrapolating data from each session (e.g. two opportunities in a 30-minute session correspond to four opportunities per hour). For comparison of workload groups within phases, a linear mixed model with random effect per hospital and fixed effects for phase, workload group, and their interaction were used. The statistical package R (The R Project for Statistical Computing, www.r-project.org/, v2.10.0) and SPSS (PASW statistics) version 17 were used for all computations.

Results

Participating ICUs (Table 1) were from France (n = 3), Greece (n = 2), Italy (n = 1), Latvia (n = 1), Luxemburg (n = 1), Portugal (n = 2), Slovenia (n = 2) and Spain (n = 1). Between May 2008 and April 2011 41,558 hand hygiene opportunities were observed (Table 2).

Discrepancies between data entered on the original observation forms and the online data entry system were 4.0%. Handrub was used in 84.9%, hand washing in 15.1% and both were used in 1% of all adequate HH actions.

Table 1: baseline ICU characteristics [a]

	Mean (SD)
Total number of beds available	13.7 (4.4)
Ventilation beds (of all beds available,%)	84.7 (18.5)
Beds occupied (of all beds available,%)	93.1 (13.2)
Mechanically ventilated patients (of all patients admitted,%)	63.5 (22.0)
Full Time Equivalents (FTE) physicians	9.1 (5.4)
Full Time Equivalents (FTE) nurses	34.0 (11.4)
Nurse to patient staffing ratio [b]	0.5 (0.2)

[a] = based on 314 point prevalence surveys in phase 1. [b] = 0.5 meaning that one nurse on average took care of 2 patients

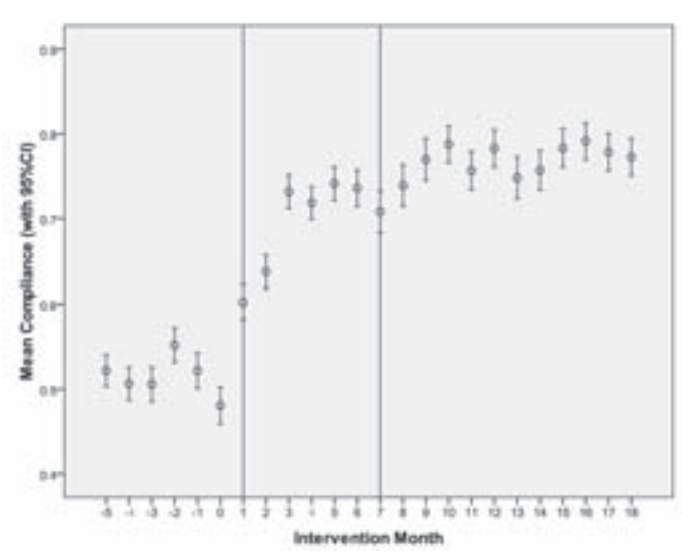
Table 2: Number of hand hygiene opportunities observed and mean hand hygiene compliance (HHC) during the three periods of the study

	Phase 1		Phase 2		Phase 3	
Hospital	Opportunities (count)	HHC (mean%)	Opportunities (count)	HHC (mean%)	Opportunities (count)	HHC (mean%)
1	648	43.7	1156	61.4	701	71.2
2	939	12.6	884	35.9	589	60.8
3	591	51.1	721	66.0	846	66.5
4	661	73.2	626	77.3	897	73.4
5	2192	49.9	1799	77.6	3445	84.2
6	618	31.2	584	72.4	592	79.1
7	746	89.8	665	82.0	716	81.6
8	1004	52.1	968	65.3	1065	74.2
9	1002	37.0	1360	63.5	1701	72.5
10	1123	6.1	890	42.7	1053	56.4
11	947	62.3	780	69.1	1051	73.4
12	375	45.6	440	63.4	452	81.2
13	3222	73.9	1821	94.5	1688	92.8
Total	**14068**	**51.5**	**12694**	**69**	**14796**	**76.7**

In the baseline, HHC varied widely between hospitals (Table 2), with compliance rates ranging from 6% to 90%. The average HHC of all observations during P1 was 52% and increased to 69% in P2, and 77% in P3. The OR for hand hygiene compliance (as compared to baseline) estimated from the mixed model was 2.68 (CI 1.75-4.11) and 3.78 (CI 2.24-6.37) in P2 and P3, respectively. The OR for compliance in P3 compared to P2 was 1.41 (CI 1.22-1.60), demonstrating a persistent

effect of the HHIP in the continued-HHIP phase. For individual ICUs, improvements in HHC were observed for twelve units in P2, and for ten units in P3 (compared to P2). We observed that improvement was absent from units with high levels (>70%) of compliance (1 unit with 89.8% compliance in P2; and 3 units with 77.3%, 82.0% and 94.5% compliance in P3). There were no significant trends for monthly HHC during baseline (OR per month = 1.00; CI 0.98-1.03). Compliance rapidly increased in P2 after initiating the HHIP, with a steep increase in both level and slope, indicating both an immediate intervention effect and a steep learning effect (Figure 1).

Figure 1: Mean hand hygiene compliance (with 95% CI) per month during the 3 phases of the study

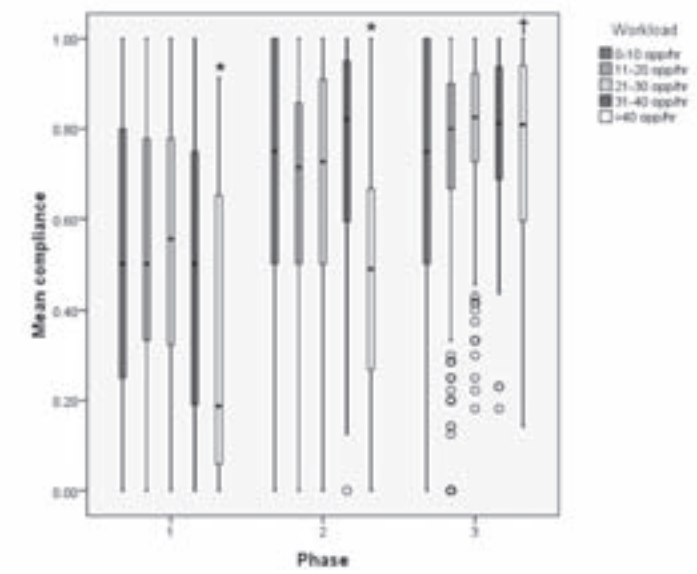


Note: Months -5 to 0 are the 6-month baseline period (P1) with no intervention; month 1 is first month of the hand hygiene improvement intervention

In this phase the OR per month for HHC was 1.14 (CI 1.12-1.17). In the continued-HHIP phase, compliance continued to increase, though only slightly (OR 1.02; CI 1.01-1.04) per month).
For all HCW categories (i.e. physicians, nurses, auxiliary nurses and others) compliance improved after the start of HHIP implementation. As compared to compliance among physicians (36%), nurses and other staff had higher HHC during baseline (57% and 56%, respectively) (Table 3). Although physicians' compliance increased after starting the intervention (OR 2.88 and 3.97, during P2 and P3 as compared to P1), the difference in OR for compliance between physicians and all other groups further increased in phases 2 and 3.
In all phases, HHC was higher for "after" indications (Table 3). The ORs between the two groups of indications increased per phase, indicating a better effect of the program on the "after" than on the "before" indications.

Both NTPSR and the number of observed opportunities for hand hygiene per hour (opp/hr) are regarded as proxy for workload. As NTPSR merely reflects the numbers of nurses present on the ICU each week and opp/hr reflects daily time spent on patient-related care, the latter is preferred. In our study, there was a small but statistically significant inversed relation between HHC and increased workload in the baseline period (r = -.05, p (one-tailed) = .03; online supplement B). The correlation became stronger in P2 (r = -.06, p (one-tailed) < .01). In P3, the correlation reversed (r = .09, p (one-tailed) = .99). Thus, there appears to be a changing effect of the HHIP program on the relation between workload and compliance. Interestingly, the largest effect of the HHIP in P3 occurred in situations with highest workload (Figure 2, eFigure 2-4). At low workload levels (<10 opp/hr), HHC never rose above 72%, and a stable compliance level was already achieved in P2. In "average" workload situations (11-30 opp/hr), compliance increased steadily during the trial. In high workload situations however, we observed a difference between the >40 opp/hr category and the other workload categories during P1 and P2. This difference was no longer observed in P3, indicating that in the last phase, a high compliance is maintained even at extreme workload. As there might have been a change in number of opportunities per hour during the trial, we assessed the effect of time within each phase on the correlation between HHC and workload, and this did not change the results (data not shown), even though highly busy episodes (> 40 opp/hr) occurred less frequently in P3 (eFigure 2-4).

Figure 2: Comparison of mean compliance between workload levels



* = p < .01 for comparison of hand hygiene compliance between >40 opp/hr and 1-40 opp/hr within phases (number of opportunities per hour, opp/hr)

† = comparison of hand hygiene compliance between >40 opp/hr and 1-40 opp/hr within phases not significant (p = .199)

Table 3: Mean hand hygiene compliance (HHC) and adjusted odds ratio's (OR) per phase, for healthcare workers and indication types [a]

		mean HHC (%)	OR (95% CI)
Phase 1	**physicians**	36.20	1*
	nurses	56.62	2.02 (1.81-2.26)
	other	55.94	1.90 (1.62-2.23)
	auxiliary	42.34	1.04 (0.89-1.21)
	before	44.70	1*
	after	58.03	2.04 (1.89-2.21)
Phase 2	**physicians**	53.98	1*
	nurses	73.47	5.89 (5.44-6.34)
	other	68.52	3.59 (3.12-4.05)
	auxiliary	67.72	4.06 (3.60-4.52)
	before	61.90	1*
	after	74.03	5.34 (4.89-5.80)
Phase 3	**physicians**	64.41	1*
	nurses	82.08	9.16 (8.62-9.70)
	other	73.05	4.49 (3.93-5.05)
	auxiliary	71.37	4.77 (4.22-5.32)
	before	71.07	1*
	after	80.57	6.96 (6.41-7.50)

* = Reference category

[a] = ORs estimated from a mixed model including fixed effects of phase, type of professional and interaction with phase, type of indication and interaction with phase; and random effects for hospital and phase

Discussion

In this multi-centre study in thirteen ICUs in eight European countries, implementation of the multifaceted, evidence-based WHO "My 5 Moments for Hand Hygiene" concept was associated with an impressive and sustained increase in HHC. To the best of our knowledge this is the first prospective international multi-centre trial evaluating the effects of the WHO "My 5 Moments for Hand Hygiene" in ICUs. Our findings support the widespread implementation of this program and offer specific aspects for further improvement.

Baseline compliance in our study (52%) was slightly higher compared to the average compliance from literature (38.7%)[2] and to ICU compliance from a previous landmark study from Geneva (36%).[3] If we compare our results to studies on multifaceted hand-hygiene campaigns in adult ICUs using handrub (not handwashing) in the last 15 years, level of compliance achieved in P3 of our study was high (77% versus previously reported 7% to 69%).[3,9-14] Median workload in our study was 14 opp/hr, with 2.1% of observations at a level of >40 opp/hr, while in studies from Geneva >50 opp/hr were not uncommon.[3,11] This is likely explained by the method of observations, rather than a difference in actual workload. Within the WHO "My 5 moments for hand hygiene" program, fewer opportunities per hour are considered than with previously used observation methods.

In our study, the HHIP improved compliance in all categories of HCW, although results were less impressive in the group of physicians, who had lowest HHC rates in all study periods. Our data confirmed previous findings that HHC is higher "after" than "before" patient contact,[3,15-18] and is generally higher among nurses than among all other HCW categories.[3,18,19] As physicians usually have fewer patient contacts per day than nurses, this difference in improvement between HCW categories may not linearly influence cross-transmission of pathogens.[20] Nevertheless, optimal infection prevention can only be achieved if all HCW, including physicians, achieve high levels of HHC.

We found a small but significant inverse association between HHC and workload in P1 and P2. An inverse relationship between workload and compliance has been reported previously, albeit for hand-washing instead of hand-rubbing.[3,11] Our findings now extend this relationship to settings where 85% of hand disinfection procedures are performed with alcohol-based hand rubbing. However, this negative association reversed in P3, suggesting that the effect of workload on HHC disappears when compliance is sufficiently high. Our interpretation of these data is that HCW reach the maximum achievable level of HHC for high workload situations (75%) in P2. In P3, this level can, as might be expected, not be increased much, but HCW maintain their optimal performance, now in extreme workload situations as well, contrary to the situation in P2. These findings underline the importance of continued HHIP programs; and suggest that HH can be improved continuously, albeit with important investments in terms of time and (personnel-) resources to implement and maintain the HHIP as was done in this study.

Strengths of this study include the large number of observations, the robust design and the duration of the study. Direct observation of hand hygiene, using the WHO concept, is based on a large body of evidence, is generalizable throughout the world, and provides detailed insight into barriers towards hand hygiene. Random assignment of time slots and beds to be observed was used to prevent selection bias. Observers were centrally trained and used identical observation and feedback methods in all ICUs. We attempted to minimize inter-observer variability (observer

bias) by providing central training of all observers combined with regular feedback, discussion between observers, and by using at most two observers per site. The international design of our study further enhances generalizability of our findings.

There are also several potential limitations. Direct observations were used to quantify HHC. Although this method is currently considered the gold standard,[2] concerns remain on the potential for inter-observer variability and induction of a Hawthorne effect. Absence of significant changes in monthly HHC rates during the six-month baseline period in our study supports a minimal, and at least stable, Hawthorne effect. We therefore strongly feel that this phenomenon did not affect our results. Recently, HHC was assessed continuously through remote video auditing,[21] which was not feasible in our study. In the aforementioned study, no data were collected on the type of opportunity, and HCW were separated into only two groups (physicians and other HCW) based on clothing. Nevertheless, remote video auditing is a useful addition to hand hygiene research, but cannot replace all aspects of direct observations and feedback.

Another limitation is the absence of observations at night or in weekends, which were omitted due to feasibility. However, we assessed a large number of observations, during both busy and quiet times, and at different NTPSR-levels.

A final limitation is that HHC observations were performed by the same persons responsible for implementing the HHIP. This creates the risk of assessment bias.[22] However, the ultimate outcome of the interventions introduced in this cluster-randomized study will be acquisition with AMRB, which will be based on surveillance culture results, thus excluding the risk of assessment bias.

Conclusions

In conclusion, implementation of a HHIP, based on the WHO concept, in thirteen relatively busy European ICUs was feasible, yielding a rapid and sustained improvement of compliance. The relatively poor performance of physicians and the poor improvement of compliance in "before" indications offer specific aspects for further improvement. A continued-HHIP led to further increase in compliance, which was maintained at a high level even during busy periods.

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The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Online Supplement

eFigure 1: Hand hygiene observation form

mosar

Hand Hygiene Observation Form

Date (dd/mm/yyyy): ___ / ___ / ____
Start time (hh :mm): ___ : ___
End time (hh :mm): ___ : ___

Observer (initials): _____
Bedspace _____

Professional 1

- ☐ Medical doctor
- ☐ Nurse
- ☐ Auxillary
- ☐ Other HCW

Opp	Indication	Action
1	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
2	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
3	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
4	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
5	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
6	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
7	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
8	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
9	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves

Professional 2

- ☐ Medical doctor
- ☐ Nurse
- ☐ Auxillary
- ☐ Other HCW

Opp	Indication	Action
1	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
2	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
3	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
4	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
5	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
6	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
7	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
8	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
9	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves

Professional 3

- ☐ Medical doctor
- ☐ Nurse
- ☐ Auxillary
- ☐ Other HCW

Opp	Indication	Action
1	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
2	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
3	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
4	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
5	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
6	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
7	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
8	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
9	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves

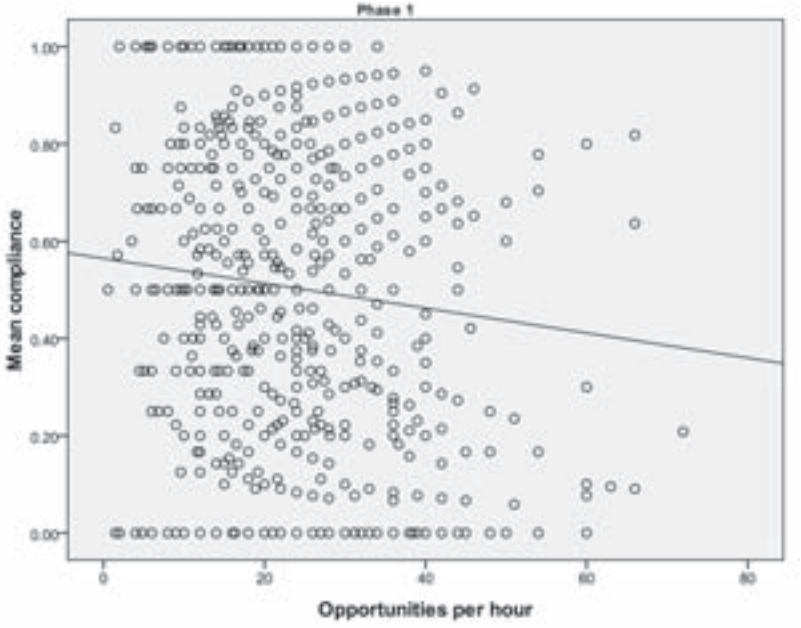
Professional 4

- ☐ Medical doctor
- ☐ Nurse
- ☐ Auxillary
- ☐ Other HCW

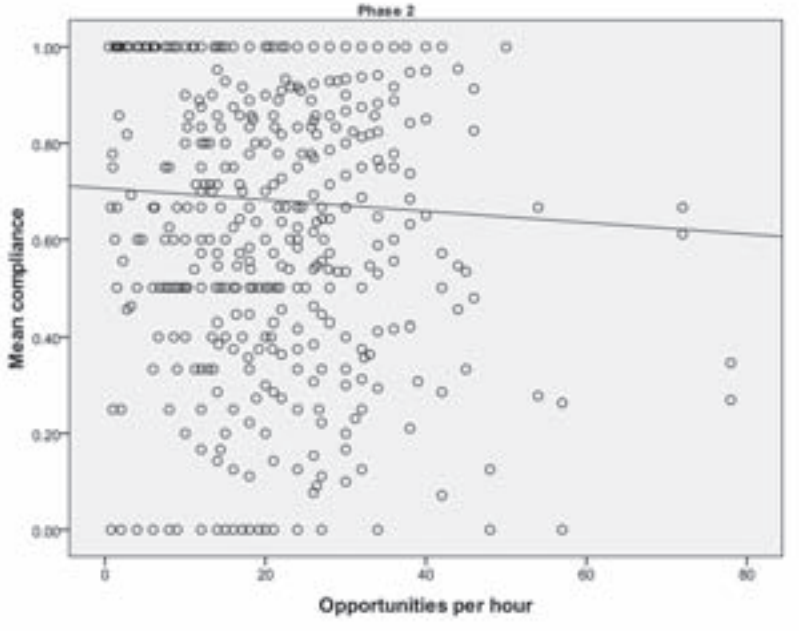
Opp	Indication	Action
1	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
2	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
3	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
4	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
5	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
6	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
7	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
8	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves
9	☐ Bef-pat ☐ Bef-asept ☐ Aft-bfluid ☐ Aft-pat ☐ Aft-surr	☐ Rub ☐ Wash ☐ missed ☐ gloves

This form was adapted from the Observation Form, Annex 34 of the Guide to Implementation (WHO/EIP/SPO/QPS/07.2, World Health Organization 2007)

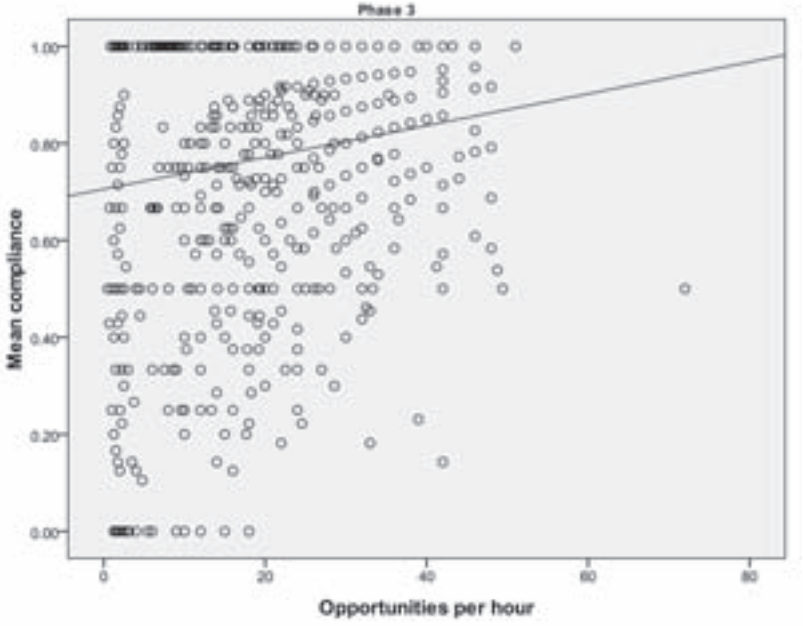
eFigure 2: Association between workload and mean compliance in phase 1



eFigure 3: Association between workload and mean compliance in phase 2



eFigure 4: Association between workload and mean compliance in phase 3



Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in thirteen European intensive care units

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Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in thirteen European intensive care units

D.J. Hetem | L.P.G. Derde | W. Hyrniewicz | J. Empel | H. Goossens | M.J.M. Bonten | on behalf of the MOSAR WP3 study group

Abstract

Background: The European epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) is changing with the emergence of community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). In this study we investigated the molecular epidemiology of MRSA during two-years in 13 ICUs in eight European countries.

Methods: Surveillance cultures for MRSA from nose and wounds were obtained on admission and twice weekly of all patients admitted for at least 3 days. First MRSA isolates per patient were confirmed in a reference laboratory and genotyped by multi-locus sequence typing (MLST), *spa*-typing and SCC*mec* (sub)typing.

Results: In all, 14,390 patients were screened of whom 8,519 stayed in ICU for ≥ 3 days. Overall MRSA admission prevalence was 3.9% and ranged from 1.0% to 6.4% for individual ICUs. Overall MRSA acquisition rate was 2.5/1,000 patient days at risk, and ranged from 0.2 to 8/1,000 patient days at risk per ICU. In all, 631 patients were colonized with MRSA, 550 isolates (87%) were submitted to the reference lab and presence of the *mec*A gene was confirmed in 510 isolates (93%). Each country had a distinct epidemiology, with ST8-IVc (UK-EMRSA -2/-6, USA500) being most prevalent (106 of 510 isolates, 21%), especially in France and Spain, and detected in ICUs in six of eight countries. ST239-III and ST368-III (the Brazilian/Hungarian clone) were dominant in Latvia and Greece. Fifteen (3%) and three isolates (0,6%) were categorized as CA-MRSA and LA-MRSA, respectively.

