Antibiotic rotation in the Intensive Care Unit

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CHAPTER 1

General introduction

Antibiotic resistance and the ICU

Infectious diseases have had a long and large impact on human morbidity and mortality. The discovery and development of antibiotics greatly reduced that burden. But in his Nobel Prize lecture for the discovery of penicillin in 1945, Alexander Fleming already warned for of the risk of selecting resistant bacteria. (1) He described both selection of resistant bacteria during antibiotic treatment, and transmission of antibiotic resistant bacteria between persons. Two processes that are still, to this day, fundamental parts of the global emergence of antibiotic resistance: Antibiotic exposure creates selective pressure for carriage and subsequent infection with antibiotic resistant bacteria, which may lead to failure of antibiotic treatment of infection.

Risk factors for carriage and infection with (antibiotic resistant) bacteria are highly prevalent among patients treated in intensive care units (ICU). Such patients suffer from critical illness which may lead to immune suppression (as do some immune modulating treatments) and the physical barriers for invasion of microorganisms may be disrupted through vascular catheters and surgical incisions, drains and other devices such as in orthopaedic external fixateurs, negative pressure wound therapy or cranial pressure monitors.

The ICU hence is a hotspot for acquisition of carriage and infection with potentially pathogenic bacteria, and infection rates in ICU can be as high as 50%. (2) Many infections are initially treated with broad-spectrum antibiotics that facilitate selection of antibiotic resistant bacteria.

Because of the frequent contact moments between medical staff (nurses and physicians) and patients, required for medical care delivery, the ICU setting is prone for the occurrence of patient-to-patient transmission of pathogens. Moreover, patients are admitted to ICU not only directly from the community, but also from other hospital wards, increasing the likelihood of introduction of resistant bacteria into the ICU. Finally, when discharged from ICU, patients typically go to regular hospital wards, further facilitating the spread of antibiotic resistant pathogens in the hospital. As a result, ICUs have been epicenters of spread for all clinically relevant antibiotic resistant bacteria, including Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococcus (VRE), Extended Spectrum

β-lactamase (ESBL) producing bacteria, and Carbapenemase Producing Entero-
bacteriacae (CPE) and Non-fermenting Gram-negative bacteria.

Infection prevention and control in the ICU

Infection prevention measures applied by healthcare staff is key for optimal control
of antibiotic resistance. Such measures can include reduction or eradication of bac-
terial loads in patients or healthcare workers, but also optimizing diagnostics and
treatment strategies to reduce antibiotic selective pressure. (3) Such interventions
have varying degrees of effectiveness in different settings, and, therefore, varying
scientific base for policy making.

This thesis will address the effectiveness of changing antibiotic selective pressures in
ICU patients. Optimizing antibiotic use, or Antibiotic Stewardship, generally implies
interventions to monitor, report or restrict antibiotics. (4) Examples are prior- or
post-hoc authorization of antibiotic prescriptions, automatic intravenous-to-oral
switch of antibiotics, dose adjustments in case of organ dysfunction (e.g. renal im-
pairment) or automatic stop orders (e.g. in surgical prophylaxis).

While antibiotic stewardship interventions mostly focus on reducing the volume of
antibiotic use, some interventions aim to *increase* antibiotic use in order to reduce
antibiotic resistant bacterial carriage and infection. These interventions include
eradication of Methicillin Susceptible *Staphylococcus aureus* (MSSA) for prevention
of post-operative wound infections, and selective decontamination of the digestive
tract (SDD) in specific patient populations, such as ICU patients.

Antibiotic rotation
Antibiotic rotation is an antibiotic stewardship intervention in which a reduction of
overall antibiotic use is not pursued, but in which the selective pressure exerted by
antibiotics is changed through the use of different types of antibiotics in large pa-
tient populations. The general dogma is that the overall volume of antibiotics used,
determines the selective pressure for antibiotic resistant bacteria. Antibiotic rotation
aims to reduce antibiotic selective pressure at population level, by systematic and
cyclic changes of antibiotic exposure, for instance through cyclic changes in pre-

ferred antibiotic use for a certain indication. This approach mimics crop-rotation, in which one field is sown with different consecutive crops over time, for optimal yield of a field, compared to mono-culturing and depletion of the fields' nutrients.

Antibiotic rotation has mostly been studied in empirical treatment, when broad-spectrum antibiotics are prescribed for a range of potential pathogens awaiting identification of a causative pathogen. Differences in rotated strategies can include the broadness of the antibiotic coverage or the inclusion, or not, of specific bacterial populations. After identification of a pathogen, the "rotated" antibiotic can be individually adjusted, for instance through de-escalation to a narrower spectrum antibiotic.

For simplicity, we distinguish two forms of antibiotic rotation; mixing and cycling. In mixing, the antibiotic exposure exerted, changes after each patient treated, thereby creating maximum heterogeneity of overall antibiotic exposure. During cycling, antibiotic exposure is changed in larger intervals, for instance in blocks of 4 weeks or 3 months, creating maximum homogeneity of overall antibiotic exposure within block periods.

There are several hypotheses for beneficial effects of antibiotic rotation. The general assumption is that the bacterial population adapts to a persistent (homogeneous) ecological antibiotic selective pressure, and these adaptations come with a cost of bacterial fitness. This reduces the capacity of bacteria to survive and being transmitted to other patients, and then reduces overall prevalence of antibiotic resistance. Another hypothesis is that with simultaneous use of diverse antibiotics at the population level (i.e., persistent heterogeneous pressure) an antibiotic resistant clone that would be transmitted between patients will have a higher chance to encounter an antibiotic to which the bacterium is still susceptible, and mixing would thus reduce the prevalence of antibiotic resistance. Similarly, it can be reasoned that during each block of cycling, one specific antibiotic exerts selective pressure towards one type of resistance, which is assumed to be associated with some fitness cost. When switching to another antibiotic the lack and cost of subsequent adaptation will increase therapeutic efficacy and reduce resistance prevalence. Naturally, emergence or introduction of multiple resistant bacteria might reduce beneficial effects of antibiotic rotation interventions.

Evaluation of the effects of antibiotic rotation has been hampered seriously by the use of heterogenous and bias-prone methodology in previous studies. In addition there is no consensus on the intervention format, how to measure ecological intervention compliance, and what endpoint to use for quantifying effects.

At the time that the studies of thesis were initiated (2010) the evidence base for antibiotic rotation strategies as a measure to control antibiotic resistance, were considered insufficient to recommend its implementation in the ICU. Based on clinical studies up till then, a large comprehensive, and methodologically sound trial for comparing antibiotic rotation strategies was felt needed. If such a trial would provide high-quality evidence of effectiveness of cycling or mixing, it would provide an intervention that could be implemented immediately without technical requirements and at no additional costs.

Outline of this thesis

The studies in this thesis aimed to strengthen the evidence base of the effects of antibiotic rotation strategies in the ICU to better inform clinical management of infections. First, the epidemiology of antibiotic resistance in European ICUs in 2010 was described – which was at the time that the intervention study of this thesis was prepared (**chapter 2**), as was the practice of using antibiotics to control antibiotic resistance in ICUs (**chapter 3**). **Chapter 4** provides the methodological design of an international cluster-randomized crossover trial to compare the effects of antibiotic cycling and rotation (with 6-week blocks) of three beta-lactam antibiotics used for empirical treatment of ICU-acquired infections. The primary analysis of that trial was an ecological analysis presented as **chapter 5**. In **chapter 6** a post-hoc analysis is presented on the individual effects (at patient level) of both interventions. In **chapter 7** the findings have been summarized together with a critical review on new developments related to antibiotic resistance epidemiology in ICUs, such as dual analysis methodology, directed evolution research, mathematical modeling, clinical trials and new crosslinks between these research fields. Altogether, this thesis provides new evidence to inform clinicians on the effects of antibiotic rotation and cycling in ICUs, as well as guidance for future studies on antibiotic rotation.