Conclusions: The molecular epidemiology of MRSA in 13 European ICUs in eight countries was homogeneous within, but heterogeneous between countries. CA-MRSA and LA-MRSA genotypes and PVL-producing isolates were detected sporadically.

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) can colonize and infect hospitalized and non-hospitalized humans. It is the leading nosocomial pathogen, and hospital-acquired MRSA infections are associated with high morbidity and mortality, and increased health-care spending.[1,2] The global epidemiology of MRSA is changing with new strains rapidly emerging

in some countries. Ten years ago MRSA was regarded as a true nosocomial pathogen, mainly affecting patients with health-care exposure, invasive medical devices, high age and undergoing surgical procedures. Since then we have witnessed a rapid increase of MRSA infections occurring in previously healthy non-hospitalized persons, so-called community-associated MRSA (CA-MRSA).[3] The risk factors for developing CA-MRSA infections differ from the traditional health-care related risk factors for health-care associated MRSA (HA-MRSA) infections and include crowding, lack of cleanliness and participation in contact sports. In the United States, CA-MRSA (predominantly USA300) became the most important pathogen for community-acquired skin and soft tissue infections and is now also emerging as a nosocomial pathogen, replacing traditional health-care associated strains.[4] In Europe most nosocomial MRSA infections are still caused by so-called health-care associated MRSA genotypes.[5] Yet, in Europe animals in the agricultural industry have become a large reservoir of livestock-associated MRSA (LA-MRSA, predominantly ST398), currently accounting for 40% of all MRSA colonization and infections in the Netherlands, a country with traditionally low prevalence of MRSA.[6]

Critically ill patients in intensive care units (ICUs) are prone to infections, mainly because of underlying medical conditions, immune-deficiencies and presence of invasive medical devices (e.g. respiratory tubes, central intravascular catheters). It is unknown to what extent the global changes in MRSA epidemiology affect ICU-populations in Europe. We, therefore, determined prevalence, acquisition and molecular epidemiology of MRSA in 13 ICUs in eight European countries that participated in a prospective trial to control transmission of antibiotic resistance in ICU. In this trial, that started with a baseline period of six months (phase 1), the implementation of universal chlorhexidine body washing together with improving hand hygiene (phase 2, six months) was associated with a statistically significant reduction of MRSA-acquisition, which persisted but did not further reduce, in phase 3 (12-15 months) in which admission screening and isolation of carriers was added as control measure.

Methods

We performed a post-hoc analysis from the ICU-trial within the Mastering Hospital Antimicrobial Resistance in Europe (MOSAR) project (www.clinicaltrials.gov number NCT00976638), that evaluated different interventions to reduce transmission of antibiotic-resistant nosocomial pathogens including MRSA, vancomycin-resistant Enterococci (VRE) and highly-resistant *Enterobacteriaceae* (HRE) in ICUs. The study was conducted in thirteen ICUs from 8 European countries: France (3 ICUs); Latvia; Portugal (2 ICUs); Italy; Greece (2 ICUs); Slovenia (2 ICUs); Spain and Luxembourg. Written approval of the study protocol was obtained from each institution's review board or national ethics committee as appropriate.

Design and data collection

Data were obtained during the clinical trial that comprised a 6 months baseline period (phase I), a 6 months period with implementation of a hand hygiene improvement program (largely based on the WHO "5 moments" program), and feedback of compliance to personnel, as well as universal chlorhexidine body washings (II), followed by a 12 to 15 month cluster-randomized intervention phase (phase III).

In phase III six ICUs were randomized to chromogenic agar-based screening for MRSA and seven were randomized to PCR-based screening for MRSA, both including feedback of screening results (from either cultures on chromogenic media or molecular tests) to personnel, and the use of contact precautions for identified carriers.

From all patients admitted for 3 days or longer nasal and wound swabs were obtained within 48 hours of ICU-admission and twice weekly thereafter. Culture frequency was reduced to once weekly after 21 days of ICU admission. Swabs were tested in local laboratories for the presence of MRSA by chromogenic agar for MRSA detection (BBL CHROMagar MRSA II, Becton, Dickinson and Company, FranklinLakes, NJ USA) throughout the trial. ICUs randomized to rapid MRSA detection by PCR additionally used a GeneXpert real-time PCR system in phase III (Cepheid, Sunnyvale, CA, USA) on the admission swabs. All participating laboratories were required to perform proficiency panels for MRSA detection.[7] Colonization was considered ICU-acquired if detected on or after the third day of ICU-admission, in the absence of colonization on admission.

Typing and definitions

The first MRSA isolate of each patient was sent to the central laboratory for confirmation and genotyping. Here, all isolates were re-identified using routine microbiological methods, including slide agglutination (Prolex, Staph Xtra Latex Kit; PRO-LAB Diagnostics, Richmond Hill, ON, Canada) in combination with coagulase. The presence of *mec*A and *luk*S/*luk*F genes, indicative of presence of Panton-Valentine leucocidin, were determined by PCR as described elsewhere.[8,9] All MRSA isolates were characterised by *spa* typing, using the Ridom's StaphsType program to allocate *spa* types.[10] Accessory gene regulator typing (*agr*) and staphylococcal cassette chromosome *mec* (SCC*mec*) typing and sub-typing were performed as previously described.[11-14] Multi-locus sequence typing (MLST) as described by Enright et al. was performed on all isolates.[15] MLST sequence types ST398 and ST130 were considered LA-MRSA.[16,17] CA-MRSA genotypes were defined as belonging to the following: ST8, *pvl* positive, SCC*mec* IVa (USA300); ST30, SCC*mec* IVc (the Southwest Pacific clone); ST1, SCC*mec* IVa (USA400); ST80, SCC*mec* IVc (the European clone); ST59, SCC*mec* V (Taiwan clone); ST93, *pvl* positive, SCC*mec* IV (Queensland clone); ST88, *pvl* positive, SCC*mec* V (Balkan clone); ST152, SCC*mec* V.[18,19] Simpson's index of diversity was calculated as previously described using Ridom's EpiCompare software (version 1.0).[20,21]

Results

Admission prevalence and acquisition

During the study period of 24-27 months 14,390 patients were screened upon admission of whom 8,976 were admitted for at least three days and 8,519 had at least one nasal or wound swab during ICU admission. MRSA prevalence on ICU admission was 3.9% across all ICUs during the study period, and was 4.3%, 4.2% and 3.7% during phase I, phase II and phase III, respectively. The highest admission prevalence (5.7%) was observed in one of the Greek ICUs, followed by a French (4.8%) and Portuguese ICU (4.4%). Admission prevalence in other ICUs ranged from 1.3% - 4% (Table 1). Patients colonized on ICU admission were admitted from a hospital ward, long-term care facility or directly from home in 69%, 4% and 26% respectively.

The overall MRSA-acquisition rate was 2.5 per 1,000 patient days at risk during the two year study period and 3.5, 3.1 and 2.0 per 1,000 patient days at risk during phase I, II and III, respectively. Acquisition rates for individual ICUs ranged from 0.5 - 8.0 per 1,000 patient days at risk, being highest in Latvia and lowest in Spain (Table 1).

Fifty patients (0,6%) had MRSA bacteremia: 17 on admission, 2 before admission and 31 acquired during ICU stay. Twenty-three (78%) of 31 patients with ICU-acquired MRSA bacteremia were colonized on admission (n=7) or acquired MRSA colonization during ICU-stay (n=16).

Genotyping

In all, 631 patients were colonized with MRSA according to local test results, and 550 putative isolates (87%) were submitted to the reference laboratory, of which 510 (93%) were confirmed as MRSA. Thirty-three (6%) were methicillin-susceptible *S. aureus* (MSSA), six (1%) were coagulase-negative staphylococci (CoNS) and one (<1%) appeared a non-staphylococcal species. Of the 510 MRSA isolates 492 (97%) were categorized as HA-MRSA, 15 (3%) as CA-MRSA and 3 (<1%) as LA-MRSA. One isolate could not be typed. The Brazilian/Hungarian clone (ST239-III (n = 41) and ST368-III (n = 100)) was most prevalent. ST368-III was dominant in the Latvian ICU (100 of 106 (94%) isolates) and ST239-III in the two Greek ICUs (34 of 65 (52% isolates) (Table 1). There were 106 ST8-IVc (UK-EMRSA -2/-6, USA500) isolates, which was the most prevalent type in ICUs in France and Spain (France: 91/166 (55%), Spain 7/18 (39%)), and this type was detected in eight ICUs in six of eight countries. ST22-IVh (UK EMRSA-15) was detected in seven ICUs in five countries. All participating ICUs had a distinct molecular epidemiology and there was little homogeneity in isolated genotypes between countries. Only ICUs from two sets of countries (France and Spain; Italy and Luxembourg) shared dominant clones. A high level of homogeneity in sequence types existed in ICUs within the same country. (Table 1)

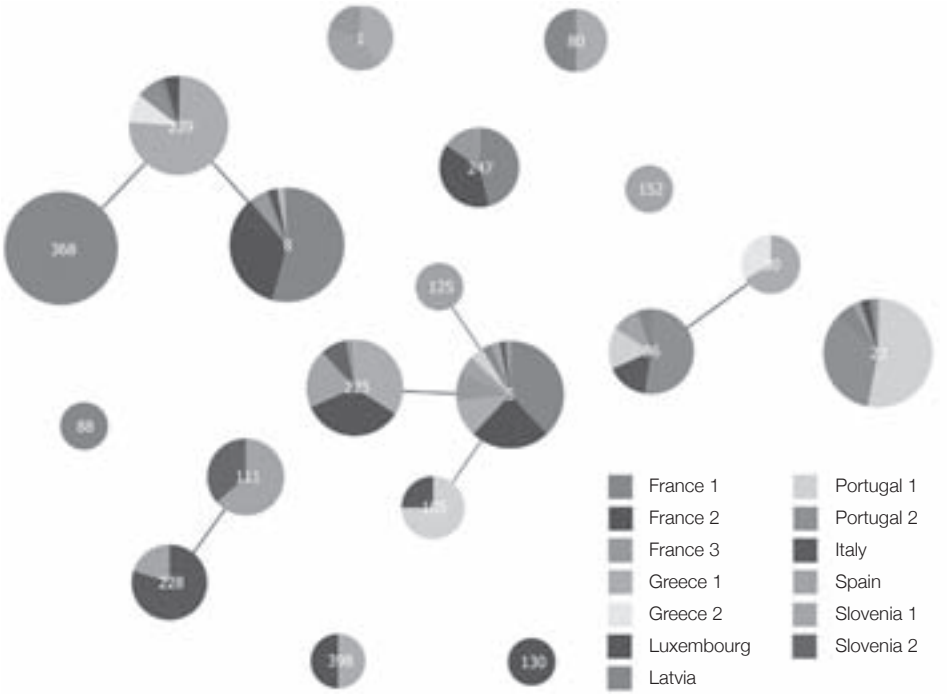
Typing of 34 of 50 (68%) MRSA bacteremia isolates from 10 ICUs revealed 10 different clones of which two were CA-MRSA (ST80-IVc and ST1-IVa). MRSA bacteremia genotypes were similar to the dominant genotypes in the ICU and 26 (87%) belonged to the six most isolated genotypes.

Table 1: Molecular epidemiology of MRSA strains across countries and hospital ICUs

Country/ Hospital	Patients screened	Colonization at admission	Colonization through acquisition	Acquisition/1000 pt.days at risk	Typed MRSA isolates	Number of ST	Most common isolated clones			Index of diversity (95% CI)
	n = 8519	n =335	n=296		n = 510		1st (%)	2nd (%)	3rd (%)	
France:										
ICU 1	1419	77 (5.4%)	22 (1.6%)	1.4	97	8	ST8-IVc 58 (59%)	ST5-VI 17 (17%)	ST247-I 6 (6%)	0.57 (0.48-0.65)
ICU 2	666	33 (5.0%)	23 (3.5%)	2.3	52	4	ST8-IVc 29 (56%)	ST5-VI 13 (25%)	ST247-I 5 (10%)	0.58 (0.49-0.67)
ICU 3	502	14 (2.8%)	5 (1.0%)	0.8	17	6	ST8-IVc 6 (35%)	ST5-VI 3 (18%)	ST247-I 2 (12%)	0.77 (0.65-0.89)
Latvia:	921	40 (4.3%)	90 (9.8%)	8.0	106	3	ST368-III 100 (94%)	ST22-IVa 4 (4%)	ST5-IVa 1 (1%)	0.11 (0.03-0.19)
Portugal:										
ICU 1	408	26 (6.4%)	28 (6.9%)	6.8	51	4	ST22-IVh 41 (80%)	ST36-II 3 (6%)	ST105-II 3 (6%)	0.32 (0.15-0.48)
ICU 2	615	19 (3.1%)	34 (5.5%)	4.5	42	5	ST22-IVh 27 (64%)	ST36-II 10 (24%)	ST239-III 3 (7%)	0.54 (0.40-0.68)
Italy:	534	20 (3.7%)	13 (2.4%)	1.9	18	6	ST228-I 8 (44%)	ST8-IVc 3 (17%)	ST36-II 3 (17%)	0.77 (0.62-0.9)
Greece:										
ICU 1	704	49 (7.0%)	52 (7.4%)	3.8	60	7	ST239-III 30 (50%)	ST225-II 12 (20%)	ST5-II 8 (13%)	0.65 (0.55-0.76)
ICU 2	268	6 (2.2%)	1 (0.4%)	0.2	5	2	ST239-III 4 (80%)	ST30-Vc 1 (20%)	--	0.4 (0.0-0.83)
Slovenia:										
ICU 1	422	17 (4.0%)	12 (2.8%)	1.7	19	6	ST111-I 7 (37%)	ST225-II 7 (37%)	ST228-I 2 (11%)	0.75 (0.63-0.87)
ICU 2	505	5 (1.0%)	4 (0.8%)	0.6	7	2	ST111-I 4 (57%)	ST225-II 3 (43%)	--	0.57 (0.47-0.68)
Spain:	638	17 (2.7%)	3 (0.5%)	0.5	18	6	ST8-IVc 7 (39%)	ST5-IVc 3 (17%)	ST22-IVh 2 (11%)	0.77 (0.62-0.91)
Luxembourg:	917	12 (1.3%)	9 (1.0%)	0.7	18	6	ST225-II 12 (67%)	ST8-IVc 2 (11%)	ST22-IVh 1 (6%)	0.56 (0.30-0.83)

Notes: MRSA: methicillin resistant *Staphylococcus aureus*, ST: sequence type, CI: confidence interval.

Figure 1: Clonal distribution of MRSA in European ICUs



In eBURST analysis MRSA isolates were grouped in eleven clonal complexes, with 262 isolates (51%) belonging to CC8. Other prevalent clonal complexes were CC5 (n = 132, 26%) and CC22 (n = 79, 16%) (Figure 1).

SCC*mec* typing revealed 218 (43%) SCC*mec* type IV, 142 (28%) SCC*mec* type III, 71 (14%) SCC*mec* type II, 40 (8%) SCC*mec* type I, 34 (7%) SCC*mec* type VI, 4 (1%) SCC*mec* type V and one SCC*mec* type XI (Table 3).

In seven (1%) isolates, obtained in five countries, *pvl* was detected, of which six were detected in CA-MRSA (Table 2). Typing of *agr* revealed type 1 in 343 (67%) isolates. In three ICUs from three countries (Italy, Slovenia and Luxembourg) *agr* type 2 was most prevalent. In two isolates *agr* was untypeable.

Community-associated MRSA and livestock-associated MRSA

Fifteen (3%) isolates were, according to the molecular epidemiological definitions used, CA-MRSA, of which seven (47%) were from the Greek ICUs. Five patients carried ST1-IVa, *pvl* negative (USA400), four *pvl* positive ST80-IVc (the European clone), three *pvl* positive ST30-IVc (the South-West Pacific clone) and one USA300 (ST8-IVa, *pvl* positive). Three patients carried LA-MRSA: two in Luxembourg (ST398-IVa and ST130-XI) and one in Greece (ST398-IVa).

Table 2: Common isolated clones and genetic characteristics

Clone		***Spa* types**	***pvl***	***agr*type**
	n=510	**Top 3**	**n (%)**	**type (%)**
HA-MRSA	492		1* (<1%)	1 (69%), 2 (27%), 3 (4%), NT (<1%)
ST8-IVc	106	t008 (76%), t304 (9%), t024 (4%)	0	1 (100%)
ST368-III	100	t425 (78%), t3563 (19%), t4410 (2%)	0	1 (98%), NT (2%)
ST22-IVh	75	t747 (59%), t032 (25%), t020 (3%)	0	1 (100%)
ST239-III	41	t037 (95%), t030 (2%), t945 (2%)	0	1 (100%)
ST225-II	35	t003 (97%), t4336 (3%)	0	2 (100%)
ST5-VI	34	t777 (97%), t179 (1%)	0	2 (100%)
ST36-II	19	t018 (95%), t012 (5%)	0	3 (100%)
ST247-I	13	t052 (69%), t024 (23%), t844 (8%)	0	1 (100%)
ST5-II	13	t002 (54%), t688 (15%), t895 (15%)	0	2 (100%)
ST111-I	11	t041 (91%), t9393 (1%)	0	2 (100%)
Other clones	**45**		**1***	
CA-MRSA	15	t127 (33%), t044 (27%), t018 (13%)	6 (40%)	3 (87%), 1 (7%), 2 (7%)
LA-MRSA	3	t011, t899, t1736	0	1 (100%)

Note: *pvl* positive isolate: ST5-IVc (1/6, 17%).

Table 3: SCC*mec* (sub)types identified

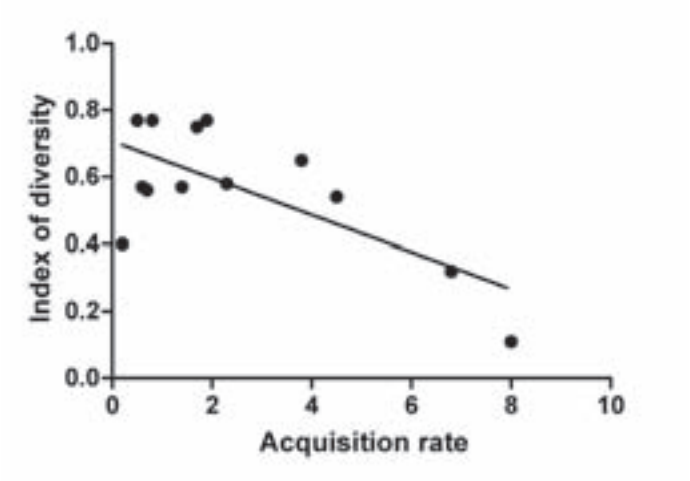
SCC*mec* type	**n = 510 (%)**
I	40 (8%)
II	71 (14%)
III	142 (28%)
IV	218 (43%)
IVa	21 (4%)
IVc	120 (24%)
IVg	1 (<1%)
IVh	75 (15%)
IV-NT	1 (<1%)
V	4 (1%)
VI	34 (7%)
XI	1 (<1%)

Note: SCC*mec*: staphylococcal cassette chromosome *mec*, NT: non-typeable.

Genetic diversity

The Simpson's index for genetic diversity was 0.86 (95% CI: 0.85-0.87) for all ICUs combined, without significant differences between the three study phases (pI: 0.87, 95% CI 0.84-0.89; pII: 0.87, 95% CI 0.86-0.89; pIII: 0.84, 95% CI 0.81-0.86). The index for individual ICUs ranged from 0.11 to 0.77 (Table 1) and genetic diversity was inversely correlated to acquisition rate (ß= -0.055, 95% CI: -0.092, -0.018, p = .007) (Figure 2). Diversity was lowest in the ICU from Latvia (0.11, 95% CI: 0.03 - 0.19) and from Portugal (0.32, 95% CI: 0.15 - 0.48). No significant differences were found between ICUs within the same country.

Figure 2: Relation between diversity and acquisition rate



Discussion

This descriptive post-hoc analysis of a multinational prospective study in 13 European ICUs across 8 countries reveals that the molecular epidemiology of MRSA was homogeneous within, but heterogeneous between countries and that CA-MRSA and LA-MRSA genotypes and PVL-producing isolates were detected sporadically.

The homogeneity of sequence types between ICUs within the same country may result from the geospatial distribution of ICUs. Participating ICUs from France were situated in the region of Île-de-France, both hospitals from Greece were in Athens and ICUs from Slovenia both around Ljubljana. Only the two ICUs in Portugal were 100 km apart. A regional distribution of MRSA in Europe has been described.[22] Our findings confirm these previous findings, but extrapolation of our results of 13 individual ICUs in 8 different countries is not possible.

During the two-year study period 15 patients with CA-MRSA and seven patients with *pvl* positive genotypes were identified among 14,390 patients. In 4 patients carriage with CA-MRSA or *pvl* positive genotypes was detected during the first 48 hours of ICU-admission. For comparison, in 18 ICUs in the United States, in which a similar surveillance strategy was applied in

2006, 626 of 5512 patients (11,3%) were colonized with MRSA at ICU-admission, of which 30 of 210 (14%) typed isolates were considered CA-MRSA. The majority of these isolates was USA300.[23] More recently, CA-MRSA, and particularly USA300, has become the dominant clone in some US hospitals with evidence of replacement of HA-MRSA.[4,24]

The lower admission rate of CA-MRSA in these European ICUs, as compared to ICUs in the US, is not well understood, and more detailed analyses are warranted. Furthermore, little is known about the transmission capacity of different MRSA genotypes in ICUs and the hospital setting. There is evidence of lower transmissibility of CA-MRSA strains in Danish hospital settings,[25] and of LA-MRSA in Dutch hospitals.[26,27] Only three LA-MRSA were detected in the current study, which might result from the localization in urbanized areas of all ICUs participating. LA-MRSA is predominantly found in rural areas with a high density of pigs and pig farmers.[28]

Strengths of this study include the rigorous screening using standardized methods in 13 ICUs and their local microbiology laboratories, and the extensive centralized genotyping. The approach of surveillance of carriage, rather than investigating clinical isolates, yields a comprehensive and more complete representation of MRSA prevalence and epidemiology in the participating ICUs. As a result we could accurately quantify missed isolates (13%) and misclassification of isolates (7%) by local laboratories. Fifty-five per cent of these misclassified isolates were from swabs taken at admission and 73% came from two ICUs. Therefore, admission rates and acquisition rates as reported, are slightly overestimated.

Yet, the surveillance schedule applied, including nasal and wound swabs only, may also have induced some underestimation of MRSA carriage, as screening of additional body sites, e.g. perineum and/or throat, may increase sensitivity of MRSA detection.[29]

In conclusion, the molecular epidemiology of MRSA in 13 European ICUs in eight countries appeared diverse and both CA-MRSA and LA-MRSA genotypes were rarely identified.

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Epidemiology of resistance in Gram-negative bacteria in European intensive care units

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Epidemiology of resistance in Gram-negative bacteria in European intensive care units

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Abstract

Introduction: There is a worldwide increased incidence of infections caused by multidrug resistant Gram-negative bacteria in intensive care units (ICUs). Here we describe the molecular epidemiology of highly resistant *Enterobacteriaceae* (HRE) in 13 European ICUs.

Methods: HRE were detected through screening (on admission and twice weekly) of perineum and wounds (if present) between May 2008 and April 2011. HRE were defined as *E. coli*, *Klebsiella / Enterobacter / Serratia / Citrobacter* (KESC) and *Proteus / Providencia / Morganella* growing on Brilliance ESBL 2 Agar and with a positive double disk synergy test (DDST), and those were subjected to species determination (Vitek 2), phenotypic detection of ESBLs and AmpC-like cephalosporinases (ESBL DDST), presence of acquired AmpC types (multiplex PCR), susceptibility to carbapenems (disks), presence of metallo-beta-lactamases (DDST) KPC types (combined-disk test) and OXA-48 types (temocillin disk test confirmed by PCR).
From six ICUs 57 consecutive isolates of *E. coli* and *K. pneumoniae* underwent pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and identification of $bla_{\text{CTX-M-1-}}$, $bla_{\text{CTX-M-2-}}$, $bla_{\text{CTX-M-9-}}$, $bla_{\text{SHV-}}$, $bla_{\text{TEM-}}$, AmpC, $bla_{\text{CMY-2-}}$like, $bla_{\text{VIM-1-}}$, and $bla_{\text{KPC-}}$like genes by specific PCRs. Simpson's index of diversity was calculated for ICUs with at least 15 isolates for both *E. coli* and *K. pneumoniae* typed by PFGE.

Results: Colonization with HRE was documented in 1,639 of 14,390 admissions, and 1,753 isolates from 1,453 admissions were included, of which 157 *E. coli* and 171 *K. pneumoniae* isolates were genotyped. HRE admission and acquisition rates were 6.3% and 8.9%, respectively, and varied from 1.1% to 13.6% for admission and from 1.3% to 30.2% for acquisition between ICUs. ESBL was detected in 1,661 isolates (94.8%). Carbapenemase-producing HRE were detected in eight ICUs, yielding 82 isolates harbouring KPC (in 3 ICUs), 75 isolates harbouring MBL (in 7 ICUs) and 2 isolates harbouring OXA-48. CTX-M-15 was the most prevalent ESBL in both *E. coli* and *K. pneumoniae*. In *E. coli* ST131 CTX-M-15 was the most prevalent clone. Based on indices of diversity there was more clonality of *K. pneumoniae* than of *E. coli* in ICUs.

Conclusion: This study reveals large variations in HRE admission rates, acquisition rates and resistance mechanisms in 13 European ICUs. Based on genotyping, there seems to be a higher transmission capacity of *K. pneumoniae* than *E. coli* in ICUs.

Introduction

Antimicrobial resistance is an emerging problem in intensive care units (ICUs) worldwide, with an increase in number of infections caused by multidrug-resistant Gram-negative bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae*. ICU patients are prone to infections, because of underlying medical conditions, immunodeficiencies and presence of invasive devices. The potential of transmitting resistance in Gram-negatives is high, as resistance genes, encoding extended-spectrum beta-lactamases and carbapenemases, are located on plasmids that can be easily exchanged between different *Enterobacteriaceae* species.

The prevalence of ESBL-producing bacteria varies considerably in Europe, and infection rates of infections caused by Gram-negative ESBL-producing bacteria are increasing. *E. coli* are most prevalent among infections with ESBL-producing bacteria, with resistance rates to third generation cephalosporins in *E. coli* bloodstream isolates ranging from 2.6% to 24.8% in 2010 across European countries.[1] Furthermore, a specific *E. coli* clone (O25b-ST131), associated with plasmid-borne CTX-M-15-genes and fluoroquinolone-resistance, has been associated with increasing infection and colonization rates.[2-4] Compared to *E. coli*, the prevalence of ESBL-producing *K. pneumoniae* among bloodstream isolates in different countries varies even more (from 1.7% to 75.6%).[1] Nosocomial outbreaks of ESBL-producing *K. pneumoniae* frequently occur across Europe, often associated with CTX-M-15.[5-7]

Carbapenems are increasingly used as treatment for infections with ESBL-producing bacteria, and a rapid increase in infections caused by carbapenemase-producing bacteria (e.g., KPC, NDM-1, VIM, and OXA-48) is currently witnessed. In Europe carbapenemases appear to be most prevalent in Greece, where, KPC and VIM are endemic, with monoclonal spread of KPC-2.[7] VIM is characterized by polyclonal spread *K. pneumoniae*.[8] Nosocomial outbreaks in other countries are culminating and are often related to patients transferred from endemic settings.[9-12]

Here we describe – in detail – the molecular epidemiology of highly resistant *Enterobacteriaceae* (HRE) in 13 European ICUs that participated in multicentre trial evaluating different approaches to control the spread of antibiotic resistance.

Materials and methods

Clinical isolates

Isolates were collected as part of the MOSAR-ICU trial conducted between May 2008 and April 2011 in European ICUs.[13] Perineum swabs (and wound swabs if present) were obtained within 48 hours of admission and twice weekly of all patients with expected length of stay of 3 days or longer, and of a sample of patients with shorter length of stay.

Swabs were plated onto the Brilliance ESBL 2 Agar (Oxoid Limited, Cambridge, UK) for 18-24h to detect *Enterobacteriaceae* resistant to third- or fourth generation cephalosporins (HRE).Only colonies from the groups of *E. coli*, *Klebsiella / Enterobacter / Serratia / Citrobacter* (KESC) and *Proteus / Providencia / Morganella* (PPM) were selected. A double disk synergy test

(DDST) was performed on Mueller-Hinton agar according to CLSI guidelines for non-fastidious bacteria. Colonies with positive DDST were considered HRE positive, and isolates (one colony of each morphotype per patient) were frozen and transported to the National Medicines Institute, Warsaw, Poland (MOSAR ESBL laboratory), for further analysis.

Species identification was done with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). The phenotypic detection of ESBLs and AmpC-like cephalosporinases was carried out using the ESBL DDST with disks containing cefotaxime, ceftazidime, cefepime and amoxicillin with clavulanate on Mueller-Hinton agar (Oxoid) non-supplemented and supplemented with 250µg/ml cloxacillin, inhibitor of AmpCs.[14] Isolates suspected of the high-level AmpC production (augmentation of inhibition zones upon cloxacillin) were tested by multiplex PCR for the presence of acquired AmpC types.[15] Susceptibility to carbapenem antibiotics was determined with disks containing ertapenem, imipenem and meropenem (Oxoid). Isolates with non-susceptibility to at least one of these compounds according to EUCAST criteria (www.eucast.org) were subjected to phenotypic detection of carbapenemases. The presence of metallo-beta-lactamases (MBLs) was assessed by the DDST with disks containing imipenem, ceftazidime and EDTA.[16] KPC types were detected by the combined-disk test with disks containing imipenem and meropenem, and both supplemented with phenylboronic acid.[17] Putative production of OXA-48 types was analyzed by the temocillin disk test,[18] confirmed by PCR.[19] Isolates without ESBL, acquired AmpC or carbapenemase, or not being *Enterobacteriaceae* were considered non-relevant isolates.

Additional molecular analyses were performed on *E. coli* and *K. pneumoniae* isolates from six hospitals located in different countries: France, Greece, Italy, Latvia, Luxembourg and Slovenia. Isolates selected were patient-unique, unless two isolates from the same patient varied by phenotype. Per hospital the first 30 consecutive isolates per species were selected.

Typing

Pulsed-field gel electrophoresis (PFGE) was performed for all selected *E. coli* and *K. pneumoniae* isolates as described,[20] using of the XbaI restriction enzyme (Fermentas, Vilnius, Lithuania). PFGE patterns were analyzed and interpreted visually using the guidelines by Tenover et al.[21] Multilocus sequence typing (MLST) was performed for isolates representing PFGE types, as described previously for *E. coli* and *K. pneumoniae*;[22,23] databases available at mlst.ucc.ie and www.pasteur.fr/mlst, respectively, were used for assigning sequence types (STs).

Beta-lactamase analysis

Beta-lactamase profiles were analyzed by isoelectric focusing as reported,[24] using a Model 111 Mini IEF Cell (Bio-Rad, Hercules, CA). Identification of the ESBL $bla_{\text{CTX-M-1}}$-, $bla_{\text{CTX-M-2}}$-, $bla_{\text{CTX-M-9}}$-, bla_{SHV}-, bla_{TEM}-, and AmpC $bla_{\text{CMY-2}}$-like genes was done by PCRs.[25-27] Sequencing of the genes was performed as reported,[25] using sets of consecutive primers specific for each gene type. For MBL- and/or KPC-producing *K. pneumoniae* isolates from the ICU in Athens, the $bla_{\text{VIM-1}}$- and bla_{KPC}-like genes were detected by specific PCRs.[28,29]

Index of diversity

For ICUs from which at least 15 isolates of both *E. coli* and *K. pneumoniae* had been typed by PFGE, the index of diversity, as defined by Simpson,[30] was determined in order to determine and compare the diversity between *E. coli* and *K. pneumoniae*. This index indicates the probability that two strains sampled randomly from a population belong to different types. This analysis was performed separately for each ICU, as PFGE types were assigned per hospital.[31,32]

Results

In all, there were 14,390 admissions included in the MOSAR-ICU trial (3,215, 3,345, and 7,830 in phase 1, 2, and 3, respectively) and colonization with HRE was documented in 1,639 admissions, yielding 2,126 isolates for further study (Figure 1). After exclusion of duplicate (n = 243) and non-relevant (n = 130) isolates, there were 1,753 isolates from 1,453 admissions for the current analyses. Thirty-seven admissions had non-identical isolates of the same species, and both isolates were included in the analysis. Proportions of isolates submitted to the reference lab per ICU ranged from 74% to 96% (overall 89%) and numbers of submitted isolates per ICU ranged from 18 to 662 (Table 1).

Figure 1: Selection of isolates

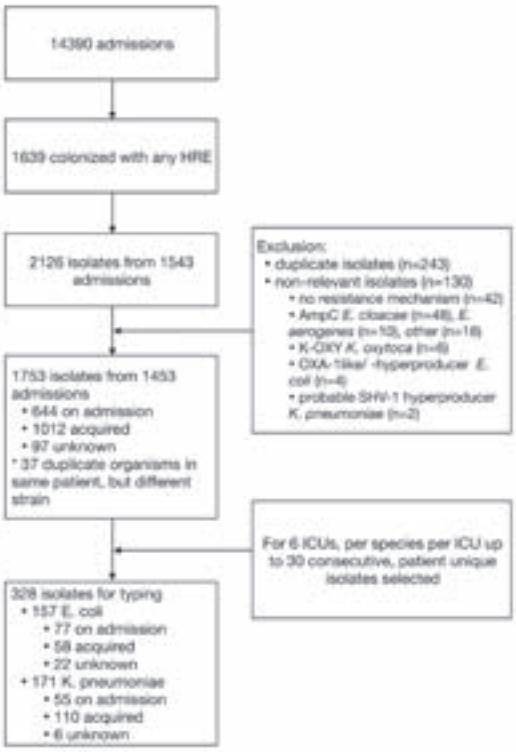

Table 1: Resistance mechanisms per ICU

ICU	Number of patients in study	Number of isolates submitted	Percentage of patients with HRE with isolate submitted	ESBL positive					ESBL negative		ESBL unknown		
				ESBL	AmpC	KPC	MBL	OXA-48	AmpC	MBL	AmpC	KPC	MBL
F1	2373	331	96	320 (96.7)	5 (1.5)	0	0	2 (0.6)	4 (1.2)	0	0	0	0
F2	1328	106	89	100 (94.3)	2 (1.9)	2 (1.9)	0	0	1 (0.9)	0	0	0	1 (0.9)
F3	1049	57	91	49 (86.0)	3 (5.3)	0	0	0	1 (1.8)	0	0	0	4 (7.0)
G1	796	141	74	607 (47.5)	3 (2.1)	42 (29.8)	2 (1.4)	0	15 (10.6)	0	0	4 (2.8)	8 (5.7)
G2	558	110	79	10 (9.1)	2 (1.8)	28 (25.5)	32 (29.1)†	0	14 (12.7)	3 (2.7)‡	0	6 (5.5)*	19 (17.3)#†
I1	788	50	77	41 (82.0)	2 (4.0)	0	0	0	4 (8.0)	1 (2.0)	1 (2.0)	0	1 (2.0)
La	1464	662	96	654 (98.8)	2 (0.3)	0	0	0	3 (0.5)	2 (0.3)†	0	0	1 (0.2)
Lu	1823	56	87	55 (98.2)	0	0	0	0	1 (1.8)	0	0	0	0
P1	910	18	86	18 (100)	0	0	0	0	0	0	0	0	0
P2	628	26	89	25 (96.2)	0	0	1 (3.8)	0	0	0	0	0	0
S1	919	41	91	40 (97.6)	1 (2.4)	0	0	0	0	0	0	0	0
S2	685	115	77	113 (98.3)	0	0	0	0	2 (1.7)	0	0	0	0
Sp	1069	40	81	40 (100)	0	0	0	0	0	0	0	0	0
Total	**14390**	**1753**	**89**	**1532 (87.4)**	**20 (1.1)**	**72 (4.1)**	**35 (2.0)†**	**2 (0.1)**	**45 (2.6)**	**6 (0.3)^**	**1 (0.1)**	**10 (0.6)***	**34 (1.9)#†**

* 4 isolates also harbour MBL

\# 4 isolates also harbour KPC

† 1 isolate also harbours AmpC

‡ 3 isolates also harbour AmpC

^ 4 isolates also harbour AmpC

Admission and acquisition rates of HRE varied widely between ICUs. Overall HRE admission rate and acquisition rates were 6.3% and 8.9%, respectively (Table 2). Admission rates ranged from 1.1% to 13.6%, and acquisition rates varied from 1.3% to 30.2%. For *E. coli* the overall admission rate was 3.2%, ranging from 0.6% to 7.2% for individual ICUs. The overall acquisition rate was 2.5%, and ranged from 0.3% to 7.9%. Admission and acquisition rates were higher for KESC group; overall admission and acquisition rates were 3.5% and 7.1%, respectively, and acquisition rates ranged from 0.4% to 26.6% for individual ICUs. Overall, admission and acquisition rates of PPM were 0.2% and 0.8%, respectively.

Table 2: Percentages of patients colonizes with highly-resistant *Enterobacteriaceae* (HRE) on admission or acquiring HRE during ICU-stay

ICU	*E. coli*		KESC		PPM		HRE total	
	On admission	Acquisition	On admission	Acquisition	On admission	Acquisition	On admission	Acquisition
F1	7.2%	3.2%	4.9%	6.3%	0.1%	0.2%	10.8%	8.7%
F2	4.0%	2.5%	2.7%	4.7%	0.1%	0.2%	5.9%	6.7%
F3	1.9%	1.3%	1.2%	2.1%	0.1%	0.0%	3.0%	3.3%
G1	1.5%	3.2%	6.4%	11.0%	1.0%	3.0%	7.8%	13.9%
G2	0.9%	0.3%	7.9%	22.8%	0.9%	7.9%	9.1%	27.2%
I1	1.9%	2.3%	2.2%	4.5%	0.2%	0.0%	3.9%	6.5%
La	5.8%	7.9%	8.5%	26.6%	0.4%	2.3%	13.6%	30.2%
Lu	1.5%	0.7%	0.8%	0.7%	0.0%	0.0%	2.3%	1.3%
P1	0.8%	1.0%	0.3%	1.1%	0.0%	0.0%	1.1%	1.8%
P2	0.6%	0.5%	1.9%	1.8%	0.0%	0.0%	2.4%	2.2%
S1	2.5%	0.8%	1.3%	2.2%	0.2%	0.5%	3.7%	3.0%
S2	3.7%	1.9%	8.7%	10.6%	0.0%	0.0%	11.5%	11.7%
Sp	2.8%	2.8%	0.5%	0.4%	0.0%	0.0%	3.2%	3.2%
Total	**3.2%**	**2.5%**	**3.5%**	**7.1%**	**0.2%**	**0.8%**	**6.3%**	**8.9%**

KESC: *Klebsiella / Enterobacter / Serratia / Citrobacter*, PPM: *Proteus / Providencia / Morganella*

HRE recovered were *K. pneumoniae* (n = 821), *E. coli* (n = 648), *E. cloacae* (n = 124), *P. mirabilis* (n = 76), *K. oxytoca* (n = 28), *C. freundii* (group) (n = 25) and others (n = 31) (Table 3). There were large differences between the ICUs in resistance mechanisms detected in HRE. ESBL was detected in 1,661 isolates (94.8%) and in 1,532 isolates ESBL was the only resistance mechanism detected.

Carbapenemase-producing HRE were detected in eight ICUs, yielding 82 isolates harbouring KPC (in 3 ICUs), 75 isolates harbouring MBL (in 7 ICUs) and 2 isolates harbouring OXA-48 (2 isolates from the same patient that was transferred from an Algerian ICU). The two Greek ICUs accounted for 141 (90%) of 156 carbapenemase-producing HRE. Such isolates were only sporadically recovered from ICUs in Italy (n = 2 MBL), Latvia (n = 3 MBL) France (n = 2 OXA-48 in ICU F1; n = 2 KPC and n = 1 MBL in ICU F2; n = 4 MBL in ICU F3) and Portugal (n = 1 MBL in ICU P2). MBL was mainly detected in *K. pneumoniae* (n = 61, 81.3% of all MBL-positive isolates), but also in *C. freundii* (n = 2), *E. aerogenes* (n = 2), *E. cloacae* (n = 4), *Enterobacter* spp. (n = 1), *E. coli* (n = 1), *P. mirabilis* (n = 2), and *P. stuartii* (n = 2). KPC was mainly detected in *K. pneumoniae* (n = 80), but also in *E. coli* (n = 1) and *E. cloacae* (n = 1). There was one patient in a French ICU that carried *E. coli* harbouring OXA-48, which was resistant to ertapenem and intermediate susceptible to meropenem and imipenem.

Table 3: Resistance mechanisms per species

Species	Number of	ESBL positive					ESBL negative		ESBL unknown		
	isolates	ESBL	AmpC	KPC	MBL	OXA-48	AmpC	MBL	AmpC	KPC	MBL
C. freundii	21	19 (90.5)	0	0	0	0	0	0	0	0	2 (9.5)
C. freundii group	4	2 (50.0)	1 (25.0)	0	0	0	0	0	1 (25.0)	0	0
Citrobacter sp.	4	4 (100)	0	0	0	0	0	0	0	0	0
C. koseri	5	5 (100)	0	0	0	0	0	0	0	0	0
E. aerogenes	3	1 (33.3)	0	0	0	0	0	1 (33.3)	0	0	1 (33.3)
E. cloacae	124	113 (91.1)	6 (4.8)	1 (0.8)	0	0	0	2 (1.6)†	0	0	2 (1.6)
E. coli	648	627 (96.8)	6 (0.9)	0	0	2 (0.3)	11 (1.7)	0	0	1 (0.2)	1 (0.2)
Enterobacter sp.	1	0	0	0	0	0	0	0	0	0	1 (100)
K. oxytoca	28	28 (100)	0	0	0	0	0	0	0	0	0
K. pneumoniae	821	677 (82.5)	2 (0.2)	71 (8.6)	33 (4.0)	0	5 (0.6)	1 (0.1)†	0	9 (1.1)*	27 (3.3)#†
K. pneumoniae ozaenae	2	2 (100)	0	0	0	0	0	0	0	0	0
Klebsiella sp./ Raoultella sp.	2	2 (100)	0	0	0	0	0	0	0	0	0
M. morganii	4	4 (100)	0	0	0	0	0	0	0	0	0
P. mirabilis	76	40 (52.6)	5 (6.6)	0	0	0	29 (38.2)	2 (2.6)‡	0	0	0
P. rettgeri	1	1 (100)	0	0	0	0	0	0	0	0	0
P. stuartii	6	4 (66.7)	0	0	2 (33.3)†	0	0	0	0	0	0
S. marcescens	3	3 (100)	0	0	0	0	0	0	0	0	0
Total	**1753**	**1532 (87.4)**	**20 (1.1)**	**72 (4.1)**	**35 (2.0)†**	**2 (0.1)**	**45 (2.6)**	**6 (0.3)^**	**1 (0.1)**	**10 (0.6)***	**34 (1.9) #†**

* 4 isolates also harbour MBL

\# 4 isolates also harbour KPC

† 1 isolate also harbours AmpC

‡ 2 isolates also harbour AmpC

^ 4 isolates also harbour AmpC

Genotyping

In all, 157 *E. coli* and 171 *K. pneumoniae* isolates were selected for additional molecular analyses (Table 4). All isolates underwent PFGE typing. ESBL/AmpC PCR was performed on 313 isolates. Sequencing of ESBL and AmpC genes was done for 95 isolates. In 67 isolates sequencing results were extrapolated from PFGE typing and beta-lactamase PCR results. MBL and KPC PCR was performed for 54 isolates that tested positive on the MBL DDST and/or combined-disk test for KPC. MLST was performed on 97 isolates, and for 135 isolates STs were extrapolated

based from PFGE type and beta-lactamase PCR data. Supplementary table 1 shows the numbers of isolates tested and results extrapolated.

Table 4: Number of isolates per species and study phase selected for genotyping and molecular analyses

ICU	Species	Phase				Colonization status		
		1	2	3	Total	On admission	Acquisition	Unknown
F1	*E. coli*	34/42	0/28	0/92	34/162	23	11	0
	K. pneumoniae	31/34	2/24	0/58	33/116	12	21	0
G2	*E. coli*	2/2	2/2	1/1	5/5	4	1	0
	K. pneumoniae	31/32	26/26	0/24	57/82	16	41	0
I1	*E. coli*	13/13	5/7	10/11	28/31	12	13	3
	K. pneumoniae	3/3	2/2	7/7	12/12	4	7	1
La	*E. coli*	30/38	0/27	0/105	30/170	8	20	2
	K. pneumoniae	30/85	0/97	0/202	30/384	8	22	0
Lu	*E. coli*	0/1	1/1	29/33	30/35	23	7	0
	K. pneumoniae	1/1	3/3	6/7	10/11	8	1	1
S2	*E. coli*	6/6	5/5	19/22	30/33	19	11	0
	K. pneumoniae	16/16	10/11	3/53	29/80	8	20	1

Klebiella pneumoniae

From the Greek ICU 57 *K. pneumoniae* were analyzed yielding three dominant genotypes; ST147 (n = 20), ST258 (n = 18) and ST36 (n = 13). All ST36 isolates contained SHV-like ESBL and VIM-1-like carbapenemase production. All 18 ST258 genotypes had SHV-like ESBL and 17 also contained KPC-like carbapenemase production. All 20 ST147 isolates had VIM-1-like carbapenemase in combination with SHV (n = 10), CMY-4 (n = 1), and KPC (n = 4). VIM-1 was detected in 36 of 57 (63.2%) *K. pneumoniae* isolates, and was present in ST147, ST17 and ST36. Twenty-two of 57 (38.6%) isolates were KPC, of which four also harboured VIM-1-like MBL, and all belonged to ST147 (with identical PFGE types).