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CHAPTER 2

Recent trends in antibiotic resistance
in European ICUs

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Abstract

Purpose of review

Antimicrobial resistance is an emerging problem in ICUs worldwide. As numbers of published results from national/international surveillance studies rise rapidly, the amount of new information may be overwhelming. Therefore, we reviewed recent trends in antibiotic resistance in ICUs across Europe in the past 18 months.

Recent findings

In this period, infections caused by methicillin-resistant *Staphylococcus aureus* appeared to stabilize (and even decrease) in some countries, and infection rates due to Gram-positive bacteria resistant to vancomycin, linezolid or daptomycin have remained low. In contrast, we are witnessing a continent-wide emergence of infections caused by multi-resistant Gram-negative bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae*, with easily exchangeable resistance genes located on plasmids, producing enzymes such as extended spectrum b-lactamases and carbapenamases. In the absence of new antibiotics, prevention of infections, reducing unnecessary antibiotic use, optimizing adherence to universal hygienic and infection control measures, and improving implementation of diagnostic tests are our only tools to combat this threat.

Summary

As the epidemiology of antibiotic resistance in ICUs is rapidly changing toward more frequently occurring epidemics and endemicity of multi and panresistant Gram-negative pathogens, better infection control and improved diagnostics will become even more important than before.

Introduction

Antibiotic resistance is a daunting phenomenon with a growing impact on patient safety, particularly in ICUs (1). Critically ill patients are prone to colonization and infection with antibiotic-resistant bacteria because of frequent exposure to antibiotics, the presence of multiple, often invasive, devices, and the occurrence of so-called immune paralysis often in combination with disrupted skin and mucosal barriers. As critical illness may affect pharmacodynamics and pharmacokinetics of antibiotics, optimal penetration in infected tissues may not always be achieved, hampering successful treatment and promoting antibiotic resistance. This dangerous array of risk factors perpetually drives a vicious circle of increased infection incidence, increasing the need for broad-spectrum antibiotics, reduced antimicrobial efficacy and increased selection of antibiotic resistance.

This review addresses recent developments of the European epidemiology of antibiotic resistance in ICUs (Table 1) (2–4,5,6,7,8,9–16) with a focus on methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria producing extended spectrum b-lactamases (ESBL) and carbapenemases.

Table 1 Epidemiological trends among multidrug-resistant organisms in Europe

MDRO	Epidemiological trends
Gram-positive	
MRSA	Stabilizing and decreasing infections rates of MRSA (2–4)
VISA	Increase among *S. aureus* isolates, but absolute numbers are still low (5)
LRSA	Sporadic isolates and a single outbreak reported from Spain (6)
Gram-negative	
ESBL-producers (mainly *E. coli* and *K. pneumoniae*)	Continent-wide increasing infection rates (7,8,9–14); suggestion of existence of a hyper-epidemic *E. coli* clone (ST131) (15,16)
Carbapenemases	Continent-wide increase of infections caused by carbapenemase-producing Gram-negative bacteria (e.g., KPC, NDM-1, VIM, OXA). Endemicity of KPCs in some countries (Greece) and cumulating numbers of reported outbreaks from other countries, often related to patient transfer from endemic settings. Community-associated acquisition of NDM-1 reported from Asian countries, with subsequent introduction in European healthcare settings

Methicillin-resistant *Staphylococcus aureus*

The prevalence of MRSA infections among *S. aureus* bacteraemia varies widely across European countries, ranging from less than 1% in Scandinavian countries to 50% in the southern European countries (17). In a prospective cohort study, performed between 2005 and 2008, of almost 120 000 patients in European ICUs (mainly in France, Spain and Austria), *S. aureus* pneumonia and bacteraemia developed in 1.3% and 0.4% of all patients, of which 34 and 38% were caused by MRSA, respectively (18). In Italy, *S. aureus* was responsible for 23% of all ICU-acquired infections in 125 Italian ICUs and 39% of ventilator-associated pneumonia (VAP)-episodes and 71% of bacteraemia episodes were caused by MRSA (19).

In most hospitals, MRSA prevalence is higher in ICUs than in general wards, most likely because of the beforementioned risk factors for MRSA colonization and infection Gram-negative bacteria (4). However, MRSA colonization and infection rates may differ extensively between different types of ICUs. For instance, incidence ratios in medical ICUs were markedly lower (incidence rate ratio, 0.42) than in other ICU types in Germany (20).

As compared to methicillin-susceptible *Staphylococcus aureus*, episodes of bacteraemia (21) and pneumonia (22) caused by MRSA have been associated with higher healthcare costs, more frequent ICU admissions (21) and a higher ICU-mortality (18,22). Whether this higher mortality in ICU is truly attributable to methicillin resistance is difficult to disentangle because of the confounding effects of, for instance, comorbidity. In the largest study in the field, antibiotic resistance appeared not to be associated with increased length of ICU stay (18).

Because of these high incidences of MRSA infections, various infection control interventions were implemented in many European countries, which were followed by stabilizing and even decreasing incidences of MRSA infections in France and the United Kingdom (17,23). In French ICUs, the incidence of MRSA infections decreased from 2.95 to 1.23 per 1000 hospital days (relative change, 58%; P¼0.001) between 1996 and 2007 (3). In the UK, a nation-wide implemented prevention program was associated with 57% reduction in MRSA bacteraemia episodes between 2006 and 2008 (2).

Vancomycin is probably still the most widely used antibiotic to treat MRSA infections, although linezolid and daptomycin are also available. Vancomycin resistance

in *S. aureus* (VRSA), through acquisition of the vanA gene from vancomycin-resistant enterococci (VRE), was first reported in the United States in 2002 (24). Yet, since then only a few other VRSA isolates have been reported from the United States, but not from Europe (25). The fear of widespread transfer of the vanA gene among *S. aureus*, therefore, has not become reality, possibly due to fitness costs associated with expression of the vanA gene (26).

S. aureus strains with intermediate vancomycin susceptibility (VISA) are far more common. VISA is associated with a thickened cell wall capable of binding, and thus, reducing the availability of vancomycin. VISA develops mainly in MRSA, by serial mutations after prolonged vancomycin exposure (27). In a global study of more than 20 000 *S. aureus* isolates, obtained between 2004 and 2009, 8.9% of European MRSA isolates had a minimum inhibitory concentration (MIC) of at least 2mg/ml for vancomycin (5). Nevertheless, more than 98% of all MRSA isolates were susceptible to vancomycin (MIC 2mg/ml) and VRSA was not encountered. There were three linezolid-resistant *S. aureus* isolates, of which only one originated from Europe. Yet, an outbreak of linezolid-resistant MRSA was recently reported in a Spanish ICU (6). Here, horizontal transmission of resistance was suspected, as different MRSA clones as well as other staphylococci were carrying the cfr gene (28).

Other multi-resistant Gram-positive micro-organisms appear less relevant in European ICUs. In the European Antimicrobial Resistance Surveillance Network, the prevalence of VRE among enterococcal bloodstream infections was less than or equal to 5%, or even absent, in 13 of 24 countries that reported at least 10 *E. faecium* isolates.

Three countries (Greece, Ireland, and the United Kingdom) reported more than 25% VRE isolates, and VRE appears to be spreading in Swedish hospitals (17,29).

Gram-negative bacteria producing extended spectrum b-lactamases Gram-negative bacteria are common pathogens in ICUs and are able to transfer resistance genes via plasmids without the necessity to replicate (horizontal transfer), which markedly increases the transmission potential of resistance. These plasmids may contain other additional genes, including virulence factors.

ESBL confer resistance to penicillins and most cephalosporins, including third generation cephalosporins and as of now, more than 700 different ESBLs have been described. Although third-generation cephalosporin resistance is frequently used as proxy for ESBL-production, such a resistance phenotype can also result from

non-ESBL AmpC enzymes, upregulation of efflux pumps, changes in membrane porins and altered penicillin binding proteins. The prevalence of ESBL-producing bacteria varies considerably in Europe. *Escherichia coli* is most prevalent among infections with ESBL-producing bacteria, with reported prevalences ranging from 1.8 to 19.2% (based on the phenotype of third generation cephalosporin resistance) among bloodstream infections in 28 countries in 2009 (7,8,9,30).

Of special interest is the epidemiology of a certain *E. coli* genotype (ST131), which appears to emerge rapidly, both among isolates associated with infections as well as with colonization (31,32). Described initially in 2008 in Europe, Asia and North America, retrospective analyses of isolates suggest that this genotype infected patients only sporadically in the 20th century. *E. coli* ST131 has been associated with plasmid-borne CTX-M-15 genes and fluoroquinolone resistance.

Klebsiella pneumoniae is the other major reservoir of ESBL genes in hospitalized patients. Compared to *E. coli*, the prevalence of ESBL-producing Klebsiella spp. among bloodstream isolates in different countries varies even more (from 0 to 70%), although this variation also results from huge variations in episodes of bacteraemia included per country (ranging from 17 to 1634). Nosocomial outbreaks frequently occur across Europe, in some cases with specific sources such as contaminated medication and endoscopes (10–14).

Carbapenemases

Carbapenems are the treatment of choice for infections caused by ESBL-producing bacteria. Yet, Gram-negative bacteria are increasingly capable of producing enzymes able to hydrolyse carbapenems, so-called carbapenemases. It is a diverse group of enzymes that can be distinguished into three classes and various subgroups, of which *K. pneumoniae* carbapenemases (KPCs) and the

New Delhi metallo-beta-lactamase enzyme (NDM-1) are currently most relevant. KPCs and NDM-1 have been associated with rapid global spread and KPCs have caused several outbreaks in ICUs. Besides the ability to hydrolyse carbapenems, carbapenemase producing Gram-negative bacteria often confer resistance to a variety of other antibiotics, such as aminoglycosides, fluoroquinoles and cephalosporins, limiting treatment options to colistin, fosfomycin and tigecycline (33), yet even panresistant Gram negatives have been described already (34). KPCs were initially described in *K. pneumoniae*, but were later also demonstrated in other species such as Enterobacter spp. and *E. coli*. The plasmids carrying KPC genes are extremely mobile allowing transfer to different species within the Enterobacteriaceae family

(35,36). The first KPC was isolated in 1996 in the United States (37). As of 2011, KPCs have been found in at least 10 countries in four continents, with notable numbers of outbreaks in Israel and the United States (38,39). In Europe, carbapenemases appear to be most prevalent in Greece, where carbapenem resistance among *K. pneumoniae* blood isolates in ICU patients increased from 1% in 2001 to 80% in 2010 (40–42). For KPCs, the epidemiology is almost completely monoclonal as 96% of 173 *K. pneumoniae* isolates obtained in 21 Greek hospitals belonged to the same pulsetype (43). Yet, the number of reported outbreaks in other European countries is

rapidly cumulating (44–50). Apparently, transfer of patients from endemic settings, such as hospitals in the United States, Israel or Greece, facilitated the dissemination of KPC-producing *K. pneumoniae* in Europe (32,50–54). Moreover, as these bacteria can be carried without signs of infection, healthy people that migrate between countries and continents may also contribute to spread (41,50,55).

NDM-1, encoded by the blaNDM-1 gene, was recently discovered in a patient in Sweden who was transferred after hospitalization in New Delhi, India (56). The patient was colonized with both *E. coli* and *K. pneumoniae* carrying plasmids containing the blaNDM-1 gene. India and other Asian countries are considered the epicentre of this new epidemic, with one Indian study reporting NDM-prevalence of more than 90% among carbapenem resistant Enterobacteriaceae and a prevalence of more than 10% of carbapenem resistance among *K. pneumoniae* in some hospitals (57). In addition, blaNDM-1 harbouring bacteria were obtained from 51 of 171 seepage water samples and from two of 50 public tap water samples in New Delhi, indicating its ubiquitous presence (58). It is assumed that over-the-counter use of antibiotics facilitates NDM-1 selection and spread through faecal–oral transmission through environmental contamination. The number of reports of infections and carriage with NDM-1 in Europe is rapidly cumulating (59,60). Cases are often related to transfer of patients from endemic areas, especially from hospitals in India, Pakistan or the Balkans (34,61–63).

Burden of disease

It is difficult to quantify the burden of disease, expressed as the excess risk of dying or the attributable length of stay (LOS), due to infections caused by these antibiotic resistant bacteria. In two French studies, infections caused by ESBL-producing bacteria were not associated with statistically significant increases of LOS or higher mortality in surgical patients (7,9). Yet, in a Spanish study of cancer patients infec-

tions caused by antibiotic-resistant Gram-negative bacteria, mostly ESBL-producing *E. coli*, were associated with higher rates of ICU admission, longer ventilation times and increased mortality. Yet, inadequate empirical antibiotic treatment because of antibiotic resistance was not associated with unfavourable outcomes (64). Although it is widely believed that antibiotic resistance negatively influences patient outcome, accurately quantifying these effects is –methodologically – challenging because of the plethora of confounders.

Infection control

Disciplined and relentless application of universal infection control measures such as hand hygiene, environmental cleaning and isolation or cohorting of colonized patients are still cornerstones of infection control practices in ICUs (Table 2) (3,65,66,67,68–71). In addition, new diagnostic tools, for instance biomarkers such as procalcitonin, may enhance our abilities to implement tailor-made antibiotic treatment durations, reducing the total volume of antibiotic exposure (67,72–74).

Furthermore, more rapid identification of antibiotic resistance in microbiology laboratories, for instance with molecular testing or chromogenic media, may enhance our abilities to identify carriers (75). However such an approach – screening of carriage on admission followed by enhanced control measures for carriers – failed to reduce acquisition rates with MRSA and VRE in American ICUs, possibly because of the long turn-around time between obtaining screening cultures and reporting results (76). A cluster-randomized trial in 13 European ICUs, evaluating a step-wise approach of increasing hand hygiene adherence in combination with universal chlorhexidine bodywashing, followed by rapid screening on admission with enhanced barrier precautions for carriers has been completed recently, and results are expected in early 2012 (77).

It is generally assumed that antibiotic resistance is associated with the quantity of antibiotic consumption, as confirmed in a large observational study in 53 German ICUs (8). Yet, the same investigators failed to demonstrate that reducing cephalosporin use during placement of cerebrospinal shunts (from 'standard' prophylaxis for 48 h to 3 weeks to a single dose of cefuroxime) reduced antibiotic resistance (68). Likewise, in a study by Nijssen et al. (69) in a Dutch ICU, a 35% reduction in the use of beta-lactam antibiotics was not associated with lower acquisition rates of cephalosporin-resistant bacteria. Yet, the replacement of b-lactam antibiotics by fluoroquinolones was associated with markedly higher acquisition rates of fluoroquinolone-resistant bacteria.