From the French ICU 34 *K. pneumoniae* isolates were analyzed and 31 (91.2%) harboured CTX-M-15, in 7 different MLST types, with 2 dominant clones; ST147 (n = 16) and ST 16 (n = 9). Three isolates were ST353 and produced CTX-M-3 (n = 1) and SHV-2 (n = 2).

In the Latvian ICU 30 *K. pneumoniae* isolates were analyzed, yielding two MLST types (ST15, n = 6 and ST199, n = 24), both producing CTX-M-15 (2 isolates produce CTX-M-1 group ESBL, not sequenced yet).

In the Luxembourg ICU, all 10 *K. pneumoniae* isolates harboured CTX-M-1 group ESBL, either being CTX-M-3 (n = 4) or CTX-M-15 (n = 6) and all seven isolates that underwent MLS-typing had different STs and different PFGE patterns.
In the Slovenian ICU, all 29 *K. pneumoniae* isolates harboured CTX-M-1-like ESBL with ST29 (n = 15) being dominant (ST29, n = 15), and several smaller clusters of isolates with ST101 (n = 4), ST147 (n = 3), and ST45 (n = 4).
In the Italian ICU CTX-M-1-like ESBL was most often detected, which were identified in ST 147, ST101 and ST37 isolates.

Escherichia coli

There were only five *E. coli* isolates from the Greek ICU; two were CTX-M-15 positive ST131. Among the 31 *E. coli* isolates from the French ICU that underwent MLS-typing there were 19 different STs, without a single ST being dominant. There was also a variety of resistance genes in these isolates, including CTX-M-15 (n = 12), CTX-M-1 (n = 9), CTX-M-14 (n = 2), SHV-12 (n = 2), CMY-2 (n = 3). From the Latvian ICU 30 isolates were selected and 15 underwent MLS-typing. Of these 15, ST131 was dominant (n = 13), producing either CTX-M-15 (n = 10), CTX-M-1 (n = 1), CTX-M-5 (n = 1), and CMY-2 (n = 1).
In Luxembourg, all 30 *E. coli* isolates harboured CTX-M-1 group ESBL; CTX-M-1 (n = 1), CTX-M-3 (n = 2), and CTX-M-15 (n = 8) (19 isolates not sequenced). No MLST typing has yet been performed on *E. coli*.
In the Slovenian ICU, all 30 *E. coli* isolates harboured CTX-M-1-like ESBL. No MLST in *E. coli* has been performed yet.
In the Italian ICU, CTX-M-1-like ESBL was most often found, with a cluster (n = 5) of ST131 isolates.

Index of diversity

Indices of diversity were calculated for three ICUs based on PFGE typing (Table 6). In all three ICUs the discriminatory index was significantly higher for *E. coli* than for *K. pneumoniae*, demonstrating that there is more clonality in the latter bacterial population.

Table 5a: MLST, PFGE, sequence and PCR results of *K. pneumoniae*

ICU	Number of isolates	MLST	PFGE *	ESBL/AmpC	KPC PCR	MBL PCR
F1	2	ST13	E1 (1), E2 (1)	CTX-M-15		
	16	ST147	A1 (12), A2 (2), A3 (1), A5 (1)	CTX-M-15		
	1	ST15	F (1)	CTX-M-15		
	9	ST16	C1 (8), C3 (1)	CTX-M-15		
	1	ST268	D (1)	CTX-M-15		
	1	ST353	G1 (1)	CTX-M-3		
	2	ST353	G2 (2)	SHV-2		
	1	ST377	B (1)	CTX-M-15		
	1	ST45	H (1)	CTX-M-15		
G2	1	-	I (1)	CMY-13	-	VIM-1-like
	5	ST147	B1 (1), B2 (1), B3 (1), B4 (2)		-	VIM-1-like
	4	ST147	B6 (3), B8 (1)		KPC-like	VIM-1-like
	1	ST147	B7 (1)	CMY-4	-	VIM-1-like
	10	ST147	B4 (4), B5 (1), H1 (3), H2 (2)	SHV-like	-	VIM-1-like
	1	ST17	C (1)	CTX-M-15, SHV-like	-	VIM-1-like
	1	ST17	F1 (1)	SHV-like	-	VIM-1-like
	1	ST17	G (1)	SHV-like	KPC-like	-
	1	ST258	D4 (1)	SHV-like		
	17	ST258	D1 (2), D2 (3), D3 (8), D5 (1), D6 (2), D7 (1)	SHV-like	KPC-like	-
	13	ST36	A1 (5), A2 (2), A3 (1), A4 (1), A5 (1), A6 (1), A7 (1), A8 (1)	SHV-like	-	VIM-1-like
	1	ST383	J (1)	CTX-M-15		
	1	ST622	E (1)	SHV-like		

* PFGE clusters were determined per ICU

Table 5a: continued

ICU	Number of isolates	MLST	PFGE *	ESBL/AmpC	KPC PCR	MBL PCR
I1	1	ST101	F (1)	CTX-M-1-like		
	3	ST14	D2 (3)	CMY-2-like		
	2	ST14	D1 (2)	CTX-M-32		
	2	ST147	B (1), C (1)	CTX-M-1		
	1	ST37	E (1)	CTX-M-1-like		
	3	ST37	A1 (2), A2 (1)	CTX-M-1		
La	6	ST15	B1 (4), B2 (1), B3 (1)	CTX-M-15		
	2	ST199	A3 (1), A4 (1)	CTX-M-1-like		
	22	ST199	A1 (18), A3 (3), A5 (1)	CTX-M-15		
Lu	2	-	A1 (1), A2 (1)	CTX-M-15		
	1	-	C (1)	CTX-M-3		
	1	NEW	F (1)	CTX-M-15		
	1	ST11	D (1)	CTX-M-3		
	1	ST147	H (1)	CTX-M-15		
	1	ST15	I (1)	CTX-M-15		
	1	ST22	E (1)	CTX-M-3		
	1	ST45	G (1)	CTX-M-15		
	1	ST659	B (1)	CTX-M-3		
S2	1	NEW1	F (1)	CTX-M-1-like		
	1	NEW2	H (1)	CTX-M-1-like		
	4	ST101	C (4)	CTX-M-1-like		
	3	ST147	B1 (1), B2 (1), E (1)	CTX-M-1-like		
	1	ST29	D1 (1)	CTX-M-1-like		
	14	ST29	D2 (8), D3 (2), D4 (1), D5 (2), D6 (1)	CTX-M-1-like		
	1	ST437	I (1)	CTX-M-1-like		
	4	ST45	A (2), G (2)	CTX-M-1-like		

* PFGE clusters were determined per ICU

Table 5b: MLST, PFGE, and sequence results of *E. coli*

ICU	Number of isolates	MLST	PFGE *	ESBL/AmpC	KPC PCR	MBL PCR
F1	3	-	AE (1), AF (1), AG (1)	CTX-M-1-like		
	3	ST10 (CC10)	J(?) (1), N(?) (1), Y (1)	CTX-M-1		
	1	ST10 (CC10)	NT (1)	CTX-M-15		
	1	ST10 (CC10)	F (1)	TEM-like		
	1	ST1122	S (1)	CTX-M-1		
	1	ST117	R (1)	CTX-M-1		
	2	ST131	D (1), Q (1)	CTX-M-15		
	1	ST1485	G (1)	CTX-M-1		
	1	ST1486	H (1)	CTX-M-15		
	3	ST1487	M1(?) (1), M2(?) (1), P (1)	CMY-2		
	1	ST155 (CC155)	U (1)	CTX-M-1		
	1	ST162 (CC469)	AA (1)	CTX-M-1		
	1	ST162 (CC469)	I (1)	SHV-12		
	1	ST38 (CC38)	A (1)	CTX-M-15		
	1	ST405 (CC405)	T (1)	CTX-M-3, TEM-52		
	3	ST410 (CC23)	AD (1), K (2)	CTX-M-15		
	1	ST57 (CC350)	C (1)	CTX-M-14		
	1	ST609 (CC46)	O (1)	CTX-M-2		
	2	ST617 (CC10)	AB (1), Z (1)	CTX-M-15		
	1	ST62	W (1)	CTX-M-14		
	2	ST648	B (1), L (1)	CTX-M-15		
	1	ST665	X (1)	SHV-12		
	1	ST88 (CC23)	AC (1)	CTX-M-1		
G2	1	-	E (1)	CTX-M-1-like		
	2	ST131	A (1), D (1)	CTX-M-15		
	1	ST224	B (1)	CTX-M-15		
	1	ST354 (CC354)	C (1)	CMY-2		
I1	2	-	S (1), U (1)			
	5	-	R (1), T (1), V (1), W (1), X (1)	CTX-M-1-like		
	3	-	A (1), I (1), K (1)	CTX-M-15		
	3	-	C1 (1), C2 (1), C3 (1)	CTX-M-32		

* PFGE clusters were determined per ICU

Table 5b: continued

ICU	Number of isolates	MLST	PFGE *	ESBL/AmpC	KPC PCR	MBL PCR
	1	ST10 (CC10)	H (1)	CTX-M-32		
	1	ST131	G (1)	CTX-M-1		
	5	ST131	F (1), N2 (1), N3 (1), O1 (1), O2 (1)	CTX-M-15		
	1	ST1434	Q (1)	CTX-M-9		
	1	ST155 (CC155)	P (1)	CTX-M-1		
	1	ST2076	E (1)	CTX-M-15		
	1	ST361	D (1)	CTX-M-1		
	2	ST405 (CC405)	L (1), NT (1)	CTX-M-15		
	1	ST46 (CC46)	J (1)	CTX-M-1		
	1	ST648	B (1)	CMY-2		
La	15	-	G1 (1), G2 (1), H (1), I (1), J (1), K (1), L1 (6), L2 (1), L3 (1), M (1)	CTX-M-1-like		
	1	ST131	F (1)			
	1	ST131	E (1)	CMY-2		
	10	ST131	A (4), C1 (5), C2 (1)	CTX-M-15		
	1	ST131	F (1)	CTX-M-5		
	1	ST1829	B (1)	CTX-M-1		
	1	ST774	D (1)	CMY-2		
Lu	19	-	J (1), K (1), L (1), M (1), N (1), O (1), P (1), Q (1), R (1), S (1), T1 (1), T2 (1), U (1), V (1), W (1), X (1), Y (1), nt (2)	CTX-M-1-like		
	1	-	Nt (1)	CTX-M-1		
	8	-	A (2), B (1), C (1), E (1), G (1), H (1), I (1)	CTX-M-15		
	2	-	D (1), F (1)	CTX-M-3		
S2	3	-	L (1), M4 (1), O (1)			
	30	-	A (1), B (1), C (1), D (1), E (1), F (1), G (1), H (1), I (1), J (1), K (2), L (1), M1 (1), M2 (1), M3 (1), N (1), P (1), Q (1), R (1), S (1), T (4), U (1), V (1) , nt (3)	CTX-M-1-like		

* PFGE clusters were determined per ICU

Table 6: Index of diversity

ICU	Species	Typing method	# Isolates	# Different types	Discriminatory index	95% Confidence interval
F1	*E. coli*	PFGE	33	32	0.998	[0.993 - 1.0]
F1	*K. pneumoniae*	PFGE	33	14	0.82	[0.719 - 0.921]
La	*E. coli*	PFGE	30	17	0.926	[0.875 - 0.977]
La	*K. pneumoniae*	PFGE	30	7	0.621	[0.44 - 0.801]
S2	*E. coli*	PFGE	27	25	0.994	[0.984 - 1.0]
S2	*K. pneumoniae*	PFGE	29	15	0.906	[0.833 - 0.98]

Discussion

This study on the molecular epidemiology of highly-resistant *Enterobacteriaceae* (HRE) in 13 ICUs in eight European countries reveals large variations in HRE admission and ICU-acquisition rates, large heterogeneity in the prevalence of antibiotic resistance mechanisms and sequence types of *E. coli* and *K. pneumoniae* and a difference in the clonality between *E. coli* and *K. pneumoniae*. In most ICUs, HRE mainly contain ESBL-producing *Enterobacteriaceae*, mostly CTX-M-15, but in two ICUs, both in Greece, carbapenemase-producing isolates were most prevalent. In these ICUs, the prevalence of KPC and MBL-producing *K. pneumoniae* accounted for 31% to 49% of HRE isolates.

Genotyping and molecular determination of resistance mechanisms of 157 *E. coli* and 171 *K. pneumoniae* HRE from six ICUs revealed that CTX-M-15 was the most prevalent ESBL gene in both *E. coli* and *K. pneumoniae*, accounting for 26-79% and 42-63% of all ESBL-containing HRE, respectively. Globally, CTX-M-15 is the most prevalent member of the CTX-M type enzymes, both caused by dissemination of plasmids and successful clones. In *K. pneumoniae*, CTX-M-15 containing plasmids have been detected in multiple sequence types, without evidence of a predominant ST. In *E. coli*, however, CTX-M-15 containing plasmids have been associated with ST131.[4] Indeed, also in this study, *K. pneumoniae* CTX-M-15 appeared present in many STs, whereas *E. coli* ST131 carrying CTX-M-15 accounted for 30% of all *E. coli* isolates typed by MLST. Moreover, ST131 was detected in all four ICUs from which *E. coli* isolates were MLS-typed. ST131 may contain a wide variety of other CTX-M genes[4,33] and CMY-type AmpC,[33] and in our study CTX-M-1, CTX-M-5 and CMY-2 were documented in *E. coli* ST131.

Although *E. coli* has emerged as the major producer of CTX-M-type ESBLs, it is less often associated with carbapenemases than *K. pneumoniae*.[10] In this study, only 0.6 % of *E. coli*, classifying as HRE, contained carbapenemase genes, compared with 17 % of *K. pneumoniae* HRE isolates.

Although initially described in *K. pneumoniae*, *Klebsiella pneumoniae* carbapenemases (KPCs) have now also been demonstrated in other species such as Enterobacter spp. and *E. coli*. The plasmids carrying KPC genes are mobile allowing transfer to different *Enterobacteriaceae*

species.[34,35] In this study most KPCs were documented in *K. pneumoniae* (98%), and only sporadically in *E. coli* (n = 1) and E. cloacae (n = 1). The previously reported endimicity of KPC producers in Greece[10] was reflected by admission prevalences of of 2.1% and 2.9% in the two Greek ICUs. KPC has been detected in a large variety of *K. pneumoniae* STs, including ST258, ST147 and ST17, which were also detected in this study.[36] *K. pneumoniae* ST258 was most prevalent and this ST has been associated with nosocomial spread.[9]

Clusters of *K. pneumoniae* with VIM carbapenemases are reported with increasing frequency from hospitals in different continents,[37,38] although still less frequent than clusters of KPC. VIM enzymes have not been associated with any particular ST.[8]

In this study there were four *K. pneumoniae* ST147 isolates that harboured both KPC and VIM. Coproduction of KPC-2 and VIM-1 in *K. pneumoniae* has been described before,[39-41] and it has been hypothesized that the VIM-1 and KPC-2 coproducer evolved by acquiring KPC-2 by a VIM-1 harbouring *K. pneumoniae* strain.[41] The presence of OXA-48 has been linked to transfer of patients from hospitals in endemic countries.[11,42] In this study, there was one patient with *E. coli* harbouring OXA-48 and this patient was transferred from an Algerian ICU.

Strengths of this study include the detailed surveillance with centralized analyses of resistance mechanisms of HRE detected in 14,390 patients in 13 ICUs in eight countries during two years of study. All ICUs used the same study protocol and local microbiology labs fulfilled quality assessment.[13] Study limitations include the limited number of ICUs precluding extrapolation of findings to all European ICUs. This is reflected by the considerable within-country and between-country variations in HRE admission and acquisition rates. Furthermore, for reasons of feasibility, MLS-typing and AmpC and ESBL sequencing were performed on representative isolates only. Finally, data were collected as part of an intervention study. However, the interventions evaluated (chlorhexidine bodywashing, hand hygiene improvement, and admission screening followed by contact precautions of HRE carriers) were not associated with lower HRE acquisition rates. It is, therefore, unlikely that these interventions influenced the molecular epidemiology of HRE.

Supplementary table 1: Numbers of isolates with PFGE, MLST, ESBL/AmpC PCR and sequencing and numbers of extrapolations in *E. coli* and *K. pneumoniae*

E. coli	50	1	0	12	1	0	5	70	14	0	1	3
K. pneumoniae	25	0	3	50	19	1	0	0	3	61	8	1
PFGE	+	+	+	+	+	+	+	+	+	+	+	+
MLST	+	+	*	*	+	+	-	-	-	*	*	-
ESBL/AmpC PCR	+	+	+	+	+	-	-	+	+	+	-	+
ESBL/AmpC seq	+	*	+	*	-	-	-	-	+	-	-	*

+ Method performed; - Method not performed; * Result extrapolated

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Clinical impact and risk factors for colonization with extended-spectrum ß-lactamase-producing bacteria in the intensive care unit

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Clinical impact and risk factors for colonization with extended-spectrum ß-lactamase-producing bacteria in the intensive care unit

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Abstract

Purpose: The changed epidemiology of extended-spectrum beta-lactamases (ESBL), the spread to the community and the need for prudent use of carbapenems require updated knowledge of risk factors for colonization with ESBL-producing *Enterobacteriaceae* (ESBL-PE).

Methods: An 8-month prospective study in the medical ICU of an 850-bed general and university-affiliated hospital.

Results: Of 610 patients admitted, 531 (87%) had a rectal swab obtained at admission, showing a 15% (82 patients) ESBL-PE carriage rate, mostly of *E. coli* (n = 51, 62%); ESBL-PE caused 9 (3%) infections on admission. By multivariable analysis, transfer from another ICU (OR = 2.56,[1,22] hospital admission in another country [OR = 5.28 (1.56-17.8)], surgery within the past year [OR = 2.28 (1.34-3.86)], prior neurologic disease [OR = 2.09 (1.1-4.0)], and prior administration of third generation cephalosporin (within 3-12 months before ICU admission) [OR = 3.05 (1.21-7.68)] were independent predictive factors of colonization by ESBL-PE upon ICU admission. Twenty-eight patients (13% of those staying for more than 5 days) acquired ESBL carriage in ICU, mostly with *E. cloacae* (n = 13, 46%) and *K. pneumoniae* (n = 10, 36%). In carriers, ESBL-PE caused 10 and 27% of first and second episodes of ICU-acquired infections, respectively.

Conclusions: We found a high prevalence of ESBLE-PE colonization on admission to our ICU, even in the subgroup admitted from the community, but few first infections. Identifying risk factors for ESBL-PE colonization may help identifying which patients may warrant empiric ESBL-targeted antimicrobial drug therapy as a means to limit carbapenem use.

Keywords

Antimicrobial agents | Community-acquired infection | Non-pulmonary nosocomial infections

Introduction

In Gram-negative pathogens, beta-lactamase production remains the most important contributing factor to antimicrobial resistance. Cases of infections with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) were first reported during the late 1980s and have subsequently spread worldwide.[1-3] The emergence of CTX-M-type ESBLs has modified the epidemiology of ESBLs since dissemination of these enzymes is not restricted to the healthcare setting but also involves the community, especially among *Escherichia coli*.[4-10] Since the beginning of the century, the prevalence of infection with ESBL, notably among *E.coli* and *Klebsiella pneumoniae*, has increased dramatically.[11-13] Infections caused by ESBL producers have been associated with severe adverse clinical outcomes, leading to increased mortality, prolonged hospital stay, and increased costs,[14-17] mostly because of delayed effective therapy. Consequently, carbapenems are increasingly used by intensivists as empiric therapy of hospital-acquired sepsis. This vicious circle of bacterial resistance already leads to a rapid worrying international dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE), especially among *K. pneumoniae* (KPC).[18-20] New agents against these multi-drug-resistant bacteria are not going to be available soon, and the intensivist's armamentarium is close to a dead end without the cautious use of carbapenems.[21] An important risk factor for nosocomial infection is prior colonization.[22] The changed epidemiology, the spread of ESBL to the community, and the need for prudent use of carbapenems require updated studies to identify current risk factors for colonization with ESBL-PE.

The primary objective of our study was to determine which factors are predictive of colonization with ESBL-PE at admission to an intensive care unit (ICU). The secondary objectives were to identify the rates of and risk factors for acquisition of ESBL-PE during the ICU stay. We also examined the occurrence and risk factors of ESBL-PE infections in relation to carriage of ESBL-PE.

Patients and methods

Setting and patients

This 8-month prospective study (1 October 2010-31 May 2011) was conducted in the medical intensive care unit of a French 850-bed general and university-affiliated hospital. The ICU includes 13 beds with 5 single rooms, and a stepdown unit of 11 beds with 3 single rooms. No specific isolation precautions were being used during the study for patients with ESBL-producing bacteria recovered from clinical or screening cultures, and contact isolation precautions were applied only for patients with carbapenemase-producing organisms, *Clostridium difficile*, methicillin-resistant *S. aureus*, and vancomycin-resistant Enterococci. However, an active hand hygiene improvement program with repeated auditing and feedback, as well as twice weekly surveillance of patients for ESBL-producing bacteria using rectal swabs, had been conducted for 2 years as part of 'MOSAR,' an ongoing EC-funded (FP-6) study.

In addition, daily body washings with chlorhexidine were routinely used for all patients. This study was approved by our Institutional Review Board (CPP Ile de France IX), and informed consent was waived.

Rectal swabs were collected from each patient within 24 h of ICU admission and twice weekly for the duration of hospitalization in the ICU. We excluded readmissions. ESBL-PE acquisition was defined as a positive rectal swab culture after a negative admission swab. Rectal swab samples were screened for ESBL-PE on chromogenic agar (Oxoid Ltd, Cambridge, UK), and ESBL production was confirmed by the double-disk diffusion method using ceftazidime or cefotaxime with clavulanic acid (see the ESM for details).[23]

Demographic, clinical, and laboratory data

A detailed clinical profile of each patient was established from the patient's medical record, referral documents (e.g., from the family physician or nursing home), interviews with each patient and/or his or her family, and previous hospital admission records. The following data were collected: demographic characteristics, the simplified acute physiology score (SAPS II), days and in hospital location before ICU admission when appropriate, main reason for admission, hospital admission and administration of antibiotics in the previous year (stratified according to within 3 months of admission or earlier), prior antibiotic exposure (class and duration), surgery in the previous year, presence of underlying diseases and Charlson comorbidity index,[24] and whether any indwelling tubes were in place for more than 24 h before ICU admission.