Table 2 Examples of infection control interventions and outcomes

Infection Control Interventions	Target MDRO	Setting	Clinical Outcome
Isolation, promotion of hand hygiene, active surveillance, feedback of epidemiological data	MRSA	Acute care hospitals and rehabilitation and long-term care hospitals	35% reduction in MRSA infection incidence (3)
Twice-daily enhanced cleaning of hand contact surfaces	MRSA	ICU	Reduced detection of environmental MRSA per bed-area day but no significant reduction of patient MRSA acquisition (65)
Admission and weekly screening cultures. Detected MRSA-positive patients were isolated using single rooms and barrier precautions.	MRSA	ICU	Best estimates were consistent with reductions in transmission associated with barrier precautions (66)
Start and stop of antibiotic therapy based on procalcitonin concentration	All	ICU	Mean reduction of 2.7 days for patients under a procalcitonin guided antimicrobial therapy; nonsignificant difference in 28 or 60-day mortality (67)
Reduction in third generation cephalosporin consumption by reducing prophylaxis for cerebrospinal shunts	Third generation cephalosporin resistant *E. coli* and *K. pneumoniae*, Imipenem resistant *P. aeruginosa*, MRSA, VRE	SICU	Intervention followed by 14.4% reduction in DDDs; all but *E. coli* resistance (for both percentages and incidence per 1000 patient days) was reduced (68)
Cross-over randomized trial with 3 month of respectively beta-lactam rotation and 3 months preferred fluoroquinolone treatment	Cephalosporin resistant Enterobacteriaceae (CRE), fluoroquinolone plus cephalosporin resistant Enterobacteriaceae (FCRE)	MICU & Neuro-SICU	After both interventions, cephalosporin usage was reduced 35–39% with no reduction in CRE acquisition; increase of fluoroquinolone use of 243% was associated with a fourfold increase in acquisition rate per 1000 patient days (69)
Three-month rotation of cephalosporins, fluoroquinolones piperacillin/tazobactam and carbapenems.	ESBL-producing Enterobacteriaceae, non-fermenters	MICU & SICU	Nonsignificant reduction of VAP incidence and resistance, no effect on mortality or length of stay (70)
Decolonization strategy including SDD and chlorhexidin	ESBL-producing Enterobacteriaceae	950-bed hospital	76% of patients lost ESBL carriage over time; 18% of the patients received the decolonization strategy of which 89% were ESBL-free at follow-up (71)

DDD, defined daily dose; ESBL, extended spectrum b-lactamase; MDRO, multidrug-resistant organism; MICU, medical intensive care unit; MRSA, methicillin-resistant Staphylococcus aureus; SDD, selective decontamination of the digestive tract; SICU, surgical intensive care unit; VAP, ventilator-associated pneumonia.

Scheduled rotation of antibiotics in ICUs is another – still controversial – measure to influence antibiotic resistance. In a two-centre Italian trial, rotation of cephalosporins, fluoroquinolones, carbapenems and piperacillin-tazobactam during 12 months was associated with a nonsignificant reduction of the incidence of VAP caused by Gram-negative bacteria, including antibiotic-resistant bacteria, without determinable effects on LOS or ICU-mortality (70). Commendable for its two-centre study design and careful monitoring of all relevant antibiotics, adherence to hand hygiene was not determined.

In addition, only clinical samples related to diagnosing VAP were used for analyses, which may underestimate the true incidence of acquisition of antibiotic-resistant bacteria. This study adds to the growing body of evidence that temporary modulation of antibiotic policies has an effect on the bacterial ecology in ICUs, but the optimal settings for this approach to reduce resistance remain to be determined.

Finally, another – controversial – approach is the use of topical antibiotics to limit the spread of antibiotic resistance in ICUs. The regimens studied most extensively in this regard are selective decontamination of the digestive tract (SDD) and selective oropharyngeal decontamination

(SOD). SDD aims to decolonize the aerobic flora in the oropharynx and gastrointestinal tract in ICU patients through application of topical antibiotics in the oropharynx and gut in combination with a 4-day course of cefotaxim. SOD only aims to eradicate potential pathogenic microorganisms from the oropharynx. In a cluster-randomized multicentre cross-over study in 13 ICUs in the Netherlands SDD and SOD were, as compared with standard care, associated with a statistically significant reduction of day-28 mortality (78). Moreover, in these ICUs with low levels of antibiotic resistance, SDD was associated with lower rates of ICU-acquired bacteraemia caused by highly resistant microorganisms (mainly Gram negatives), as compared with SOD and standard care (79).

Furthermore, effective decontamination of the gut appeared associated with a lower risk of developing ICU-acquired Gram-negative bacteraemia, underscoring the critical role of the gut as a source for bacteraemia in these patients (80). Both SDD and SOD were associated with lower rates of ICU-acquired respiratory tract colonization with highly resistant microorganisms (79). In a

longitudinal analysis of the bacterial ecology in these 13 units, it was apparent that prevalences of antibiotic resistant Gram negatives were lowest during periods in which long-stay patients received topical antibiotics (81). Yet, ceftazidime resistance

in the intestinal flora appeared to increase after a period of SDD and ceftazidime resistance in respiratory samples tended to

increase during SDD and SOD (81). Therefore, controversy remains about the safety of SDD and SOD and its efficacy in high endemicity settings remains to be determined (82). Interestingly, though, a decolonization strategy including SDD was successful for ESBL-eradication in 16 of 18 patients (71).

Conclusion

Antibiotic resistance is now deferring the treatment of a significant and still growing proportion of infections in ICU patients across Europe. Although incidences of MRSA infections seem to be stabilizing (or decreasing) in some countries, multi-resistant Gram-negative bacteria are now most cumbersome. In the absence of new antibiotics, prevention of infections, reducing unnecessary antibiotic use, optimizing adherence to universal hygienic and infection control measures, and improving implementation of diagnostic tests are our only tools to combat this phenomenal threat.

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CHAPTER 3

Fighting antibiotic resistance in the intensive care unit using antibiotics

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Abstract

Antibiotic resistance is a global and increasing problem that is not counterbalanced by the development of new therapeutic agents. The prevalence of antibiotic resistance is especially high in intensive care units with frequently reported outbreaks of multidrug-resistant organisms. In addition to classical infection prevention protocols and surveillance programs, counterintuitive interventions, such as selective decontamination with antibiotics and antibiotic rotation have been applied and investigated to control the emergence of antibiotic resistance. This review provides an overview of selective oropharyngeal and digestive tract decontamination, decolonization of methicillin-resistant Staphylococcus aureus and antibiotic rotation as strategies to modulate antibiotic resistance in the intensive care unit.

Intensive care units (ICUs) are the hot spots for emergence of multidrug-resistant microorganisms (MDRO), frequently causing infections in critically ill patients. Severe morbidity, presence of indwelling devices, high antibiotic exposure and frequent contacts of healthcare workers with patients all contribute to transmission of and colonization with MDRO, with the risk of subsequent development of infection. Preventive measures such as hand hygiene, skin decolonization with antiseptics and screening for MDRO carriage followed by isolation of carriers are widely used in ICU settings to prevent the spread of MDRO between patients. In addition, antibiotic stewardship is applied to reduce antibiotic-induced selection, which probably also reduces transmission of MDRO. However, despite all these measures, the prevalence of antibiotic resistance is increasing and is not counterbalanced by development of new antibiotics.

The paradigm that antibiotic resistance can only be controlled by reducing antibiotic use, though, is challenged with increasing frequency. In this review, we summarize the current evidence of three approaches for controlling antibiotic resistance in ICUs, in which either more antibiotics are used or in which different prescription strategies are applied without the aim to reduce overall antibiotic use. In selective decontamination of the digestive tract (SDD) or selective oropharyngeal decontamination (SOD) high concentrations of prophylactic topical antibiotics are used to eradicate and prevent colonization with so-called potentially pathogenic microorganisms (PPMO), in order to reduce infections with these pathogens. Topical antibiotics are also used to specifically eradicate carriage with methicillin-resistant Staphylococcus aureus (MRSA). Finally, protocolized changing

of the preferred empirical antibiotic treatment of infections, called antibiotic rotation, has been propagated as a measure to control antibiotic resistance.

Selective decontamination

The digestive tract harbors commensal bacteria, such as Enterobacteriaceae and glucose non-fermenters, that may cause infections in critically ill patients, such as ventilator associated pneumonia (VAP). SDD and SOD aim to eradicate these bacteria from the gut (in SDD) and oropharynx (in SDD and SOD). The term 'selective' reflects the choice of antibiotics that do not affect anaerobic bacteria, as an intact anaerobic flora has been assumed to protect the host against overgrowth of PPMO, a principle called colonization resistance.

In ICUs with low levels of antibiotic resistance, such as in The Netherlands, SOD and SDD have been associated with absolute reductions in 28-day mortality of 2.9–3.5%, corresponding to numbers needed to treat of 34 and 29, as well as reductions in the ICU length of stay, ICU mortality and hospital mortality (1–3).

As a result, SDD and SOD are currently considered standard care for ICU patients in The Netherlands and in some settings in other countries. In settings with higher levels of antibiotic resistance (i.e., almost all other European and non-European countries), SDD and SOD are less frequently used due to clinicians preference and fear for increased antibiotic resistance (4). Yet, this perceived fear of antibiotic resistance due to using SDD or SOD is poorly supported by scientific evidence. Although only few studies determined long term effects of SDD and SOD, none of them reported an increase in the prevalence of antibiotic resistance (5–8). Moreover, a recently published meta-analysis of 64 studies failed to demonstrate that SDD or SOD were associated with more infections or higher carriage rates with MDRO in patients that received these interventions (9). In Dutch studies, the use of SDD and SOD was associated with lower prevalence of antibiotic resistance and with a 10% reduction in total intravenous antibiotic use, which might also beneficially influence ICU ecology (1–2,8). Moreover, longitudinal analysis of the antibiotic susceptibilities of respiratory tract isolates from 38 Dutch ICUs revealed a significant decline in antibiotic resistance in those ICUs that introduced SDD or SOD, and a trend toward decreasing resistance during its use (8). These reductions during SDD or SOD might result – indirectly – from the observed reduction in systemic antibiotic use, alleviating selective antibiotic pressure, or – directly – from the bactericidal effects, even for MDRO, exerted by the topical antibiotics.

Following this line of reasoning, SDD has been used to contain outbreaks of multi-drug resistant Gram-negative bacteria (MDR-GNB) (10,11), but here the evidence for efficacy is less clear. In a Dutch ICU SDD was used during an outbreak of extended-spectrum β-lactamase (ESBL) producing *K. pneumoniae* that could not be controlled with more traditional infection control measures. The use of SDD was associated with an increased prevalence of colistin-resistant *K. pneumoniae*, largely caused by patient to patient spread of one resistant clone. In addition, the prevalence of tobramycin resistance among species intrinsically resistant to colistin increased. In this setting with failing infection control, SDD seemed to augment cross-transmission with highly resistant bacteria (12).

In an ambulatory study of 58 patients persistently colonized with ESBL-producing Enterobacteriaceae, patients were randomized to either a decolonization regimen with colistin and neomycin or placebo. Although there was a decrease in the proportion of patients colonized with ESBL-producing Enterobacteriaceae during treatment, this effect disappeared 7 days after cessation of the intervention (13). SDD, or similar approaches, have been used to control outbreaks with carbapenemase-producing Enterobacteriaceae. In an attempt to eradicate KPC-2-producing *K. pneumoniae* (KPC) from the digestive tract in 14 patients, SDD (colistin and gentamicin) failed in eight patients, of which four carried colistin-resistant KPC. Secondary resistance to colistin and gentamicin was acquired in two and five patients, respectively.

Overall, the KPC decolonization rate was not significantly different between the SDD and control group (six out of 14 patients decolonized after a mean observation of 21 days vs 23/76 patients decolonized after a mean observation of 53 days; $p = 0.102$) (14).

In an Israeli randomized controlled trial among 40 patients colonized with carbapenem resistant *K. pneumoniae*, a 7-day SDD regimen was compared with placebo. After 2 weeks follow-up, carbapenem-resistant *K. pneumoniae* were no longer detected in rectal cultures of 61.1% and 16.1% of the patients receiving SDD or placebo, respectively (odds ratio: 0.13; 95% CI: 0.02–0.74).

However, after 6 weeks of follow-up, the difference between both study groups was smaller and no longer statistically significant (58.5% in the SDD arm vs 33.3% in the placebo arm) (15).

In another Israeli study among 152 patients intestinal decontamination with gentamicin, colistin or both was associated with a statistically significant reduction in colonization with carbapenem resistant Enterobacteriaceae compared with a wait

and see policy (11/26 [42%] decolonized with gentamicin, 8/16 [50%] with colistin, 3/8 [37.5%] with colistin/gentamicin and 7/102 [7%] with the wait-and-see policy). Resistance to the administered antibiotics occurred in one of the 16 patients treated with colistin monotherapy, and in six of 26 patients treated with gentamicin monotherapy, but in none of the eight patients treated with the combination of colistin and gentamicin (16). Based on these findings we consider the use of SDD to eradicate MDR-GNB experimental, with undetermined efficacy and the possibility of secondary resistance.

In some ICUs with a high prevalence of MRSA (Table 1), vancomycin has been added to the classical SDD regimen (polymyxin E, tobramycin, amphotericin B and 4-day course of parenteral cefotaxime), either for identified MRSA carriers (targeted decolonization) or for all eligible patients (universal decolonization).

Spanish investigators implemented, after an observation period (10 months), targeted decolonization with enteral vancomycin (during 17 months), followed by universal decolonization with vancomycin and SDD (during 22 months). Both interventions were associated with reduced occurrence of MRSA in clinical samples of blood, tracheal aspirates and intravascular catheters, and universal decolonization was more effective than targeted decolonization. However, the diagnostic sampling frequency was significantly lower during universal decolonization. Furthermore, semiquantitative analyses of surveillance samples revealed that the bacterial load in MRSA carriers was significantly lower during universal decolonization ($p < 0.0001$), while patients colonized with a high bacterial load (≥ 105 CFU/ml MRSA) more frequently had positive clinical samples with MRSA as compared with patients with lower bacterial counts in surveillance samples ($p < 0.0001$) (17).

During a MRSA outbreak in an Italian ICU where classical SDD was standard of care (polymyxin E, obramycin, amphotericin B, 4-day course of parenteral cephalosporin), enteral vancomycin was added to the SDD suspension for targeted treatment of MRSA-carriers. The addition of vancomycin was associated with a reduction in MRSA carriage in the gut (from 89 to 62%; $p < 0.05$) and MRSA infections (urinary tract infections (UTI), bacteremia and lower respiratory tract infections (LRTI), from 50 to 9.5% [$p < 0.05$]), although less surveillance and clinical samples were taken during the intervention (18).