We defined colonization pressure as the sum of the daily proportion of patients in the unit colonized with ESBL-PE during the days preceding acquisition or ICU discharge.[25]

The clinical impact of ESBL-PE colonization in the ICU was assessed from the rate of positive clinical samples until hospital discharge, mortality rate and length of stay, and comparing ESBL-PE carriers with non-carriers, and the primary outcome was defined as the rate of infection with ESBL-PE, contrasting early and late infection in carriers and non-carriers.

Statistical analysis

Results are reported as medians and interquartile ranges (25th-75th percentiles) or numbers with percentages. Associations between each variable and colonization with ESBL-PE at ICU admission were tested using the χ^2 or Fisher's exact test for categorical data and by the Mann-Whitney *U* test for continuous data. We used multivariable logistic regression with a backward procedure to identify patients' characteristics associated with ESBL-PE colonization at ICU admission. Variables selected by bivariate analysis ($p < .10$) and those considered clinically relevant were entered in a logistic regression model. Considering the number of events, a maximum of eight variables was entered in a two-step model, first including baseline characteristics, then antibiotic exposures.[26] Results are expressed as crude and adjusted odds ratios (OR) with their 95% confidence intervals (CI). A p value $< .05$ was considered statistically significant.

A similar analysis was conducted for the subgroup of patients admitted from the community, defined as those admitted directly from home or having stayed in the hospital for <48 h before ICU admission.

To examine risk factors for ESBL acquisition, we selected patients without ESBL colonization at admission, staying 5 days or more in the ICU and having at least two screening samples obtained before ICU discharge. Additional analyses were performed to identify risk factors for subsequent culture positivity with ESBL-PE among all the patients and among the subgroup of patients colonized with ESBL-PE. Statistical analyses used the Stata software, version 10.1 (StataCorp, College Station, TX, USA).

Results

ESBL-PE colonization at ICU admission

During the study period, 610 patients had at least one ICU admission, of which 531 (87%) had a screening sample obtained within 24 h of ICU admission. The remaining 79 patients had similar characteristics (data not shown) but a much shorter length of ICU stay (2 days [2, 3] vs. 5 days [3-10]) and were not screened for ESBL carriage.

The median age of the 531 patients was 64 years (50-75); 198 (37%) had a Charlson comorbidity index of > 2. Within the previous year, 231 (43%) patients had been hospitalized for more than 24 h and 242 (46%) had been exposed to antibiotics.

Eighty-two (15%) patients were detected to be ESBL-PE carriers on the admission screening sample, mostly of *E. coli* (n = 51; 62%) or *K. pneumoniae* (n = 15; 18%). Table 1 shows the main characteristics of the patients and those associated with colonization at ICU admission. By multivariable analysis (Table 2), transfer from another ICU, previous hospital admission in another country, surgery within the past year, prior neurologic disease, and prior administration of third generation cephalosporin (within 3-12 months before ICU admission) remained associated with colonization by ESBL-PE upon ICU admission; exposure to fluoroquinolones within the past 3 months fell short of statistical significance (p = .062). The final model showed a good calibration (Hoshmer- Lemeshow X^2 = 2.13; p = .54), but limited discrimination (area under the curve, 0.68).

In the subgroup of 394 (74%) patients admitted from the community, 49 (12%) were found colonized by an ESBL-PE on the admission screening sample, 41 (84%) of which were *E. coli*. Independent predictive factors of colonization by ESBL-PE at ICU admission in this subgroup included a previous hospital admission within 3-12 months before admission, prior urinary tract disease, and exposure to third generation cephalosporin (Table 2); again treatment with fluoroquinolone within the past 3 months fell short of statistical significance (p = .077). The final model again showed good calibration (X^2 = 0.19; p = .91) but limited discrimination (area under the curve, 0.69).

Table 1: Univariate analyses of variables associated ESBL colonization at ICU admission in 531 patients screened

Variables	ESBL- (n = 449)	ESBL+ (n = 82)	OR (95% CI)	*p value*
Male gender	274 (61%)	46 (56%)		0.40
Age, median [IQR]	64 [50-75]	64 [49-76]		0.66
Age >75 years	113 (25%)	22 (27%)		0.75
Medical admission	399 (89%)	75 (91%)		0.48
Main reason for ICU admission:				
Acute respiratory failure	206 (46%)	31 (38%)		0.18
Metabolic/AKI	21 (5%)	3 (4%)		1
Neurologic disorder/coma	45 (10)	9 (11%)		0.76
Cardiac arrest	33 (7%)	1 (1%)	0.16 (0.2-1.17)	0.04
Drug intoxication	27 (6%)	2 (2%)		0.3
Severe sepsis/septic shock	73 (16%)	24 (29%)	2.1 (1.2-3.6)	0.005
Hemorrhagic shock	13 (3%)	4 (5%)		0.31
Other shock	17 (4%)	1 (1%)		0.33
Diffuse dermatitis	5 (1%)	3 (4%)		0.1
Others	10 (2%)	3 (4%)		0.43
SAPS II, median [IQR]	34 [23-49]	35 [25-46]		0.70
Admission during previous year	185 (41%)	50 (61%)	2.2 (1.4-3.6)	0.001
Hosp.< 3 months	125 (28%)	33 (40%)	1.7 (1.1-2.8)	0.024
3 months < Hosp < 1 year	121 (27%)	38 (46%)	2.3 (1.4-3.8)	<0.001
Both < 3 months and > 3 months	61 (14%)	21 (26%)	2.2 (1.2-3.9)	0.006
In another country	6 (1%)	7 (9%)	6.9 (2.25-21.0)	0.001
Location before ICU admission:				
Hospital days before ICU	0 [0-2]	1 [0-10]		0.001
Another ICU	30 (7%)	18 (22%)	3.9 (2.1-7.5)	<0.001
Emergency/home	280 (62%)	38 (46%)	0.5 (0.32-0.84)	0.006
Nursing home	9 (2%)	1 (1%)		1
Other	130 (29%)	25 (30%)		0.78
Urinary catheter >24 h	42 (9%)	20 (24%)	3.2 (1.75-5.78)	<0.001
Venous catheter > 24 h	36 (8%)	18 (22%)	3.3 (1.76–6.14)	<0.001
Mechanical ventilation > 24 h	12 (3%)	6 (7%)	2.9 (1.06-8.02)	0.042
Comorbidities:				
Charlson comorbidity index	2 [0-3]	3 [1-4]		0.003
Charlson >2	156 (37%)	42 (52%)	2.0 (1.26-3.27)	0.003

Table 1: continued

Variables	ESBL- (n = 449)	ESBL+ (n = 82)	OR (95% CI)	*p value*
Chronic pulmonary disease	84 (19%)	13 (16%)		0.54
Diabetes mellitus	121 (27%)	22 (27%)		0.98
Neurologic disease	51 (11%)	17 (21%)	2.0 (1.1-3.7)	0.02
Immunodeficiency	113 (25%)	29 (35%)		0.055
Liver cirrhosis	30 (7%)	9 (11%)		0.17
Chronic renal insufficiency	45 (10%)	13 (16%)		0.12
Dialysis	13 (3%)	2 (2%)		1
Congestive heart failure	124 (28%)	31 (38%)		0.062
Urinary tract disease	8 (2%)	6 (7%)	4.4 (1.49-13.1)	0.011
Prior surgery:				
Surgery < 1 year	69 (15%)	26 (32%)	2.6 (1.5-4.3)	<0.001
3 months < Surgery < 1 year	31 (7%)	14 (17%)	2.8 (1.4-5.4)	0.002
Surgery < 3 months	42 (9%)	15 (18%)	2.2 (1.14-4.1)	0.016
Prior antibiotics:				
Antibiotic therapy < 1 year	190 (42%)	52 (63%)	2.45 (1.5-4.01)	<0.001
Ab < 1 year and broad-sp.	161 (36%)	46 (56%)	2.36 (1.46-3.81)	<0.001
Ab < 3 months	150 (33%)	44 (54%)	2.38 (1.47-3.84)	<0.001
Ab < 3 months and broad-sp.	126 (28%)	40 (49%)	2.47 (1.53-4.01)	<0.001
Ab < 3 months and > 10 days, broad-sp.	76 (17%)	34 (42%)	3.5(2.13-5.85)	<0.001
Aminopenicillins	29 (6%)	10 (12%)		0.059
Penicillin + iBL	66 (15%)	22 (27%)	2.17 (1.25-3.8)	0.005
Fluoroquinolones	38 (8%)	17 (21%)	2.9 (1.54-5.44)	0.001
3GC	39 (9%)	17 (21%)	2.81 (1.5-5.27)	0.001
Carbapenem	8 (2%)	8 (10%)	6.05 (2.2-16.6)	<0.001
3 months < Ab < 1 year	81 (18%)	25 (31%)	2.03 (1.2-3.45)	0.008
3 months < Ab < 1 year and broad-sp.	67 (15%)	21 (26%)	2.01(1.15-3.54)	0.013
Aminopenicillins	9 (2%)	4 (5%)		0.11
Penicillin + iBL	33 (7%)	7 (9%)		0.68
Fluoroquinolones	12 (3%)	7 (9%)	2.45 (1.31-9.06)	0.016
3GC	15 (4%)	8 (10%)	3.18 (1.3-7.76)	0.015
Carbapenem	2 (0.5%)	1 (1%)		0.39

Ab antibiotic, *AKI* acute kidney injury, *broad-sp.* broad-spectrum, *3GC* third generation cephalosporin, *hosp.* hospital, *iBL* beta-lactamase inhibitor, *[IQR]* interquartile range, *MV* mechanical ventilation

Table 2: Adjusted odds ratio (aOR) for ESBL-PE colonization at ICU admission in 531 patients, irrespective of their prior location (All), and in the subgroup of 394 patients admitted from the community

Patients	**All (n = 531)**	**Admitted from the community (n = 394)**
Variable	**aOR [95% CI]**	
Surgery within past year	2.28 [1.34-3.86]	-
Hospital admission in another country	5.28 [1.56-17.8]	-
3 months< hospital admission < 1 year	-	2.83 [1.46-5.45]
Prior neurologic disease	2.09 [1.10-4.00]	-
Transfer from another ICU	2.56 [1.26-5.22]	-
Prior urinary tract disease	-	6.03 [1.44-25.1]
Fluoroquinolones < 3 months	1.95 [0.96-3.95]*	2.59 [0.90-7.45]**
3GC >3 months	3.05 [1.21-7.68]	3.58 [1.18-10.8]

* p = .062, ** p = .077

ESBL-PE-acquired carriage in ICU

At least two screening samples were obtained in 212 patients without detectable colonization with ESBL-PE on admission and staying at least 5 days in the ICU. Twenty-eight (13%) acquired ESBL carriage, detected a median of 9 days [8-20] after ICU admission. Thirteen (46%) were *Enterobacter cloacae*, ten (36%) were *K. pneumoniae*, and only two (7%) were *E. coli* (see the ESM Table). Two patients (7%) had several ESBL-PE species identified (*K. pneumoniae* with *E. coli* or *C. diversus*). No carbapenemase-producing *Enterobacteriaceae* was detected. Factors associated with ESBL acquisition in the ICU found by univariate and multivariable analyses are shown in Tables 3 and 4, respectively. Prior exposure to third generation cephalosporins or to a beta-lactam + inhibitor (within 3 months of ICU admission) were both strongly associated with ESBL-PE acquisition. The final model showed both a good calibration (X^2 = 2.32; p = .97) and discrimination (area under the curve, 0.89).

Table 3: Univariate analyses of variables associated with ESBL acquisition during ICU stay in 212 patients staying for 5 days or more and non-colonized on ICU admission

Variables	No acquisition (n = 184)	ESBL acquisition (n = 28)	Odds ratio (95% CI)	*p value*
Male gender	109 (59%)	24 (87%)	4.1 (1.4-12.4)	0.007
Age > 75 years	37 (20%)	13 (46%)	3.4 (1.5-7.9)	0.004
Medical admission	155 (84%)	24 (86%)		0.84
Main reason for ICU admission:				
Acute respiratory failure	95 (52%)	11 (39%)		0.22
Neurologic disorder/coma	18 (10%)	3 (10%)		0.75
Cardiac arrest	11 (6%)	1 (4%)		0.68
Severe sepsis/septic shock	31 (17%)	10 (36%)	2.7 (1.1-6.5)	0.019
Other shock	13 (4%)	2 (4%)		0.57
Others	16 (6%)	1 (0%)		0.35
SAPS II, median [IQR]	36 [27-49]	48 [37-56]		0.004
Comorbidities:				
Charlson > 2	66 (36%)	14 (50%)		0.15
2-bedded room	150 (81%)	25 (89%)		0.4
Colonization pressure, total days[a]	2.5 [1.7-4]	5.8 [4.3-10]		<0.001
Antibiotic therapy within 1 year	94 (51%)	16 (57%)		0.55
Ab < 1 year and broad-spectrum	79 (43%)	16 (57%)		0.16
Ab < 3 mo.	75 (41%)	14 (50%)		0.36
Ab < 3 mo. and broad-spectrum	63 (35%)	14 (50%)		0.12
Ab < 3 mo., broad-sp.and > 10 days	36 (20%)	11 (39%)	2.6 (1.1-6)	0.021
Aminopenicillins	13 (7%)	1 (4%)		0.7
Penicillin + iBL	30 (17%)	10 (36%)	2.7 (1.1-6.4)	0.02
Fluoroquinolones	11 (6%)	6 (21%)	4.2 (1.4-12)	0.015
3GC	21 (12%)	9 (36%)	3.6 (1.4-9)	0.01
Imipenem	2 (1%)	1 (4%)		0.3
Ab in ICU before acquisition:				
Days of therapy	7 [5-11]	8.5 [4.5-19]		0.052
Aminopenicillins	39 (21%)	7 (25%)		0.64
Duration	6 [3-9]	6 [3.5-7.5]		
Penicillin + iBL	99 (54%)	21 (75%)	2.5 (1-6.3)	0.035
Duration	5 [3-7]	7 [4-9]		
Fluoroquinolones	14 (8%)	4 (14%)		0.27

Table 3: continued

Variables	No acquisition (n = 184)	ESBL acquisition (n = 28)	Odds ratio (95% CI)	*p value*
Duration	5 [3.3-12.3]	2.5 [1-4.5]		
3GC	60 (33%)	11 (39%)		0.48
Duration	5 [3-7]	6 [4-8]		
Imipenem	26 (14%)	11 (39%)	3.9 (1.7-9)	0.003
Duration	2.5 [2-5]	3 [2.5-4.5]		
ICU-acquired infection	44 (24%)	20 (71%)	8.0 (3.3-19)	<0.001
Outcomes:				
Duration of ICU stay	9 (7-14)	24 (17-34)		<0.001
Mechanical ventilation	91 (50%)	22 (79%)	3.7 (1.4-9.7)	0.004
Duration of MV	7.5 [5-14]	19 [10-27]		0.001
Dialysis for acute renal failure	15 (8%)	6 (21%)	3.1 (1.1-8.7)	0.04
Alive	154 (84%)	18 (64%)	0.35 (0.14-0.8)	0.014

Ab antibiotic, *AKI* acute kidney injury, *broad-sp.* broad-spectrum, *3GC* third generation cephalosporin, *iBL* beta-lactamase inhibitor, *[IQR]* interquartile range (25-75%); *MV* mechanical ventilation

Colonization pressure is expressed as the sum of the daily proportion of all other patients colonized during the stay of a given patient

Table 4: Adjusted odds ratio for ESBL acquisition among 212 patients staying in ICU for 5 days or more

Predictor	Odds ratio	[95% CI]
Age > 75 years	6.3	[2.17-18.6]
Male gender	3.5	[1.03-11.7]
Colonization pressure[a]	1.3	[1.18-1.49]
3GC within past 3 months	4.8	[1.52-15.0]
B-lactam + inhibitor within past 3 months	3.5	[1.22-10.1]

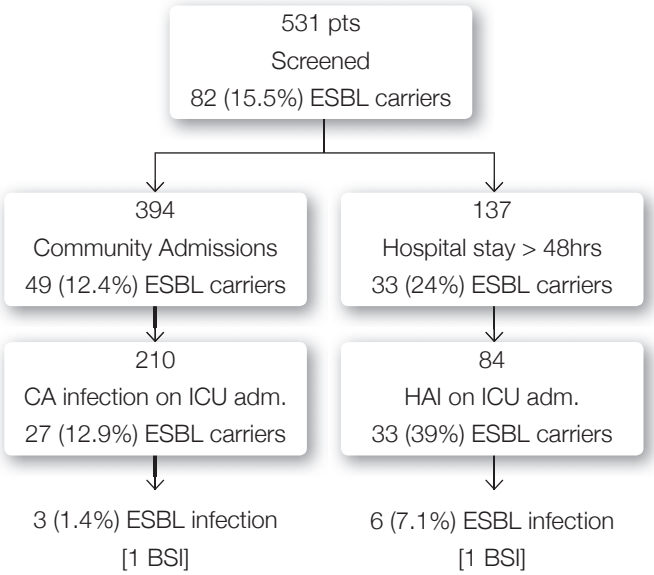
3GC third generation cephalosporin

Colonization pressure is expressed as mean (daily number of colonized patients/beds occupied) × number of days in ICU prior to ESBL colonization

Impact of ESBL-PE on infections in the ICU

During the 8-month study period, 210 patients had community-acquired infection on admission (Figure 1). Only three such patients (1.4%, or 6.1% of ESBL-PE carriers) were infected with ESBL-PE on admission (all urinary tract infection), one of whom received a delayed effective therapy. Of 84 patients with hospital-acquired infections identified at ICU admission, 6 (7.1%) were caused by ESBL-PE, including pulmonary infection (n = 3), and urinary tract infection, catheter-related bloodstream infection or septic arthritis (one each); one received delayed effective therapy.

Figure 1: ESBL colonization and infection on ICU admission.



Breakdown of patients by carriage status of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* (ESBL-PE) on ICU admission among those admitted from the community (N = 394) or having stayed in the hospital for 2 days or more before ICU admission (N = 137) and corresponding number of patients having infection on ICU admission (or within 48 h of admission) with an ESBL-PE microorganism, including those having bloodstream infection (BSI). CA community-acquired, HAI hospital-acquired infection.

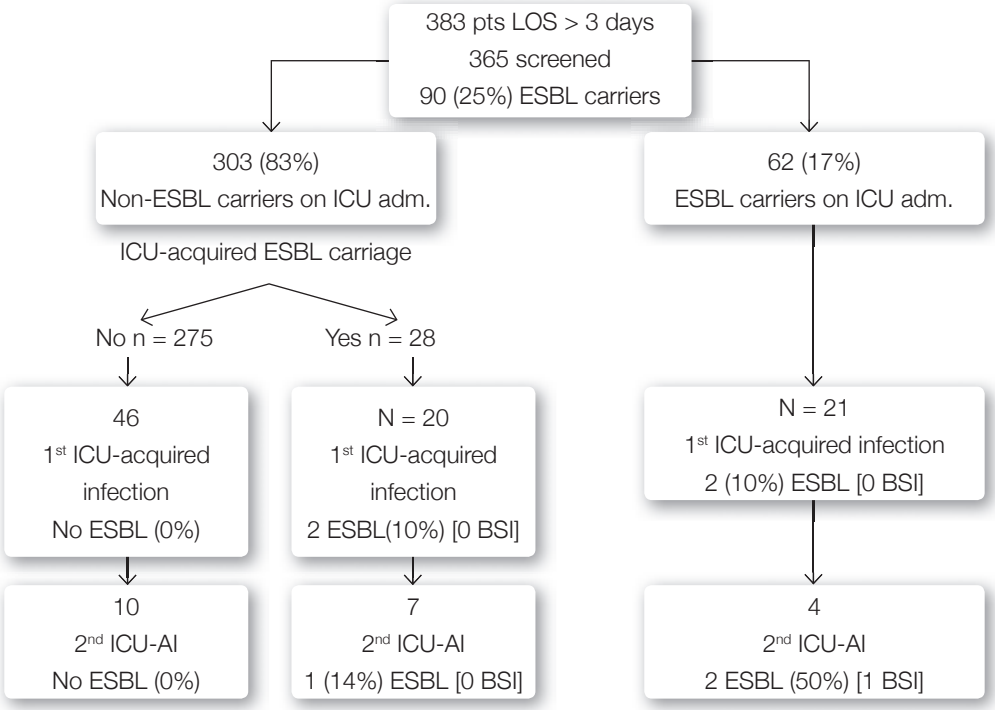
Among the 365 patients staying in the ICU for more than 3 days, we found 108 ICU-acquired infections in 87 patients (Figure 2). Seven (6.5%) of these were caused by ESBL-PE, including urinary tract infection (n = 5), or pulmonary infection and intra-abdominal infection with bacteraemia (1 each). None of these patients received delayed effective therapy. All patients with ICU-acquired infection caused by ESBL-PE also had rectal carriage, including 14 (82%) with the same species; only one patient had infection before detection of carriage.

The median length of stay before the occurrence of ICU-acquired infections caused by ESBL-PE was 10 days.[6-11] Among the 90 patients staying in the ICU for more than 3 days and having ESBL-PE carriage either on admission or acquired during the ICU stay, ESBL-PE caused 4/41 (10%) of first episodes and 3/11 (27%) of the second episodes of ICU-acquired infection (Figure 2). Eight further patients having clinical samples growing ESBL-PE were considered to have colonizations, which were not treated with antibiotics.

The median time elapsed between the detection of ESBL carriage in rectal swabs and detection of ICU-acquired infection was 5 days.[3-9] The few ESBL infections did not allow multivariate analysis of variables associated with infection caused by ESBL-PE.

The overall in-ICU mortality rate was 18%, and the median length of stay was 5 days.[3-10] The mortality rate of the 110 ESBL-colonized patients and of the 16 patients having ESBL-PE infection was similar (19%), as was their length of ICU stay (9 days[4-19] and 9 days,[7-15] respectively).

Figure 2: ICU-acquired ESBL colonization and infection.



Number (%) of patients with first and secondary infections caused by ESBL-PE among those acquiring infection during the ICU stay contrasting carriers and non-carriers and patients colonized on admission or acquiring ESBL-PE carriage.

AI acquired infection, *BSI* bloodstream infection.