Table 1. Vancomycin containing decolonization strategies for MRSA

Study (year)	Study duration (months)	Patients (n); location	Design	Inclusion criteria	Control (duration, patients [n])	Intervention (duration, patients [n])	MRSA outcome	Ref.
					Table 1. Vancomycin-containing decolonization strategies for methicillin-resistant *Staphylococcus aureus* in (nonburn unit) intensive care unit populations.			
Silvestri et al. (2002)	8	65; IT	Before–after study during an MRSA outbreak	Ventilation ≥72 h	Standard care: hand disinfection, gloves and aprons, cohort isolation of MRSA carriers, CHX-BW, SDD (5 months, n = 44)	SC + enteral vancomycin for MRSA carriers (3 months, n = 21, 13 received intervention)	LRTI, UTI, BSI: 22/44 (50%) vs 2/21 (9.5%), p < 0.05	[18]
Silvestri et al. (2004)	12	84; IT	Randomized trial	Age >18 years and expected ventilation ≥72 h	Standard care: hygiene standards according to CDC, SDD (n = 42)	Standard care + universal oropharyngeal vancomycin (n = 42)	ICU-acquired oropharyngeal colonization: 15 (36%) vs 0 (0%), p < 0.01 ICU-acquired LRTI: 7 vs 1 episodes, p < 0.001	[19]
De la Cal et al. (2004)	49	799; SP	Prospective observational study	Expected ventilation ≥72 h	Period 1; standard care: hand hygiene with CHX, single isolation of MRSA carriers, protective clothing, care of equipment, cleanliness of environment (10 months, n = 140)	Period 2; SC + enteral vancomycin for MRSA carriers (17 months, n = 258, 51 received intervention) Period 3; SC + universal enteral vancomycin + SDD (22 months, n = 401)	ICU-acquired colonization (5 body sites): 51/258 (20%) vs 40/401 (10%) in period 2 vs 3, p < 0.001 ICU-acquired infection: 44/140 (31%) vs 37/258 (14%) vs 9/401 (2%), p < 0.001	[17]
Silvestri et al. (2010)	36	191; IT	Prospective observational study	Age >18 years and expected ventilation ≥72 h	Standard care: hand hygiene with CHX, single/cohort isolation of MRSA carriers, protective clothing, care of equipment, cleanliness of environment, SDD + oropharyngeal vancomycin for MRSA carriers (18 months, n = 98, 40 received intervention)	SC + universal oropharyngeal vancomycin (18 months, n = 93)	ICU-acquired oropharyngeal colonization: 29/98 (30%) vs 0/93 (0%) ICU-acquired LRTI: 21/98 (21%) vs 5/93 (5%), p = 0.001	[20]

BSI: Blood stream infection; CDC: Centers for Disease Control and Prevention; CHX: Chlorhexidine; CHX-BW: Chlorhexidine body washing; ICU: Intensive care unit; IT: Italy; LRTI: Lower respiratory tract infection; MRSA: Methicillin-resistant *Staphylococcus aureus*; SC: Standard care; SDD: Selective digestive decontamination; SP: Spain; UTI: Urinary tract infection.

LEGEND: BSI, blood stream infection; CDC, Centers for Disease Control and Prevention; CHX, chlorhexidine; CHX-BW, chlorhexidine body washing; ICU, intensive care unit; IT, Italy; LRTI, lower respiratory tract infection; N, number of patients; RCT, randomized controlled trial; SC, standard care; SDD, selective digestive decontamination; SP, Spain; UTI, urinary tract infection.

In a second study in the same ICU, patients were randomized to SDD (n = 42) or SDD with vancomycin oropharyngeal gel (n = 42). Decolonization with vancomycin was associated with a statistically significant lower number of patients with respiratory tract MRSA colonization (15 vs 0) and less episodes of ICU-acquired LRTI caused by MRSA (7 vs 1) (19).

The third study from this ICU was a 36-month prospective observational study, in which oropharyngeal vancomycin was first added to SDD as a targeted therapy for MRSA, and second as a universal therapy for MRSA. The latter strategy led to substantial reductions in MRSA oropharyngeal carriage, ICU-acquired MRSA LRTI and all MRSA LRTI episodes (20). Of note, MRSA carriage was defined as at least two consecutive positive surveillance cultures over a period of at least 1 week, and for ICU-acquired MRSA infection a negative admission sample for MRSA was a prerequisite. These – perhaps strict – definitions may have led to an underestimation of both ICU-acquired colonization and infections caused by MRSA in this Italian ICU.

Secondary benefits reported in studies using decolonization with vancomycin include a reduced consumption of parenteral vancomycin, a decrease in total systemic antibiotic use and a diminished expenditure for total antibiotic use. However, the use of vancomycin is not free of risks and in one out of four of the aforementioned studies an outbreak of vancomycin-resistant enterococcus occurred among 13 patients (17).

All in all, the evidence for effectiveness of topical vancomycin application to control MRSA spread and reduce MRSA infections is limited, with most observations coming from a few ICUs only, and careful analysis of its ecological safety and its effectiveness compared with classical infection control measures is needed before its use can be recommended. Moreover, the added value of decolonization with vancomycin compared with implementation of other infection control measures is unknown. For instance, a program of hand hygiene improvement together with universal chlorhexidine body washing (CHX-BW) was also associated with reduced acquisition of MRSA in a European multicenter ICU study (21).

Another approach for decontamination of Gram-positive bacteria, in particular for MRSA, is topical use of mupirocin. In an American multicenter ICU study of 122,646 patients in 74 ICUs in 43 hospitals, universal application of nasal mupirocin together with chlorhexidine body washing reduced the occurrence of clinical cultures with MRSA with 36% and the incidence of all-cause ICU-acquired bacteremia with 45% (22). The latter effect was mainly attributable to a reduction in bacteremia from

skin commensal organisms; there was no difference in the occurrence of MRSA or MSSA bacteremia. The universal approach appeared more effective than targeted treatment for MRSA carriers identified through screening. The effects of mupirocin on MRSA-colonization and infection in ICU patients have been determined in four other, smaller studies (Table 2), with considerable heterogeneity between studies with regard to study design, study population, surveillance methods and interventions (targeted vs universal, with/without CHX-BW). In these studies, decolonization with mupirocin and CHX-BW (n = 3) or mupirocin only (n = 1) were associated with significant reductions in MRSA colonization and infection (23–26), and in the incidence of all-cause pneumonia, *S. aureus* pneumonia and nosocomial *S. aureus* colonization and infection (23,24).

Although all these studies were conducted in settings with high prevalence of MRSA carriage, reported numbers of ICU-acquired colonization and infection were low. Only few studies reported the occurrence of mupirocin resistance in MRSA isolates, which ranged from 0.05% in a MRSA-endemic setting where identified carriers received mupirocin (24) to 20.2% low-level resistance in a French RCT in which two of four study arms universally received mupirocin (26). Although not specifically in the ICU, an increase in mupirocin resistance amongst MRSA blood isolates over time has been observed in a large university hospital where targeted mupirocin and CHX-BW had been used for a period of 9 years (27). Moreover, in this setting carriage of MRSA with both low-level mupirocin resistance and genotypic chlorhexidine resistance before start of decolonization was associated with persistent MRSA carriage (28). Similar to decontamination with vancomycin, the attributable effect of mupirocin above regular infection control measures with CHX-BW remains unknown, and monitoring resistance is necessary.

Table 2: Mupirocin-containing decolonization strategies for MRSA in ICU populations