Discussion

The main finding from this 8-month study of 531 patients is the high rate of rectal carriage of ESBL-PE on ICU admission (15% overall and 12% in patients admitted from the community), with *E. coli* representing the most common ESBL-PE species recovered (62%). Second, infections caused by ESBL-PE were rarely (3%, Figure 1) observed on ICU admission despite this high carriage rate; conversely, such infection was more common (10%) among patients with ICU-acquired infections, especially during second episodes (14%, Figure 2). This study is, to our knowledge, the first to analyze risk factors for ESBL-PE carriage at ICU admission and acquisition, based on prospectively collected and comprehensive information on patients' characteristics and exposures to antibiotics and other risk factors prior to and during ICU admission.

Most studies conducted in the past decade report an increasing incidence of ESBL-PE isolates recovered from both clinical and surveillance samples. Recent studies of colonization rates in ICU patients are sparse,[27-30] with rates varying from 2%[28] to as high as 49%.[30] Although ESBL rates reported differ according to the regional area and patient populations studied, the overall incidence of ESBL-PE has markedly increased in Europe during the past decade, including in the community.[3,5,10,12,13] Of concern, *E.coli* has emerged as the most common microorganism recovered from ESBL-PE carriers at admission; however, *K. pneumonia* and *E. cloacae* remain the most common ICU-acquired ESBL-PE microorganisms.

The increasing prevalence of ESBL-PE carriage on ICU admission raises important questions on empiric therapy policies in patients presenting with infection, which may include the use of a carbapenem as first-line therapy.

However, carbapenemase-producing *Enterobacteriaceae* have now emerged (notably among *Klebsiella pneumoniae*) as a group of highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality.[32] Since the emergence of antibiotic resistance is associated with widespread broad-spectrum antibiotic use, intensivists may now be close to a dead end without the cautious use of carbapenems.[21] In this context, examining the incidence and risk factors for colonization and infection with such microorganisms might help better targeting empiric therapy. Our results suggest that, despite the high colonization rate at ICU admission, infections caused by ESBL-PE remain very infrequent (only 3% of infected patients in our study). Thus, limited use of empiric treatment with carbapenems targeting ESBL-PE can still be recommended, even in a setting with a high endemic rate of ESBL-PE carriage. Restricting their use to selected patients having risk factors for colonization with ESBL-producing bacteria at ICU admission is likely to limit unnecessary exposure to carbapenems in the ICU.

Previous studies have shown that patients infected with ESBL-producing bacteria have identifiable clinical characteristics that can be used readily upon ICU admission,[28,29,31,33,34] such as male gender, being elderly and/or a nursing home resident, recent hospitalization, or exposure

to any antibiotic.[10] We identified prior hospital admission in another country, transfer from another ICU, surgery within the past year, and prior neurologic disease as independent risk factors for colonization with ESBL-PE at ICU admission. Predictive factors in the subgroup of patients admitted from the community included a previous hospital admission within 3-12 months before ICU admission and a history of urinary tract disease. Interestingly, our study confirms that exposure to third generation cephalosporin within months before ICU admission was an independent risk factor for colonization with ESBL-PE at ICU admission, irrespective of the patient's location before ICU admission.

Despite the high colonization pressure with ESBL-PE, the rate of ICU-acquired colonization with these microorganisms was relatively low, at 6% overall and 9% in the population staying in the ICU for > 3 days. This is probably explained mostly by the high hand hygiene compliance rate (> 80%) of personnel in our unit during the study period resulting from the ongoing hand hygiene improvement program and possibly from the routine use of daily chlorhexidine body washings. Among the 212 patients staying in the ICU for > 5 days and non-colonized on admission, only 28 (13%) acquired ESBL-PE carriage, mostly with *E. cloacae* (n = 13, 46%) and *K. pneumoniae* (n = 10, 36%), but rarely with *E. coli*. Acquisition of ESBL-PE during the ICU stay was associated with age > 75 years, male gender, colonization pressure, and administration of a third generation cephalosporin or a beta-lactam/inhibitor combination within 3 months before ICU admission. Intriguingly, exposure to various antibiotic classes during the ICU stay did not remain associated with ESBL-PE acquisition after multivariable analysis.

However, colonization pressure remained associated with acquisitions, and a substantial proportion of these were due to *K. pneumoniae* and *Enterobacter* spp, for which horizontal transmission may predominate over selection of resistance.[35]

Important features of our study include its prospective design, accounting for antibiotic exposure before and during the ICU stay, in the unique setting of a sustained controlled high hand hygiene compliance rate and absence of additional contact precautions, which helps interpreting the results. First, despite the high level of standard precautions, ESBLE-PE acquisitions still remained substantial and related to colonization pressure. Standard precautions alone thus do not appear sufficient to control the spread of ESBL-PE within ICUs. Second, the use of third generation cephalosporins should be limited, as prior exposure to these drugs was a risk factor both for carriage on admission and for acquisition.

Third, a different control policy may be justified against *E.coli* on one hand and *K. pneumoniae* and *Enterobacter* spp. on the other. While there are no universal guidelines concerning infection control measures for ESBL-PE carriers, our data suggest that additional measures may be warranted to control the spread of the latter species. Indeed, *K. pneumoniae* and *Enterobacter* are frequently involved in hospital outbreaks; in addition, environmental contamination is more frequent with ESBL-producing *Klebsiella* than with *E coli.*[36] Therefore, intensifying control measures might prove useful for these two species.

Overall, there were 294 infections at admission and 108 ICU-acquired infections, of which 3 and 6.5% were caused by ESBL-PE, respectively. Infections caused by ESBL-PE were thus relatively infrequent in our study despite the high carriage rate. In a prospective study of 455 consecutive episodes of *K. pneumoniae* bacteremia in 12 hospitals from seven countries conducted in 1996-1997, 85 (19%) were due to an ESBL-producing strain.[37] This rate was higher among the 253 nosocomial infections (31%), particularly those acquired in the intensive care unit (43%). In our patients, ESBL-PE caused 3% of all ICU-acquired bacteremias and 5% of ICU-acquired bloodstream infections caused by Gram-negative bacilli. Although ventilator-associated pneumonia (VAP) is the main source of ICU-acquired infections,[40] only one of our patients developed a VAP caused by an ESBL-PE.

A major risk factor for nosocomial infection is prior colonization.[22] All but one of the patients who had an ICU-acquired infection caused by ESBL-PE were found to have been previously colonized a median of 5 days[3-9] before infection. ESBL-PE caused only 4.6% of 87 first episodes but 14% of 21 secondary episodes of ICU-acquired infection. Among the 90 ESBL-PE carriers staying in the ICU for more than 3 days, these organisms caused 10% of the first episodes and 27% of the second episodes of ICU-acquired infections. In previous studies, performed in the context of much lower prevalence rates on ICU admissions, 9-25% of ESBL-colonized patients acquired ESBL-PE infection.[28,31] In our environment, carbapenems can thus be viewed as drugs of choice for empiric therapy of late ICU-acquired infections, especially in known carriers, thus providing coverage for ESBL-PE, as well as for drug-resistant *Pseudomonas aeruginosa*, another major cause of late ICU-acquired infection. Indeed, 73 patients received carbapenems during the 8-month period, mostly for empiric therapy of ICU-acquired infections.

Our study has some limitations. Because the study was monocentric, the results cannot be extrapolated to other ICUs with different epidemiologies and infection control policies and standards, especially regarding the acquisition of ESBL-PE. Colonization relied on rectal swabbing, which does not provide 100% sensitivity for the detection of ESBL carriage, thus possibly resulting in misclassification of patients. Confronting epidemiological information with the results of molecular typing of isolates could have provided a better insight into the risk of cross-transmission between different species. A final limitation of our study is the relatively small number of infections, thus limiting our ability to identify specific risk factors for infection. In addition, despite careful examination of patients' records, it is likely that we did not retrieve all antimicrobial drugs that patients may have received as outpatients before their hospital admission. However, the information collected in our study reflects the information available to the intensivist in real-life practice when confronted with decisions on antibiotic therapy in patients presenting with sepsis in the ICU.

To conclude, since improving carbapenem use is currently a major challenge for intensivists, our analyses of risk factors for colonization on admission and acquisitions of ESBL-PE may be useful for identifying which patients may warrant empiric therapy targeting these organisms in the context of high endemic rates, even on ICU admission. They may also contribute to future antibiotic stewardship programs and/or interventional studies to help control ESBL-PE.[38,39] Larger scale studies are needed to identify risk factors for ICU-acquired ESBL infection and to assess patterns of use of empiric therapy with carbapenems according to the knowledge of ESBL colonization status of the patient.

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Conflicts of interest All authors report no conflict of interest relevant to this study.

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Electronic supplement material

2012-00132 (R1)

Clinical Impact and Risk Factors for Colonization with Extended-Spectrum β-Lactamase-Producing Bacteria in an Intensive Care Unit.

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This electronic supplement includes details on the methods used for detection of ESBL-producing *Enterobacteriaceae* in rectal swab samples, and one Table, detailing ESBL-producing *Enterobacteriaceae* recovered during the study, whether on admission or during the intensive care unit (ICU) stay.

Methods: Detection of ESBL in rectal swab samples:

The rectal samples were first screened for potential ESBL-producing bacteria by growing, at 24 hours, on Oxoid Brilliance™ ESBL agar (Oxoid Ltd, Cambridge, UK). This medium contains a combination of chromogens that produced different colours for each ESBL-producing bacteria species. If any coloured colonies were observed on the medium, a single colony per colour was picked and sub cultured on blood agar plates, and incubated overnight in air. All potential ESBL-producing isolates underwent ESBL detection by the double-disk diffusion method using ceftazidime or cefotaxime with clavulanic acid and their susceptibility was determined by disk diffusion testing according to the Clinical Laboratory Standards Institute's guidelines.[25]

Table: ESBL-Producing *Enterobacteriaceae* species associated with colonization on ICU admission, ESBL acquisition, and recovered from clinical samples.

Parameter	ESBL at admission	ESBL acquisition	All
	(n = 82)	(n = 28)	(n = 110)
Colonizing species			
E. coli	51 (62%)	2 (7%)	53 (48%)
K. pneumoniae	15 (18%)	10 (36%)	25 (23%)
E. cloacae	7 (9%)	13 (46%)	20 (18%)
Others	5 (6%)	1 (4%)	6 (5%)
Polymicrobial	4 (5%)	2 (7%)	6 (5%)
Days before acquisition:	-	9 [8-20]	-
ESBL clinical sample, no.	21	5	26 (24 in ICU)
ESBL infection, no.	13	4	17 (16 in ICU)
Clinical isolates			
E. coli	12 (57%)	0	12 (46%)
K. pneumoniae	5 (24%)	3 (60%)	8 (31%)
E. cloacae	3 (14%)	2 (40%)	5 (19%)
Other	1 (5)	0	1 (4%)

Duration of colonization with antimicrobial-resistant bacteria after ICU discharge

eight

M.R. Haverkate, M.C.J. Bootsma, L.P.G. Derde, C. Brun-Buisson, M.J.M. Bonten, on behalf of the MOSAR Work Packages 3 and 7

Submitted

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Duration of colonization with antimicrobial-resistant bacteria after ICU discharge

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Keywords

Antimicrobial-resistant bacteria | ICU | colonization | survival function | interval censored data

Abstract

Purpose: Readmission of patients colonized with antimicrobial-resistant bacteria (AMRB) is important in the nosocomial dynamics of AMRB. We assessed the duration of colonization after discharge from the intensive care unit (ICU) with highly resistant *Enterobacteriaceae* (HRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant Enterococci (VRE).

Methods: Data were obtained from a cluster-randomized trial in 13 ICUs in 8 European countries (MOSAR-ICU trial, 2008-2011). All patients were screened on admission and twice weekly for AMRB. All patients colonized with HRE, MRSA, or VRE and a readmission to the same ICU during the study period were included in the current analysis. Time between discharge and readmission was calculated and the colonization status at readmission was assessed. Because of interval-censored data, a maximum likelihood analysis was used to calculate the survival function, taking censoring into account. A nonparametric two-sample test was used to test for differences in the survival curves.

Results: The MOSAR-ICU trial included 14,390 patients, and a total of 64,997 cultures were taken from 8,974 patients admitted for at least three days. 127 unique patients had 143 episodes with AMRB colonization and at least one readmission. 32 patients were colonized with two or more AMRB. Median times until decolonization were 3.4 months for all AMRB together, 1.4 months for HRE, and less than one month for MRSA and VRE. There were no significant differences between the survival curves.

Conclusion: Fifty percent of the patients had lost colonization when readmitted two or more months after previous ICU discharge.

Introduction

The increasing prevalence of antimicrobial-resistant bacteria (AMRB) in healthcare facilities poses a heavy load on infection control policies. Readmission of patients colonized with AMRB is an important factor in the nosocomial dynamics of AMRB. It creates a 'feedback loop' where pathogens are reintroduced into the ward and can colonize or infect new patients.[1] Yet, AMRB carriage disappears after some time in most patients after discharge. In many hospitals AMRB-positive patients are 'flagged' in patient systems, in order to allow quick identification at readmission.[2-4] Naturally, the feasibility of this infection control measure would be enhanced if patients could be safely 'deflagged'. However, little is known about duration of colonization with AMRB after hospital discharge.

Several studies have assessed the duration of carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in varying settings,[2,4-10] yielding large differences in colonization duration. Results vary from 94% clearance in less than one day after short-term occupational exposure to livestock-associated MRSA[9] to a half-life of MRSA persistence of 40 months.[4]

For highly resistant *Enterobacteriaceae* (HRE) and vancomycin-resistant Enterococci (VRE) estimates of colonization duration are even scarcer, but also non-consistent.[11-20] In Thailand[12] a median duration of outpatient colonization with ESBL-producing bacteria of 98 days was observed, while a mean duration of carriage of 9 months was reported among adopted children from Mali.[15] For VRE, reported median colonization time was 7 weeks[17] and in another study VRE was still recovered from 60% of carriers of a particular outbreak strain, but only from 20% of carriers of non-epidemic strains after six months.[16]

Knowledge about the time until decolonization of AMRB is of great importance for understanding nosocomial dynamics and for predicting effects of interventions. Colonization is a better indicator for bacterial dynamics than infection, since colonization only leads to infection in a small group but contributes significantly to the epidemiology of these bacteria.[21] Therefore, we assessed the duration of colonization after discharge from the intensive care unit (ICU) with HRE, MRSA, and VRE.

Methods

Study design and study population

Data were obtained from a cluster-randomized trial in 13 ICUs in 8 European countries (MOSAR-ICU trial): France (3 ICUs), Greece (2 ICUs), Italy, Latvia, Luxembourg, Portugal (2 ICUs), Slovenia (2 ICUs), and Spain. Data were collected from May 2008 until April 2011. The trial consisted of a 6-month baseline period, followed by implementation of a hand hygiene improvement program and unit-wide chlorhexidine body washing in month 7 to 26, and surveillance screening for AMRB carriage at ICU-admission (followed by contact precautions for AMRB carriers) with ICUs randomized to PCR-based or chromogenic agar-based screening methods in month 13 to 26. During the whole study period, all patients expected to stay for more than two days were screened on admission and twice weekly for HRE, MRSA, and VRE.

Written approval of the study protocol was obtained from each institution's review board or national ethics committee. A waiver for informed consent was granted for all participants since the study was considered to involve no more than minimal risk of harm to patients (ClinicalTrials.gov Identifier: NCT00976638).

All patients colonized with HRE, MRSA, or VRE in at least one of the two last swabs during admission and at least one readmission to the same ICU were included in our study. HRE included *Enterobacteriaceae* suspected to harbour extended-spectum beta-lactamase (ESBL): *Escherichia coli* (*E.coli*), *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* (KESC), and *Proteus*, *Providencia*, and *Morganella* (PPM) species. The date of discharge was taken as the start of the 'at risk' period for decolonization. This 'at risk' period for decolonization was calculated as the time between discharge and readmission. At readmission, we analyzed the first two available cultures for every patient and labelled a patient as 'colonized' if at least one of these cultures was positive, or 'decolonized' if both cultures were negative. We assumed admission was non-informative with regard to colonization status. We performed analyses for all bacteria together (looking at 'colonization' as the event of interest, regardless of the type of bacteria the patients were colonized with) and for all different bacterial types separately.

Microbiological tests

Swabs were obtained from the anterior nares (for detection of MRSA), the perineal area (for detection of HRE and VRE), and wounds (if present; for detection of HRE, MRSA, and VRE). Samples were frozen at -70/-80°C for a maximum of 2 months before processing in the baseline period. The media used for detection of AMRB were Brilliance 2 ESBL for detection of HRE, BBL CHROMagar MRSA II for detection of MRSA, and ECCV with 8 µg/ml vancomycin for detection of VRE. Resistance phenotypes detected from chromogenic media were shipped to central laboratory facilities for genetic confirmation.

Statistical analyses

The time between discharge and readmission varied between patients. Naturally, it was impossible to determine exact times until decolonization for patients non-colonized upon readmission, resulting in interval-censored data.[22] In Mathematica 7.0 (Wolfram Research, Inc., Mathematica, Version 7.0, Champaign, IL), we used a maximum likelihood analysis to calculate the survival function, taking censoring into account. A more detailed description of the method can be found in the paper of Goggins and Finkelstein.[22] Graphs were constructed to visualize the survival functions for the different bacteria. Furthermore, a nonparametric two-sample test was used to test for significant differences in the survival curves. This method was developed by Andersen and Rønn and a more detailed description can be found in their paper.[23]

In sensitivity analyses, we determined the effects of detection bias, by assuming that colonization remained until discharge and thus including all cases with at least one positive culture anytime during admission, and through analyzing the first readmission culture instead of the first two.

Also, we analyzed the data excluding wound cultures, since they were taken infrequently and were dependent on the presence of wounds. Furthermore, we investigated different study phases separately (baseline versus intervention period) to determine whether decolonization therapy (chlorhexidine body washings), improved hand hygiene, and surveillance screening with contact precautions of identified carriers influenced colonization duration. Finally, we checked whether there was a difference between patients who were readmitted from their home and those coming from health care facilities (including hospital wards and long term care facilities).

Results

The MOSAR-ICU trial included 14,390 patients, and a total of 64,997 cultures were taken from 8,974 patients admitted for at least three days. There were 926 patients with at least one readmission, who had - in all - 2111 admissions during the study period. 127 of these 926 patients were colonized with AMRB in (at least) one of the last two swabs during their first admission and had a subsequent readmission during the study period; and were included in the analysis (Table 1).

Table 1: Baseline characteristics (n = 127)*

	At first admission	At readmission
Age, median (IQR)	63.0 (51.0-75.0)	64.0 (51.0-75.0)
Male, N (%)	79 (62.2%)	79 (62.2%)
Length of stay at ICU, median (IQR)	12.0 (6.0-22.0)	9.0 (5.0-18.0)
Location prior to ICU admission, N (%)		
Home/private residence	29 (22.8%)	14 (11.0%)
Healthcare facility	90 (70.9%)	105 (82.7%)
Unknown/other	8 (6.3%)	8 (6.3%)
Had surgery in 12 months before ICU admission, N (%)	38 (31.1%)	59 (48.0%)

* of all unique patients, data from their first admission and readmission in the study period was used. Including subsequent admissions gave similar results (data not shown). 5 cases had missing data on some variables.

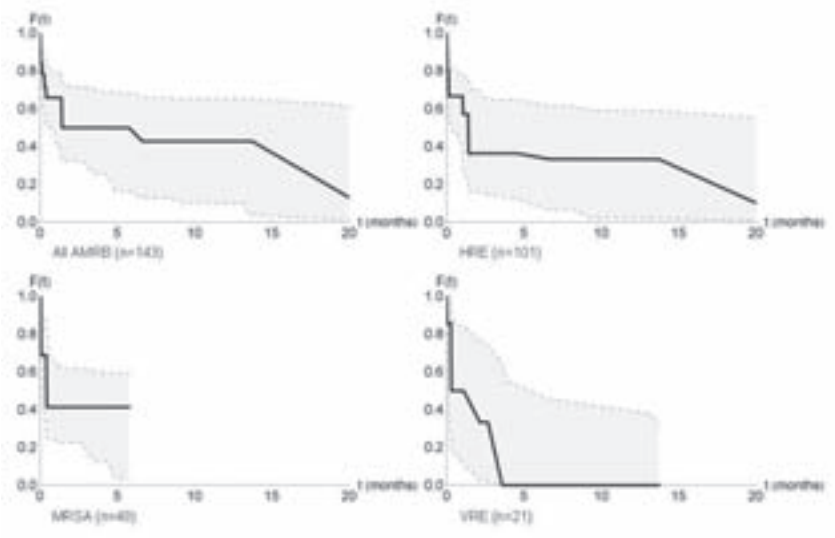
IQR: interquartile range

When analyzing all AMRB together, 143 episodes of colonization were recorded, including 32 episodes of colonization with two or more organisms; 101 episodes of HRE colonization (further divided into 34 for *E. coli*, 73 for the KESC-, and 8 for the PPM-group), 48 episodes of colonization with MRSA, and 21 episodes of colonization with VRE. The median times to decolonization were around one to two months for all bacterial types (Table 2 and Figure 1).
The nonparametric two-sample test revealed no significant differences between the survival curves of the different bacteria.

Table 2: Median times to decolonization for antimicrobial-resistant bacteria

Bacteria	Number of episodes	Median time to decolonization in months (95% CI)
All AMRB	143	3.4 (0.67 - ∞)
HRE	101	1.4 (0.32 - ∞)
E. coli	34	1.0 (0.03 - 7.6)
KESC	73	1.3 (0.08 - ∞)
PPM	8	0.4 (0.02 - ∞)
MRSA	48	0.4 (0.05 - ∞)
VRE	21	0.6 (0.04 - 5.4)

Figure 1: Survival functions of time to decolonization for antimicrobial-resistant bacteria and 95% confidence intervals



For AMRB, the median time to decolonization was 3.4 months, which was longer than for individual species. This can be explained by the fact that, in this case, we looked at 'colonization' as the event of interest, regardless of the type of bacteria the patients were colonized with. When a patient was readmitted and colonized with another type of bacterium than in his/her previous admission, this was still counted as 'colonized'. In our study, in 88 out of 98 cases in which a patient was still colonized at readmission, this was with (at least) one of the bacteria with which he/she was colonized in the previous admission. 10 cases were colonized with another type of bacterium. The same applies to HRE: we regarded a patient, colonized with HRE at discharge,

as 'still colonized' at readmission if he/she was colonized with any type of HRE. In 62 out of 65 cases in which a patient was still colonized with an HRE at readmission, this was with (at least) one of the bacteria with which he/she was colonized in the previous admission.

Including all cases with at least one positive culture anytime during admission instead of only looking at the last two samples of the first admission period did not significantly influence the results (data not shown). Restricting the analyses to the first culture of the readmission (instead of the first two cultures) also did not change results (data not shown). Furthermore, separate analyses of the different study phases or excluding wound cultures did not significantly change results (data not shown). Finally, there were no significant differences between patients being readmitted from home or from a health care facility.