Study (year)	Study duration (months)	Patients (n); location	Design	Inclusion criteria	Control (duration, patients [n])	Intervention (duration, patients [n])	Outcome (MRSA and other pathogens)	Ref.
						Table 2. Mupirocin-containing decolonization strategies for methicillin-resistant *Staphylococcus aureus* in intensive care unit populations.		
Nardi *et al.* (2001)	16	223; IT	Double blind placebo-controlled RCT	Ventilation and ICU survival ≥48 h, no infection at ICU admission	SDD (n = 104)	SDD + universal nasal/oropharyngeal mupirocin (n = 119)	MRSA pneumonia: 7 vs 1 episodes; S. aureus pneumonia: 9 vs 1 episodes, p < 0.05; All-cause pneumonia: 20/104 (19%) vs 9/119 (8%), p < 0.02	[23]
Sandri *et al.* (2006)	60	2200; BR	Prospective observational study	All admitted patients	N/A	Nasal mupirocin/CHX-BW for MRSA-carriers (n = 2200), 364 received intervention	MRSA colonization (% of nasal swabs): 17% in 1999 vs 11% in 2003, p = 0.006; ICU-acquired MRSA infection: 35/424 (8.2%) in 1999 vs 13/458 (2.8%) in 2003, p = 0.001; S. aureus colonization (% of nasal swabs): 26% vs 18%, p = 0.001; ICU-acquired S. aureus infection: 42/424 (9.9%) in 1999 vs 16/458 (3.4%) in 2003	[24]
Ridenour *et al.* (2007)	19 (including 1 month phase-in before intervention)	1581; USA	Observational study	All admitted patients	Standard care: contact isolation precautions for MRSA carriers (9 months, n = 845, 114 received contact isolation precautions)	SC + nasal mupirocin/CHX-BW for MRSA carriers (9 months, n = 736, 80 received intervention)	ICU-acquired MRSA colonization: 15/845 (1.8%) vs 10/736 (1.4%); ICU-acquired MRSA infection: 6/845 (0.7%) vs 10/736 (0.1%); ICU-acquired MRSA colonization/infection: incidence density 8.45 vs 4.05 per 1000 patient days, p = 0.048	[25]
Huang *et al.* (2013)	33 (including 3 months phase-in before intervention)	122,646; USA	Multicenter CRT	All admitted patients	Standard care: Contact precautions for MRSA carriers (12 months, n = 15,816 vs 15,218 vs 17,356)	Continuation of SC (18 months, n = 23,480); SC + nasal mupirocin/CHX-BW for MRSA carriers (18 months, n = 24,752); SC + Universal nasal mupirocin/CHX-BW (18 months, n = 26,024)	MRSA positive clinical cultures: aHR 0.92 (0.77–1.10); ICU-acquired all-cause bacteremia: aHR 0.98 (0.84–1.15); MRSA positive clinical cultures: aHR 0.74 (0.62–0.88); ICU-acquired all-cause bacteremia: aHR 0.77 (0.65–0.90); MRSA positive clinical cultures: aHR 0.64 (0.53–0.77); ICU-acquired all-cause bacteremia: aHR 0.55 (0.48–0.64)	[22]
Camus *et al.* (2014)	39	515; FR	2 × 2 multicenter placebo-controlled double-blinded RCT	Age >18 years, intubation since <48 h, expected ventilation ≥48 h	Patients not having received mupirocin/CHX-BW (n = 256): • SDD + placebo (n = 130) • Two placebo's (n = 126)	Patients having received mupirocin/CHX-BW (n = 259): • Nasal mupirocin/CHX-BW + placebo (n = 130) • Nasal mupirocin/CHX-BW + SDD (n = 129)	ICU-acquired MRSA colonization (per number of patients not colonized at ICU-admission): 29/227 (12.8%) vs 18/228 (7.9%), p = 0.15; ICU-acquired MRSA infection: 17/256 (6.6%) vs 7/259 (2.7%), p = 0.04; MRSA decolonization rate: 13/31 (41.9%) vs 18/26 (69.2%), p = 0.04	[26]

aHR: Adjusted hazard ratio; BR: Brazil; CHX-BW: Chlorhexidine body washing; CRT: Cluster randomized trial; FR: France; ICU: Intensive care unit; IT: Italy; MRSA: Methicillin-resistant *Staphylococcus aureus*; N/A: Not applicable; RCT: Randomized controlled trial; SC: Standard care.

LEGEND: aHR, adjusted hazard ratio; BR, Brazil; CHX-BW, chlorhexidine body washing; CRT, cluster randomized trial; FR, France; ICU, intensive care unit; IT, Italy; N, number of patients; N/A, not applicable; RCT, randomized controlled trial; SA, S. aureus; SDD, selective digestive decontamination; US, United states of America.

Antibiotic rotation

Antibiotic empiric treatment guidelines generally rely on one predominant first-line antibiotic. On an ecological level, this creates an antibiotic selective pressure that may select for one antibiotic resistance characteristic. Antibiotic rotation aims to disrupt this monotonic selection pressure in order to reduce resistance and preserve antimicrobial treatment options. There are two extremes for such a strategy: rotating first line empirical antibiotics for every next patient (also called antibiotic mixing), or after a predefined time period (also called antibiotic cycling; Figure 1). Both rotation strategies allow individual treatment adaptations, including de-escalation, to guarantee best medical practice for individual patients. If proven effective, antibiotic rotation would be a flexible and directly implementable intervention.
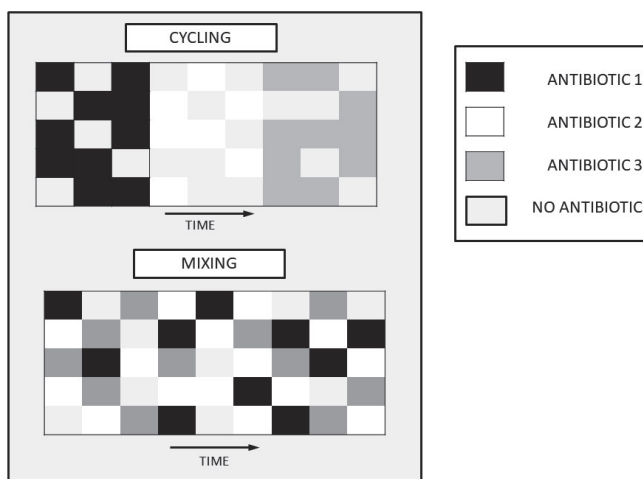
Figure 1

Antibiotic rotation strategies have been evaluated hospital-wide, but also in ICUs, which will be the focus of this review (Table 3) (29–45). The current evidence base for this approach is, however, limited due to heterogeneity in outcome measures, results and study design (Table 3). Studies reported results on infection (n = 9), colonization (n = 2), infection/colonization (n = 5) and clonality (n = 1). Compared with baseline, only nonsignificant results were found for mixing (n = 3). For cycling, decreased (n = 4), stable (n = 8) and increased (n = 3) antibiotic resistance was described. For three studies, a baseline comparison period was not available.

Table 3. Summary of antibiotic rotation studies

Study (year)	Location	Design/ setting	Study arms (duration; months)	Intervention (indication; antibiotic schematic)	Rotation length	Reported primary outcome	Reported conclusion	Ref.
Gruson et al. (2000)	FR	Before–after Single ICU	SC (24), cycling (24)	Early VAP: A-B-C-D; Late VAP: E-F-G-H	1 month	VAP attributed to antibiotic-resistant Gram-negative bacteria	"Potentially antibiotic-resistant Gram-negative bacilli (in) VAP ... decreased from 140 to 79 isolated bacilli" (p = 0.06)	[29]
Gruson et al. (2003)[1]	FR		SC (24), cycling (60)				Potentially resistant Gram-negative bacteria of isolates from VAP episodes: Standard care 140/332 (42.2%), first 24 months cycling 79/229 (34.5%), second 36 months cycling 78/187 (41.7%)	[31]
Raymond et al. (2001)	USA	Before–after Single ICU	SC (12), cycling (12)	Pneumonia: A-B-C-D; Peritonitis, sepsis eci: C-E-A-B	3 months	Antibiotic resistant pathogens from nosocomial infections	Incidence of resistance decreased from 25.4 to 17.9% (p = 0.015) and from 13.4 to 5.8% (p = 0.0006) for Gram-positive and Gram-negative infections, respectively	[30]
Warren et al. (2004)	USA	Before–after Single ICU	SC (5), cycling (24)	Gram-negative infections, empirical and targeted treatment: A-B-C-D	3–4 months	Infection or colonization with antibiotic resistant Gram-negative bacteria	"Rate of acquisition of enteric colonization with either P. aeruginosa or Enterobacteriaceae resistant to ... the target drugs ... did not significantly change"	[32]
Tsukayama et al. (2004)	USA	Intervention only Single ICU	Cycling (16)	Empirical therapy: A-B	4 months	"Evolution of antibiotic-resistance and spread of P. aeruginosa"	"Correlation of antibiotic use with antibiotic resistance and ... a large number of ribotypes suggested ... de novo development of antibiotic resistance ... in P. aeruginosa." "Ribotypes associated with antibiotic resistance appeared to have a survival advantage"	[33]
van Loon et al. (2005)	NL	Intervention only Single ICU	Cycling (16)	Gram-negative empirical therapy: A-B-A-C	4 months	"Colonization with antibiotic-resistant Gram-negative bacteria"	"Acquisition rates with resistant bacteria were highest during levofloxacin exposure (vs nonexposure periods) (RR 3.2, 95% CI: 1.4–7.1) and piperacillin/tazobactam exposure (RR 2.4, 95% CI: 1.2–4.8) (and lowest during cefpirome exposure [RR 0.9, 95% CI: 0.4–1.7, p = 0.85])"	[34]