Discussion

For all antimicrobial-resistant bacterial species, 50% of the patients had lost colonization when readmitted two or more months after the previous ICU admission. In view of these findings, interventions targeted at recently readmitted patients, for example preventive isolation or contact isolation, may be most effective. Although this study was performed on a selection of hospital patients, i.e. patients admitted to ICUs, the results are of critical importance since these patients are especially prone to colonization and (subsequent) infection.[21,24]

The analysis of all AMRB can be seen as a special case. Here, we did not assess the type(s) of bacterium a patient was colonized with, but only colonization status with any AMRB. Naturally, longer times to decolonization were found, since the type of bacterium at readmission is not necessarily the same as the one at discharge from the previous admission. This analysis shows that there might be a risk group consisting of patients who are prone to get colonized with any AMRB. What the specific risk factors are was not addressed in this study, since it was only applicable to 10 patients.

Our study revealed shorter decolonization times than previously reported, especially for MRSA,[4-8] which may have resulted from several methodological differences. For example for the calculation of the colonization duration of MRSA, Scanvic et al.[5] only included patients readmitted more than three months after discharge, while we included all readmitted patients, regardless of time since discharge. And although Larsson et al.[6] found a median time to decolonization of MRSA of 179 days (5.9 months), they also demonstrated that 43% of the cases were colonized less than 2 months, which is more similar to our results. In one of the first studies on colonization duration of MRSA, Sanford et al.[4] estimated the half-life of MRSA persistence in readmitted carriers to be 41 months. However, plasmid analysis and information on phage types indicated that only a part of the cases of persistent MRSA carriage had continued carriage of the same

strain and that the remainder represented acquisition of a new strain. Mattner et al.[7] indicate that they tend to overestimate the duration of colonization by regarding a patients as 'still colonized' if the readmission swabs were missed. This could partly explain the differences between their median time to decolonization (549 days) and the time found in this study.
Though, our results are consistent with the study of Robicsek.[2] They also found that 50% of the people lost colonization quickly (within one month). However, the colonization rate thereafter decreased slowly.

Another reason for the differences with these studies is that four of them used the Kaplan Meier method to estimate the median time to decolonization.[5-8] As we pointed out in the methods section, the exact time of decolonization is usually unknown, especially when readmission cultures are used or when sampling is infrequent. As this will result in interval-censored data, we have used a maximum likelihood analysis to calculate the survival function, with censoring taken into account. This will yield lower decolonization times than a Kaplan Meier estimate. Also, we used a very specific patient population. Moreover, as our results are based on the results of growth of cultures on selective media, patients colonized upon readmission with a new strain might be misclassified.

Our estimates on colonization duration with HRE were lower than findings in another study.[20] This can be explained by differences in study populations (ICU versus whole hospital), the fact that only patients readmitted after 3 months or more were included in the other study and the use of the Kaplan Meier method. Our estimates of duration of colonization are probably more accurate for the first months, but since the other study covered a period of 14 years, colonization and readmission after more than one or two years might have been captured better.

For the readmission swab, we focussed on the first two swabs. We required one or both of the readmission swabs to be positive, in order to label a patient (still) colonized. This strategy was chosen to prevent false-negative results of the first readmission swab, which may occur due to sampling errors depending on the swabbing technique used and the site swabbed.[4,25,26] Furthermore, the samples were frozen and stored during baseline (although for a maximum of two months) which might have influenced the results.
Survival of Gram-positive and Gram-negative bacteria during freezing and storage seems quite good,[27,28] although the amount of bacteria recovered from frozen suspensions may be reduced.[29] In the intervention period of the MOSAR trial, decolonization therapy (body washings with chlorhexidine) was implemented. No mupirocine was used. Body washing with chlorhexidine eradicates Gram-positive bacteria from the skin.[30-34] In the MOSAR trial, patients were washed from the neck down. Since we mainly used swabs taken from the nose for MRSA, one could expect that chlorhexidine would not influence colonization at this site. However, chlorhexidine

may remove colonization or temporal contamination with MRSA at other body sites. Hence, chlorhexidine may lower the colonization pressure of Gram-positive bacteria and could be effective in prevention of transmission. Little is known about the influence of chlorhexidine on colonization with Gram-negative bacteria.[30] However, eradication seems unlikely given that the gut, the main reservoir of Gram-negative bacteria, is not targeted with this decolonization therapy. To prevent potential influence of chlorhexidine washings on colonization duration, we only included patients with at least one positive swab in the last two swabs of their 'first' admission. In this way, patients in which carriage was eradicated were excluded. Furthermore, when we analysed the different study phases separately, no significant differences in colonization duration were found.

A limitation of this study is the unknown reason for readmission of patients. We have no information whether this reason is correlated to the colonization status of the patients. Therefore, we assumed admission was non-informative with regard to colonization status. We did have data on the 'most specific reason for ICU admission', and there was no difference in prevalence of sepsis or in the type of sepsis (urinary tract or other origin) in patients admitted to the ICU for the first time in the study period or readmitted patients (data not shown). Furthermore, there were no structured follow-up cultures taken of discharged patients. Therefore, we do not know exactly when colonization disappeared.

The prevalence and incidence of AMRB is different per hospital and country, but this did not seem to influence the results. The maximal contribution of one hospital to all cases was 18.7% for AMRB, 21.8% for HRE, 26.2% for *E. coli*, 25.9% for KESC, 62.5% for PPM (note: there were only 8 cases), 21.0% for MRSA, and 29.4% for VRE.

To our knowledge, this is the first study to assess the duration of colonization of ICU patients with HRE. Further studies with more readmitted colonized patients could be of help to assess the time to decolonization more precisely. As shown, approximately 50% of the patients were no longer colonized with a specific type of bacteria when readmitted after two months, implying that interventions, such as preventive isolation or contact precautions, should mainly be targeted at recently readmitted patients.

Acknowledgments

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Summary and general discussion

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Introduction

The aim of this thesis was to assess effects of interventions to control antibiotic resistance in 13 intensive care units (ICUs) across Europe. Furthermore, we investigated the published evidence on the effectiveness of chlorhexidine body-washing to prevent transmission of multi-resistant bacteria in ICUs, and investigated the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) and highly-resistant *Enterobacteriaceae* (HRE) in these 13 ICUs, factors predictive of colonization with HRE at admission in one of these ICUs, and the duration of colonization with MRSA, vancomycin-resistant Enterococci (VRE) and HRE.

Summary of results

Effectiveness of chlorhexidine body-washing

Seven studies investigating the use of chlorhexidine body-washing in the ICU were included in our systematic review.[1] Most studies had quantified infection rates (five quantified MRSA,[2-6] and one VRE infection rates),[3] and there were few data on the effects of this intervention on infections with HRE available.[2,4,5,7] Overall, included studies had low to medium risk of bias, but the reported evidence was weakened by inter-study differences in intervention, co-interventions, and patient case mix.

Importantly, decontamination of body surfaces may not only prevent infections but may also reduce cross-transmission, and thus may influence the risk of non-treated patients to acquire bacterial carriage (i.e., colonization pressure).[8] Not incorporating this patient dependency in the statistical analysis may lead to wrong inferences.[9] Thus, effects of chlorhexidine body-washing are best evaluated when applied universally in a unit. In our systematic review, only one study evaluated the effectiveness of chlorhexidine body-washing on the unit level.[7] In that study, the intervention reduced primary BSI after 5 or more days of stay in a medical ICU.[7] Hand hygiene compliance was not systematically assessed in any of the included studies. We concluded that – based on these seven studies - there is some evidence that chlorhexidine body-washing is effective in preventing carriage, and possibly bloodstream infections (BSI), with MRSA and VRE in ICU patients, but that there is no evidence (or lack of evidence) that chlorhexidine body-washing reduces carriage or infections with HRE.[1] Furthermore, the effectiveness of universal chlorhexidine body-washing had not been rigorously determined for any of these endpoints, and it is unknown to what extent its effectiveness depends on the level of hand hygiene in the participating units.

Reducing colonization and transmission of antibiotic resistant bacteria in intensive care units

The main part of this thesis is an international multicenter study in thirteen adult ICUs with at least 8 beds and endemic MRSA, VRE and/or HRE infection rates in which we have combined an interrupted time-series and a cluster-randomized intervention: the MOSAR-ICU trial.

After a baseline phase of 6 months, we implemented unit-wide chlorhexidine body-washing in all units, combined with a hand hygiene improvement program. In the third phase of the trial, ICUs were additionally randomized to universal screening for MRSA, VRE or HRE (with PCR-based tests for MRSA and VRE and chromogenic screening for HRE); or to universal screening for MRSA and VRE with chromogenic methods (without screening for HRE). In all ICUs, carriers detected were to be treated with contact precautions.

In all 14,390 patients were assessed for eligibility, of whom 8,519 (at risk for acquisition and with at least one surveillance swab) were included in the analysis. ICU and patient characteristics were typical for European ICUs, with bed occupancy rates above 85%, a mean length of ICU stay (LOS) of patients at risk for acquisition of 8 days and a mean nurse-to-patient staffing ratio of 0.54.

Compliance to the study protocol was high and all interventions were implemented successfully. Optimization of hand hygiene together with universal chlorhexidine body-washing was associated with a 3.6% weekly reduction of MRSA-acquisition. There was no evidence for an increase in chlorhexidine resistance of MRSA isolates during the trial. There was a trend towards a similar effect of these interventions on acquisition with VRE, but no such trend was observed for HRE acquisition.

The lack of effect on HRE-acquisition might be explained in part by differences in bacterial epidemiology. HRE mainly colonize the digestive tract, whereas MRSA and VRE also colonize the skin and the environment. Improved hand hygiene and universal chlorhexidine body-washing may synergistically reduce transmission of skin colonizing pathogens. These findings suggest that patient-to-patient transmission may not be the dominant acquisition route for HRE in ICU patients.

The intervention in phase 3 (universal screening for MRSA, VRE or HRE (with PCR-based tests for MRSA and VRE and chromogenic screening for HRE); or universal screening for MRSA and VRE with chromogenic methods (without screening for HRE)) was added to the optimized hand hygiene and universal chlorhexidine body-washing. It increased the proportions of patients treated with barrier precautions, especially in the ICUs using PCR-based screening tests, but failed to further reduce acquisition rates.

Based on this study we recommend that all ICUs implement the hand hygiene improvement program and universal chlorhexidine body-washing; and we do not recommend universal screening to control antibiotic resistance in ICUs.

Improving hand hygiene compliance in 13 European intensive care units

Between May 2008 and April 2011 we observed 41,558 hand hygiene opportunities within the MOSAR-ICU trial. During baseline, there was no evidence that performing observations increased compliance with hand hygiene requirements, i.e. there was no evidence of a Hawthorne effect. Implementation of the hand hygiene improvement program based on the World Health Organization's (WHO) concept in 13 European ICUs appeared feasible, yielding a rapid and sustained improvement of compliance from 52%, to 69% and eventually 77% in phase 1 (P1), phase 2 (P2) and phase 3 (P3) respectively. Compliance in P3 (77%) was high compared to the reported 69% achieved in other studies with comparable campaigns since the start of this millennium.[10-16] It has been suggested that feedback of hand-hygiene compliance improves the effectiveness of the WHO "5 moments" method.[17] In our study, we used a combination of the WHO "5 moments" method with monthly feedback, conference calls and newsletters, which seems to support this opinion. However, we did not assess any behavioral aspects of our program.

Secondly, in our hand hygiene analyses, we observed a small but significant inverse association between compliance and workload in P1 and P2. This association reversed in P3, suggesting that the negative effect of high workload on compliance disappeared when compliance was sufficiently high. A similar negative association was demonstrated previously,[10,13] in the era of hand washing, instead of hand rubbing. Our interpretation of these findings is that HCW cannot improve their performance after they reach a certain "maximum achievable level" of compliance. When the hand-hygiene improvement program continues, HCW will achieve the high level of compliance, even in extremely high workload situations.

Lastly, our data confirmed previous findings that compliance is generally higher among nurses than among other HCW categories, including physicians, and is higher in "before" than "after" opportunities for hand hygiene.[18] These findings offer specific aspects for improvement of the WHO "5 moments" method, as optimal infection prevention can only be achieved if all HCW achieve high levels of compliance. However, as nurses usually have by far the most patient contacts per day, a difference in hand hygiene compliance between HCW categories may not linearly influence cross-transmission.[19]

In conclusion, the WHO "5 moments" method offers a standardized, nowadays extensively tested program, that can be implemented in ICUs successfully. Adding feedback of results to HCW appears to increase the effectiveness of the program and specific aspects for improvement were identified.

Molecular epidemiology of MRSA in intensive care units

During the MOSAR-ICU trial, 631 patients were colonized with MRSA according to local test results, and 550 isolates (87%) were submitted to the reference laboratory, of which 510 (93%) were confirmed to be MRSA.

Almost all MRSA isolates were healthcare associated MRSA (HA-MRSA); community-acquired MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA) accounted for <4%. The Brazilian/Hungarian clone was most dispersed, being dominant in Latvia and the two Greek ICUs. ST8-IVc (UK-EMRSA-2/-6, USA500) was the most prevalent type in ICUs in France and Spain, and detected in eight ICUs in six countries. It was followed by ST22-IVh (UK EMRSA-15), detected in seven ICUs in five countries. All participating ICUs had a distinct molecular epidemiology and only ICUs from two sets of countries (France and Spain; Italy and Luxembourg) shared dominant clones. In countries with more than one ICU participating, the molecular epidemiology was homogeneous between ICUs, but not between countries. In all cases, these ICUs were not more than 100 kilometers apart.

In eBURST analysis MRSA isolates were grouped in eleven clonal complexes, with 51% belonging to clonal complex eight (CC8). Other prevalent clonal complexes were CC5 (26%) and CC22 (16%). There were only seven isolates with the Panton-Valentine leucocidin gene.

The Simpson index for genetic diversity, used to quantify genetic similarity of different MRSA isolates, was 0.86 for all ICUs combined, and varied from 0.11 to 0.77 for individual ICUs. The genetic diversity was inversely related to the MRSA-acquisition rate, which suggests that clonal spread was important in acquisition of MRSA. There were no significant differences in Simpson index between the three study phases.

In conclusion, this study revealed that MRSA epidemiology was homogenous within ICUs of the same country, but heterogeneous between countries. It is important to notice that a reduction in MRSA acquisition in the MOSAR-ICU trial was achieved in all ICUs, irrespective of the genetic diversity demonstrated in this study. LA-MRSA and CA-MRSA were nearly absent in these European ICUs.

Epidemiology of HRE in intensive care units

After exclusion of duplicate and non-relevant isolates, there were 1,753 HRE isolates from 1,453 admissions of the MOSAR ICU-trial available for molecular analyses. The average proportion of isolates submitted to the central laboratory per ICU was 89% (range 74%-96%). HRE were isolated from chromogenic agar (n = 1753) and *Klebsiella pneumoniae* (n = 821) and *Escherichia coli* (n = 648) were most prevalent. Extended-spectrum beta-lactamase (ESBL) was detected in 1,661 isolates (94.8%).

This study revealed large variation in the molecular epidemiology of HRE, and in particular of *E. coli* and K. pneumonia in ICUs in different countries. There was a difference in clonality - based on pulsed field gel electrophoresis (PFGE) patterns - between *E. coli* and *K. pneumoniae*, with more

clonality of *K. pneumoniae* strains. This suggests a more important role for cross-transmission in acquisition of *K. pneumoniae*. The importance of cross-transmission for *K. pneumoniae* has been reported before,[20] and this pathogen is a frequent cause of hospital outbreaks. Moreover, contamination is more frequent with ESBL-producing *Klebsiella* than with *E. coli*.[21] If further analyses confirm differences in transmission capacity of *K. pneumoniae* and *E. coli*, this might justify a pathogen-specific infection control approach for HRE.

Although reported HRE rates differ between studies, the overall incidence of HRE has markedly increased worldwide during the past decade.[22,23] Moreover, carbapenemase-producing *Enterobacteriaceae* (notably *K. pneumoniae*) have recently emerged in different parts of the world, and have imposed even bigger constraints on controlling antibiotic resistance.[24-26] Carbapenemase-producing HRE was detected in eight ICUs, and in the two Greek ICUs such bacteria accounted for 90% of all HRE. Metallo-beta-lactamase (MBL) and KPC resistance mechanisms were mainly detected in *K. pneumoniae*. In the Greek ICUs, the prevalence of KPC and MBL-producing *K. pneumoniae* accounted for 31% to 49% of all HRE isolates. In eleven of 13 ICUs, HRE mainly contained ESBL-producing *Enterobacteriaceae*, mostly CTX-M-15.

In summary, this study revealed large heterogeneity in the prevalence of antibiotic resistance mechanisms and sequence types of *E. coli* and *K. pneumoniae* and a difference in the clonality between these two pathogens. If confirmed with more analyses and in other studies, this would demand different control policies for *E. coli* and *K. pneumoniae*. Carbapenemase-producing HRE was most prevalent in both Greek ICUs, where the prevalence of KPC and MBL-producing *K. pneumoniae* amongst HRE was 31% to 49%. The clinical consequences for patient outcomes remain to be determined.

Risk factors for colonization with HRE in intensive care units

This sub-study was conducted during and after the third phase of the trial, in one of the French medical ICUs that participated in the MOSAR-ICU trial.[23] During the study period, 15 percent of all patients carried HRE, mostly *E. coli* (62%) or *K. pneumoniae* (18%). In patients admitted from the community, 12% were colonized with HRE on admission to ICU, most with *E. coli* (84%). Transfer from another ICU, previous hospital admission in another country, surgery within the past year, prior neurologic disease, and prior administration of third generation cephalosporin within the year before ICU admission were associated with HRE colonization at ICU admission. Independent predictive factors for colonization at ICU admission in the subgroup admitted from the community were recent hospital admission (within 3-12 months), prior urinary tract disease, and exposure to third generation cephalosporins.

Of all patients at risk for acquisition 13% acquired HRE carriage during their ICU stay, detected a median of 9 days after ICU admission. No carbapenemase-producing *Enterobacteriaceae* were detected in this ICU. Prior exposure to third generation cephalosporins or to a beta-lactam inhibitor (within 3 months of ICU admission) was strongly associated with acquisition.

These factors may be targeted by new interventions, such as “flagging” of patients with one or more risk factors.

Among the 365 patients staying in the ICU for more than 3 days, there were 108 ICU-acquired infections in 87 patients, of which seven (6.5%) were caused by HRE. Infection with HRE was associated with HRE carriage, and 82% of the patients with ICU-acquired HRE infection had rectal carriage with the same HRE species. The ICU mortality rate of the total population was 18%, with a median length of stay of 5 days. The mortality rate of HRE-colonized and infected patients was 19%, with a median length of ICU stay of 9 days.

Thus, we would not recommend empirical treatment with carbapenems for all patients admitted to the ICU with infection, but to restrict such treatment to patients with risk factors, such as documented carriage with HRE.

Duration of colonization

In the MOSAR-ICU trial, there were 926 patients with at least one readmission in ICU, and these patients had - in all - 2,111 admissions during the study period. 127 of these patients were colonized with antimicrobial-resistant bacteria (AMRB) in at least one of the last two swabs during their first admission and were included in this study, in which the duration until decolonization was determined. The median times to decolonization were 1-2 months for all bacterial types, with no significant differences between the survival curves of the different bacteria. Almost all patients (90%) that were still colonized at readmission were colonized with (at least) one of the bacteria with which they were colonized in the previous admission.

Our study revealed shorter decolonization times than previously reported, which may have resulted from several methodological differences between ours and other studies. We included ICU patients only; we assumed admission was non-informative with regard to colonization status and there were no structured follow-up cultures taken of discharged patients. Our estimates of duration of colonization are probably accurate for the first months. However, colonization and readmission after more than one or two years could not be reliably assessed in our study. Our estimates are consistent with results from two recent studies.[27,28] In the first study 50% of the patients had lost colonization within one month, and the colonization rate decreased more slowly thereafter.[27] In the second study 43% of the patients were colonized less than 2 months.[28] In view of these findings, interventions targeted at recently readmitted patients may be most effective.

Perspectives for future studies

Controlling antimicrobial resistance in ICUs remains challenging, even with the knowledge obtained in this thesis. We demonstrated that, in a background of optimal hand hygiene and universal chlorhexidine body-washing, screening and isolation of patients failed to reduce AMRB acquisition in ICUs with endemic resistance levels. Based on this study we recommend that

all ICUs implement the hand hygiene improvement program and universal chlorhexidine body-washing; and we do not recommend universal screening to control antibiotic resistance in ICUs. Our interventions were mainly effective for MRSA and VRE. However, controlling the emergence of HRE, especially carbapenemase-producing strains that are increasingly causing infections worldwide,[23-26] will be a major challenge in the coming years. The results of this thesis identified – at least – five areas for future research to optimize (or improve) our infection control practice for these bacteria in ICUs.

1. Accurate risk stratification of patients with HRE carriage and at risk for HRE infection is currently unknown. Recent studies have identified previous hospital admission, age, and recent use of third generation cephalosporins as important risk factors.[23] A better understanding of risk factors would allow for development of more targeted infection control interventions and empirical treatment strategies.

2. The dynamics of HRE epidemiology are not well understood. Cross-transmission can (and will) occur, but may not be the dominant route of acquisition for all species. Exogenous sources (such as animals and food) have been considered to be important for infections in humans, but these associations are currently based on (limited) observational studies only. Apart from these uncertainties, ICU patient populations have specific dynamics, with high rates of colonization on admission, short length of stay and many different risk factors for HRE carriage, creating enormous stochastic variation in prevalence. For such complex systems mathematical modelling using input from existing databases might help to gain insight in the transmission parameters of different HRE types. Modeling also allows scenario analyses of different interventions, without the immediate need for lengthy, costly trials, and allows for model-based predictions of future trends.

3. Resistance in Gram-negatives is easily transferred, and spreads between species. Resistance mechanisms can act together, and this synergistic activity enhances antimicrobial resistance and broadens its spectrum expressed by Gram-negatives.[29] This results in infections with pan-resistant pathogens, which can be devastating in ICU patients.[30] This stresses the need for basic research in the field of molecular biology to better understand the processes of horizontal gene transfer and to identify targets for new antibiotics, preferably new antimicrobial classes. Additionally, alternative approaches like targeting virulence genes, genomic-based approaches and 'natural' anti-bacterials need to be explored.[30] The interest of pharmaceutical companies to develop such antibiotics is low, because of substantial costs involved, technical difficulties, long development times and the fact that antibiotics can only be prescribed to patients for a short period of time.[29]

4. Apart from sophisticated research aimed at understanding the dynamics of HRE spread and resistance mechanisms, there is an urgent need for pragmatic solutions. The overall prevalence of HRE may soon be too high, to allow a holistic (one size fits all) infection control approach. The evidence of pathogen-specific transmission capacities amongst HRE may require a more pathogen-targeted approach. For example, there is evidence of less clonality in *E. coli* compared to *K. pneumoniae*, suggesting more clonal transmission of the latter species and – if confirmed – justifying different control policies for these pathogens. Clinical evaluation of such approaches is now warranted.

5. Antibiotic stewardship programs, including the rational use of decolonization with non-absorbable antibiotics, could be effective against HRE, including carbapenemase-producing *Enterobacteriaceae*.[31-33] Although considerable beneficial effects of oro-pharyngeal and intestinal decolonization (SOD and SDD) have been demonstrated in Dutch ICUs,[31] the ecological safety and effectiveness in ICUs with endemic levels of HRE remains to be determined.[34,35] Obviously, meticulous monitoring of resistance is an essential part of such interventions. There is growing evidence that interventions aimed at optimizing antimicrobial use can reduce antimicrobial resistance while reducing associated costs.[36,37] However, studies demonstrating not only reduced resistance, but improvement of patients' outcomes are needed.[38]

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Dutch summary (Nederlandse samenvatting)

L.P.G. Derde

Kolonisatie en infectie van intensive care patiënten

Patiënten die opgenomen zijn op de intensive care (IC) dragen vaak (antibioticaresistente) bacteriën bij zich. Dit noemen we kolonisatie. Sommige patiënten dragen deze bacteriën al bij opname bij zich, anderen worden tijdens opname op de IC gekoloniseerd. Kolonisatie tijdens opname treedt vaak op door overdracht van bacteriën van patiënt op patiënt, via de handen van personeel (transmissie). Kolonisatie kan leiden tot infectie, waarbij de bacteriën die de patiënt bij zich draagt ziekteverschijnselen veroorzaken. IC patiënten zijn zeer vatbaar voor infecties, vooral met antibioticaresistente bacteriën (ARB). Onderliggende immuundeficiënties, co-morbiditeit en het gebruik van invasieve materialen en behandelingen (b.v. beademing, centrale lijnen) zijn de belangrijkste risicofactoren voor het krijgen van zo'n infectie.[1] Deze risicofactoren, samen met veelvuldig gebruik van antibiotica, zorgen ervoor dat IC patiënten vaak gekoloniseerd raken en infecties krijgen met ARB. Het voorkómen van kolonisatie (en dus infectie) is een uitdaging.

Antibioticaresistente bacteriën

Voorbeelden van ARB zijn methicilline-resistente *Staphylococcus aureus* (MRSA), vancomycine-resistente Enterococcen (VRE) en multiresistente *Enterobacteriaceae* (HRE). Deze bacteriën komen steeds meer voor in ziekenhuizen over de hele wereld.[2-4]

Hoewel de laatste decennia meerdere typen MRSA zijn beschreven in Europa en de Verenigde Staten, wordt het merendeel van de MRSA infecties die in het ziekenhuis worden opgelopen nog steeds veroorzaakt door de "klassieke" MRSA genotypes.[3,5,6] Risicofactoren voor kolonisatie met MRSA zijn bijvoorbeeld hogere leeftijd, eerdere ziekenhuisopname (vooral IC opname), recente chirurgische ingrepen, recent of langdurig antibioticagebruik, het hebben van (chirurgische) wonden, co-morbiditeit en het gebruik van invasieve materialen.[4,7,8]

Resistentie tegen het antibioticum vancomycine onder Enterococcen werd voor het eerst beschreven in Europa in 1988.[9] De risicofactoren voor VRE zijn globaal dezelfde als die voor MRSA. VRE heeft daarbij de potentie om maandenlang op oppervlakken te persisteren.[10]

Het aantal infecties dat veroorzaakt wordt door ARB neemt toe en is geassocieerd met vertraagde start van adequate antibiotica, falen van antibiotische behandeling, langere opnameduur in het ziekenhuis en een hogere morbiditeit en mortaliteit.[11-14] De laatste jaren wordt vooral een toename van HRE gezien, hetgeen nieuwe uitdagingen op het gebied van infectieziekten-bestrijding met zich meebrengt.

HRE is een groep van bacteriën met allerlei resistentie-mechanismen, waaronder extended-spectrum beta-lactamase (ESBL)- en carbapenemase-productie.

ESBL-resistentie is plasmide-gemedieerd, wat betekent dat het gen dat codeert voor resistentie zich op een los DNA-element (plasmide) in de bacterie bevindt. Hierdoor kan deze resistentie zich efficiënt binnen, maar ook tussen verschillende Gram-negatieve bacteriën verspreiden.[15] Dit bemoeilijkt de bestrijding van HRE. De laatste jaren neemt het aantal carbapenemase-producerende Gram-negatieve bacterien (resistent voor bijna alle antibiotica) toe in verschillende delen van de wereld, hetgeen de controle van HRE nog moeilijker maakt.[16-18]
Het toenemend aantal infecties met resistente bacteriën zoals MRSA, VRE en HRE maakt dat de bestrijding van deze bacteriën, vooral op de IC, steeds belangrijker wordt.[11]

Huidige maatregelen om antibioticaresistente bacteriën te bestrijden

Om ARB te bestrijden worden bijvoorbeeld handhygiëne verbeterprogramma's gebruikt, worden patiënten gewassen met chloorhexidine of worden andere decontaminerende middelen gebruikt, zoals mupirocine-zalf in de neus om MRSA kolonisatie te voorkomen. Daarnaast worden patiënten bij opname soms gescreend op kolonisatie, en worden isolatie-maatregelen gebruikt als patiënten ARB bij zich dragen.
Het verbeteren van handhygiëne onder personeel wordt gezien als de hoeksteen van infectie-preventie.[19] Handhygiëne in ziekenhuizen is over het algemeen matig, met name op de IC. Op de achterliggende redenen hiervoor zullen we hier niet ingaan. We weten dat het verbeteren van handhygiëne mogelijk is, maar de variatie in gebruikte methoden en programma's in de literatuur is enorm. In 2009 heeft de World Health Organization (WHO) haar "Guidelines on Hand Hygiene in Healthcare" gepubliceerd.[19] Hierin wordt gebruik gemaakt van een multimodaal handhygiëne verbeterprogramma ("My 5 Moments for Hand Hygiene"), dat geschikt is voor training, observatie, en feedback over handhygiëne in bijvoorbeeld ziekenhuizen. Hoewel deze strategie gebaseerd is op uitgebreide evaluatie van de beschikbare literatuur, en er een test- en evaluatiefase van ongeveer 3 jaar is geweest, zijn de haalbaarheid van het programma en de effectiviteit in het reduceren van kolonisatie en infectie niet klinisch geëvalueerd.
Het gebruik van chloorhexidine in plaats van water en zeep voor het wassen van patiënten is geassocieerd met minder infecties, vooral op de IC.[20-27] Er zijn echter geen grote, goed uitgevoerde studies, en de verschillen tussen de studies in termen van co-interventies, type patiënten en protocol voor chloorhexidinegebruik zijn groot.
Ook isolatie-maatregelen voor patiënten die ARB bij zich dragen, of behandeling in een eenpersoons kamer is geassocieerd met minder transmissie van bacteriën.[28,29] Helaas richten de meeste van deze studies zich alleen op MRSA en niet op de in opkomst zijnde HRE.

Het doel van dit proefschrift is om interventies, gericht op de bestrijding van ARB, te evalueren in een *multi-center*, cluster gerandomiseerde trial. Daarnaast hebben we de huidige literatuur betreffende wassen met chloorhexidine op ICs geanalyseerd, en de moleculaire epidemiologie, risicofactoren en duur van dragerschap met MRSA, VRE en HRE bekeken op Europese intensive cares.

Resultaten in dit proefschrift

Na de introductie in **hoofdstuk 1**, beschrijven we in **hoofdstuk 2** van dit proefschrift de resultaten van een systematische review van de bestaande literatuur over het wassen van IC patiënten met chloorhexidine. De geïncludeerde studies hebben een redelijke tot goede kwaliteit. Helaas wordt de interpretatie van de resultaten van de studies bemoeilijkt doordat de studies sterk verschillen in co-interventies, type patiënten en exacte uitvoering van het chloorhexidine protocol. Daarnaast werd slechts in 1 studie naar het effect op unit-niveau gekeken.[21] Omdat dekolonisatie met chloorhexidine waarschijnlijk ook kruis-transmissie van bacteriën voorkomt, beïnvloedt het behandelen van een patiënt met chloorhexidine ook het risico van niet-behandelde patiënten op het verkrijgen van kolonisatie (kolonisatiedruk).[30] Daarom kan evaluatie van het effect van deze interventie het best op unit-niveau plaatsvinden. We concluderen dat, gebaseerd op de 7 studies in onze systematische review, er enig bewijs is dat wassen met chloorhexidine effectief is in het voorkómen van dragerschap, en wellicht ook bacteremieën, met MRSA en VRE in IC patiënten, maar dat er geen bewijs (of ontbrekend bewijs) is dat deze behandeling ook effectief is in het voorkómen van dragerschap en infecties met HRE.[31]

Het belangrijkste deel van dit proefschrift bestaat uit een internationale *multi-center* studie in dertien (volwassenen-) ICs, met minstens 8 bedden en endemisch MRSA, VRE en/of HRE. In deze studie hebben we een *interrupted time-series* design gecombineerd met een cluster-gerandomiseerde interventie. De belangrijkste resultaten van deze trial, de MOSAR-ICU trial, worden beschreven in **hoofdstuk 3**. Na een baseline fase van 6 maanden, hebben we chloorhexidine *"body-washing"* met een handhygiëne verbeterprogramma geïmplementeerd in alle deelnemende ICs. In de derde fase werden ICs daarbij gerandomiseerd voor ofwel universele screening voor MRSA, VRE en HRE (PCR testen voor MRSA en VRE; en chromagar testen voor HRE); ofwel voor universele screening voor MRSA en VRE met chromagar testen (zonder HRE screening). Op alle ICs werden patiënten die ARB bij zich droegen behandeld met isolatiemaatregelen in deze fase. In totaal werden 14.390 patiënten gevolgd, van wie er 8.519 (die risico op verkrijgen van dragerschap hadden en minstens 1 surveillance kweek) geïncludeerd in de analyses. Het wassen van patiënten met chloorhexidine, gecombineerd met optimaliseren van handhygiëne, leidde tot een wekelijkse reductie van 3.6% in verkregen MRSA kolonisatie. Voor VRE leek er eenzelfde effect te zijn, maar patiënten kregen niet minder HRE kolonisatie door deze interventie. Dit zou verklaard kunnen worden doordat HRE zich anders gedragen dan MRSA en VRE. HRE vinden we vooral in de darm, terwijl MRSA en VRE ook de huid en de omgeving van patiënten koloniseren. Betere handhygiëne en dekolonisatie van de huid met chloorhexidine zouden synergistisch kunnen zijn in het verminderen van kolonisatie met deze bacteriesoorten. Voor HRE daarentegen is overdracht van patiënt op patiënt waarschijnlijk niet de dominante verspreidingsroute. De hierboven beschreven screenings-interventie in fase 3 werd toegevoegd aan de strategie van handhygiëne-verbetering en chloorhexidine behandeling.

Hierdoor werden meer patiënten, zoals verwacht, behandeld met isolatiemaatregelen, maar dit verminderde het aantal mensen dat gekoloniseerd raakte met ARB niet. Op basis van deze resultaten bevelen wij aan dat alle ICs een handhygiëne verbeterprogramma starten, gecombineerd met chloorhexidine *"body-washing"* en zouden we universele screening met isolatiemaatregelen voor dragers van ARB niet willen aanbevelen.

In **hoofdstuk 4** beschrijven we in detail de resultaten van het in de trial gebruikte handhygiëne verbeterprogramma. In totaal werden 41.558 observaties van handhygiëne verricht gedurende de trial. Het implementeren van het WHO *"5 Moments"* programma leidde tot een snelle en persisterende toename van handhygiëne, van 52% in de baseline fase, tot 69% in fase 2 en 77% in fase 3. Het programma was erg succesvol, hetgeen verklaard zou kunnen worden door het toevoegen van maandelijkse *feedback* van resultaten en het gebruik van nieuwsbrieven en teleconferenties. In een recente studie leek het toevoegen van soortgelijke gestructureerde *feedback* de effectiviteit van het programma te verbeteren.[32] In onze studie verslechterde de handhygiëne bij toenemende werkdruk, zoals ook bekend is uit de literatuur.[33,34] Opvallend was echter dat deze associatie verdween in fase 3. Onze interpretatie van deze gegevens is dat ziekenhuispersoneel na het bereiken van een maximaal haalbaar niveau van handhygiëne (100% is in de praktijk onhaalbaar), wel nog verbetering van handhygiëne laten zien bij hoge werkdruk, waar deze eerst achterbleef. Onze studie bevestigt eerdere bevindingen dat handhygiëne van verpleegkundigen beter is dan handhygiëne van andere groepen personeel, inclusief artsen; en dat handhygiëne voorafgaand aan patiënt-gerelateerde handelingen slechter is dan handhygiëne nadien.[19] Samenvattend is de WHO *"5 Moments"* methode een uitvoerig getest en gestandaardiseerd programma, dat met succes geïmplementeerd kan worden op de IC. Het toevoegen van gestructureerde feedback lijkt de effectiviteit te verhogen.

In **hoofdstuk 5** beschrijven we de moleculaire epidemiologie van MRSA in Europese ICs. Gedurende de MOSAR-ICU studie werden 631 patiënten geïdentificeerd als gekoloniseerd met MRSA in de lokale laboratoria. Van deze patiënten werden 550 (87%) isolaten naar het centrale laboratorium gestuurd. Van 93% werd bevestigd dat er sprake was van MRSA. Vrijwel alle isolaten (>96%) waren "klassieke" MRSA (HA-MRSA). De zogeheten Braziliaans/Hongaarse kloon kwam het meest voor, en was dominant in Letland en de twee Griekse ICs. Alle ICs hadden een eigen moleculaire epidemiologie en ICs van slechts twee sets van landen (Frankrijk en Spanje; Italië en Luxemburg) deelden hun dominante kloon. In landen waar meer dan 1 IC aan de studie deelnam was de epidemiologie homogeen tussen ICs, maar niet tussen landen. De Simpson index voor genetische diversiteit wordt gebruikt om genetische overeenkomsten tussen verschillende MRSA isolaten te vergelijken. De waarden liggen tussen 0 en 1, waarbij 0 identieke isolaten aanduidt (clonale verspreiding) en 1 maximale diversiteit. De Simpson index was 0.86 voor alle ICs samen, maar wisselde tussen 0.11 en 0.77 voor individuele ICs.

Deze index was niet significant verschillend tussen de studie fasen. De genetische diversiteit was omgekeerd geassocieerd met het verkrijgen van MRSA kolonisatie, wat suggereert dat clonale verspreiding belangrijk is bij verkregen MRSA kolonisatie tijdens IC opname. Samenvattend laat deze studie zien dat MRSA epidemiologie homogeen is binnen ICs in hetzelfde land, maar heterogeen tussen landen. Opvallend is dat een reductie in verkregen MRSA kolonisatie werd gezien in alle ICs in de MOSAR-ICU studie, onafhankelijk van de genetische diversiteit die in dit hoofdstuk wordt beschreven.

In **hoofdstuk 6** van dit proefschrift beschrijven we de epidemiologie van een andere groep van ARB, de multiresistente *Enterobacteriaceae*, op intensive cares. In totaal waren 1.753 isolaten van 1.453 patiënten beschikbaar voor moleculaire analyses. Gemiddeld werden van 89% (range 74% - 96%) van de met HRE gekoloniseerde patiënten isolaten ingestuurd naar het centrale laboratorium. De meest voorkomende HRE waren *Klebsiella pneumoniae* (n = 821) en *Escherichia coli* (n = 648). ESBL werd in 1,661 (94.8%) isolaten gevonden.
Er was een opvallend verschil tussen *E. coli* en *K. pneumoniae* in clonaliteit, met meer clonaliteit in de *K. pneumoniae* isolaten. Dit suggereert dat bij *K. pneumoniae* kruis-transmissie een grotere rol speelt. Indien dit verschil in nieuwe studies bevestigd kan worden dan zou dit een verschillende controle-strategie voor deze bacteriën rechtvaardigen.
Op acht ICs werden gedurende de MOSAR-ICU trial carbapenemase-producerende HRE gevonden. Bij de twee Griekse ICs omvatte dit type HRE 90% van alle isolaten. Dit is een belangrijke bevinding omdat carbapenemase-producerende HRE nauwelijks nog met antibiotica kunnen worden behandeld, een snel groeiend en wereldwijd probleem.[16-18] Twee belangrijke resistentie-mechanismen, metallo-beta-lactamase (MBL) en KPC, werden vooral gevonden in *K. pneumoniae*. In de Griekse ICs was de prevalentie van KPC en MBL producerende *K. pneumoniae* respectievelijk 31% en 49%. In de andere 11 ICs waren voornamelijk HRE met het ESBL-resistentie mechanisme gevonden, met name het CTX-M-15 type. Concluderend was er sprake van grote heterogeniteit in de prevalentie van HRE resistentie mechanismen, en was er een verschil in clonaliteit tussen *E. coli* en *K. pneumoniae*. Dit verschil zou, indien bevestigd, verschillende maatregelen ter controle van deze pathogenen rechtvaardigen. Carbapenemase-producerende HRE kwam met name voor in de Griekse ICs.

In **hoofdstuk 7** beschrijven we de resultaten van een sub-studie in een van de Franse ICs die deelnam aan de MOSAR-ICU trial. Deze studie werd uitgevoerd tijdens en na fase 3 van de trial en onderzocht risicofactoren voor HRE dragerschap. Op deze IC was 15% van de patiënten HRE drager bij opname, vooral van *E. coli* (62%) of *K. pneumoniae* (18%). In de groep patiënten die vanaf thuis werd opgenomen op de IC was 12% HRE drager bij opname, het merendeel droeg *E. coli* bij zich (84%). Overplaatsing vanaf een andere IC, eerdere ziekenhuisopname in het buitenland, chirurgie in het laatste jaar, eerdere neurologische ziekte en eerder gebruik van

derde-generatie cefalosporinen (3GCS; een groep antibiotica) waren in de hele groep patiënten geassocieerd met HRE kolonisatie bij opname. In de groep patiënten die opgenomen werd vanaf thuis waren ziekenhuisopname in het laatste jaar, eerdere urineweginfecties en blootstelling aan 3GCS risicofactoren om tijdens IC opname gekoloniseerd te raken met HRE. Van alle patiënten raakte 13% gekoloniseerd met HRE gedurende IC opname, na een mediane duur van 9 dagen op de IC. Er werden geen carbapenemase-producerende HRE gevonden. Van de 365 patiënten die minstens 3 dagen waren opgenomen werden bij 87 patiënten in totaal 108 infecties vastgesteld die op de IC waren ontstaan. Hiervan werd slechts 6.5% veroorzaakt door HRE. Gemiddeld overleed 18% van de patiënten op de IC en was de mediane opnameduur 5 dagen. Voor patiënten die gekoloniseerd en geïnfecteerd waren met HRE was de mortaliteit hetzelfde (19%), en de mediane opnameduur 9 dagen.
Op basis van bovenstaande gegevens zouden wij niet willen adviseren om alle patiënten die met een infectie op de IC worden opgenomen empirisch te behandelen met carbapenems. We zouden willen adviseren om deze groep antibiotica te reserveren voor patiënten met risicofactoren, zoals hierboven genoemd.

Hoofdstuk 8 beschrijft de duur van kolonisatie met ARB gebaseerd op data van 926 patiënten uit de MOSAR-ICU trial die minstens eenmaal heropgenomen werden. Deze patiënten werden samen in totaal 2.111 keer opgenomen gedurende de studie periode. Van deze patiënten waren 127 gekoloniseerd geweest met ARB tijdens hun eerste opname. De mediane tijd tot verlies van kolonisatie (dekolonisatie-tijd) was 1-2 maanden, zonder verschillen tussen de verschillende typen ARB. Onze studie liet kortere dekolonisatie-tijden zien dan eerder gerapporteerd is. De oorzaak hiervoor ligt waarschijnlijk in de methodologie van deze studie. In deze studie werden alleen IC-patiënten geïncludeerd; we hebben aangenomen dat de aan- of afwezigheid van kolonisatie niets te maken had met de heropname(s) en we hebben niet de beschikking over kweken die afgenomen zijn na ontslag van de IC. Al met al zijn onze schattingen waarschijnlijk adequaat voor de eerste maanden. Kolonisatie en heropname na meer dan 1 of 2 jaar kon niet betrouwbaar gemeten worden in deze studie. Gezien de resultaten van deze studie zouden interventies die gericht zijn op recent opgenomen patiënten de meest effectieve kunnen zijn.

In **hoofdstuk 9** vindt u een Engelse samenvatting en discussie van de resultaten van dit proefschrift.

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Lennie

Curriculum Vitae

Lennie Derde was born in 's-Hertogenbosch on August 10th, 1976, and grew up in Hintham, the Netherlands. After graduating from secondary school in 1994 (Sint Janslyceum (Gymnasium), 's-Hertogenbosch), she started medical school at the VU University of Amsterdam. During her study she performed a research project at the Raymond Purves Bone & Joint Research Laboratories of the Royal North Shore Hospital, Sydney University, in St. Leonards, Australia. During this research project she worked on the cellular uptake and metabolic effects of heparin and pentosan polysulfate in ovine articular chondrocytes. After receiving her doctorate in 1998, she graduated (cum laude) in March 2001.

After graduating she worked in the Deboray Retief Memorial Hospital in Mochudi, Botswana, as an Internal Medicine and Paediatrics resident. Lennie started working as a resident Internal Specialties in the BovenIJ Hospital in November 2001, and started her formal training in Internal Medicine in September 2002 (supervisor dr. H. Muller), in the Gooi Noord Hospital (currently Tergooiziekenhuizen). She continued her training in the UMC Utrecht from May 2005 (supervisor prof. dr. D.W. Erkelens†, prof. dr. E. van der Wall, prof. dr. D.H. Biesma and prof. dr. M.M.E. Schneider), and started her fellowship Infectious Diseases in January 2006 under supervision of prof. dr. I.M. Hoepelman.

In September 2007, the author started working on the MOSAR project, specifically the MOSAR-ICU trial, at the Julius Center for Health Sciences and Primary Care of the UMC Utrecht, under supervision of prof. dr. M.J.M. Bonten. After a period of three years, she registered as an internist-infectiologist, and interrupted her research project to start her fellowship Intensive Care (supervisor prof. dr. J. Kesecioglu) for one year. In March 2011 she started the last year of her research project, afterwards returning to finish her last year of Intensive Care fellowship in the UMC Utrecht, where she will remain to work as a staff member after finishing her fellowship. Lennie Derde lives together with Noortje van Wissen, and their cat, El Guappo.