[1] Continuation of Gruson 2000 study; 24 months study, 36 months routine use.
[2] Identical protocol to Sandiumenge 2006, different outcome pathogens compared to Sandiumenge 2006.
[3] One antibiotic removed after 17 months (Warren) and 12 months (Hedrick) of cycling.
[4] Extension of Bennett 2007 study.
[5] Same protocol as study by Warren 2004 under supervision of the United States Center for Disease Control and Prevention.
APP-beta: Antipseudomonal penicillin beta-lactamase inhibitor combinations; AU: Austria; BE: Belgium; FR: France; GNF-GNB: Glucose nonfermenting Gram-negative bacteria; IT: Italy; MRSA: Methicillin-resistant Staphylococcus aureus; NL: The Netherlands; POWI: Post-operative wound infection; RR: Relative risk; SC: Standard care; SP: Spain; VAP: Ventilator-associated pneumonia.

Table 3. Summary of antibiotic rotation studies (cont.).

Study (year)	Location	Design/ setting	Study arms (duration; months)	Intervention (indication; antibiotic schematic)	Rotation length	Reported primary outcome	Reported conclusion	Ref.
Evans *et al.* (2005)	USA	Two-intervention comparison Single ICU	Cycling I (12), SC (6), cycling II (12)	Cycling I = dual antibiotic rotation for sepsis/peritonitis: A-B-C-D; pneumonia: C-D-A-B; Standard care; Cycling II = single antibiotic rotation for sepsis/peritonitis/ pneumonia: D-A-B-C	3 months	"Characterize the evolution of Gram-negative antibiotic resistance"	Resistant Gram-negative bacteria of isolates from ICU-infection (% isolates): cycling I 22/286 (11%), Standard care 17/154 (16%), cycling II 55/362 (22%), p = 0.003 (cycling I vs II)	[35]
Martinez *et al.* (2006)	SP	Two-intervention comparison with crossover Single hospital, 2 ICUs	Cycling (4), mixing (4)	Antipseudomonal therapy: A-B-C-D	Cycling: 1 month; Mixing: every patient	"Proportion of patients acquiring enteric or nonfermentative Gram-negative bacilli resistant to the antibiotics under intervention"	"No differences were observed in the aggregated rate of acquisition of relevant microorganisms, incidence of ICU acquired infections, and prevalence of infection due to PRGNB (potentially resistant Gram-negative bacilli), ARGNB (antibiotic-resistant Gram-negative bacilli)"	[36]
Sandiumenge *et al.* (2006)	SP	Before–after three-intervention comparison Single ICU	SC (10), cycling I (12), cycling II (12), mixing (10)	Treatment of VAP: Cycling I (prioritization): A-B-C; Cycling II (restriction): C-B-A; Mixing: A-D-E-C	Cycling: 4 months; Mixing: every AB course	"Acquisition of resistant microorganisms" "Incidence rate of resistant microorganisms and their susceptibility patterns"	Cycling I and II, compared with standard care were "associated with increases in carbapenem-resistant *Acinetobacter baumannii* (CR-Ab) (relative risk [RR] 15.5; 95% CI: 5.5–42.8), (and) ESBL-producing Enterobacteriaceae (RR 4.2; 95% CI: 1.9–9.3)" "During the restriction period (Cycling II), incidence of ESBL-producing Enterobacteriaceae … returned to patient-specific rates (Standard care or Mixing) but CR-Ab remained higher"	[37] [43]

¹Continuation of Gruson 2000 study, 24 months study, 36 months routine use.
²Identical protocol to Sandiumenge 2006, different outcome pathogens compared to Sandiumenge 2006.
³One antibiotic removed after 17 months (Warren) and 12 months (Hedrick) of cycling.
⁴Extension of Bennett 2007 study.
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Table 3. Summary of antibiotic rotation studies (cont.).

Study (year)	Location	Design/setting	Study arms (duration; months)	Intervention (indication: antibiotic schematic)	Rotation length	Reported primary outcome	Reported conclusion	Ref.
Sandiumenge et al. (2011)[a]		Before–after three-intervention comparison. Single ICU	SC (10), cycling I (12); cycling II (12); mixing (10)	Treatment of VAP: Cycling I (prioritization): A-B-C; Cycling II (restriction): C-B-A; Mixing: A-D-E-C	Cycling: 4 months; Mixing: every AB course	Resistance of E. faecium, S. aureus, Klebsiella spp., A. baumannii, P. aeruginosa and Enterobacter spp. (ESKAPE) pathogens in ventilator-associated pneumonia (VAP)	"In the present study, those periods in which greater antimicrobial diversity was attained showed a lower rate of VAP due to resistant ESKAPE pathogens"	
Damas et al. (2006)	BE	Intervention-only. Single hospital, 3 ICUs	Cycling (24)	Pneumonia, UTI, catheter-related infection, intra-abdominal infection, and POWI: A-B-C, per ICU different sequences of the rotated antibiotics	8 months	"Susceptibility (percentage) of bacteria" in E. coli, Enterobacter spp. and P. aeruginosa	"No significant change in antibiotic susceptibility was observed over time". "A decrease in the susceptibility of several species was observed for antibiotics used as the first-line (cycling) therapy in the unit"	[38]
Bennett et al. (2007)	USA	Before–after with parallel control-ICU. Single hospital, 2 ICUs	ICU I: SC (12), cycling (24); ICU II: SC (36)	Pneumonia, abdominal sepsis: A-B-C-D[§]	1 month	"Changes in the antibiotic susceptibility profiles"	"Significant increase in the percentage of P. aeruginosa isolates sensitive to ceftazidime (67% in 2002 vs 92% in 2004, p = 0.002) and piperacillin/tazobactam (78% in 2002 vs 92% in 2004, p = 0.043)"	[39]
Sarraf-Yazdi et al.[†] (2012)	USA		ICU-I: SC (24), cycling (72); ICU-II: SC (96)			"Antibiotic susceptibility profiles of predominant Gram-negative pathogens"	Pseudomonal isolates showed improvements in susceptibility to "ceftazidime (66% vs 81%; p = 0.003) and piperacillin/tazobactam (75% vs 85%; p = 0.021), susceptibility of E. coli isolates to piperacillin/tazobactam improved (46% vs 83%; p < 0.0005)"	[44]
Hedrick et al.[‡] (2008)	USA	Before–after. Single ICU	SC (4), cycling (18)	Gram-negative empirical therapy: A-B-C-D[§]	3–4 months	"Resistant Gram-negative pathogens identified through … surveillance or clinical cultures"	"Per 100 patients enrolled, P. aeruginosa was isolated at a similar rate in the baseline and aggregate cycling periods (37.3 vs 37.2; p = 0.999)"	[40]

[a] Continuation of Gruson 2000 study; 24 months study, 36 months routine use.
[b] Identical protocol to Sandiumenge 2006, different outcome pathogens compared to Sandiumenge 2006.
[c] One antibiotic removed after 17 months (Warren) and 12 months (Hedrick) of cycling.
[d] Extension of Bennett 2007 study.
[§] Same protocol as study by Warren 2004 under supervision of the United States Center for Disease Control and Prevention.
APP-beta: Antipseudomonal penicillin beta-lactamase inhibitor combinations; AU: Austria; BE: Belgium; FR: France; GNF-GNB: Glucose nonfermenting Gram-negative bacteria; IT: Italy; MRSA: Methicillin-resistant Staphylococcus aureus; NL: The Netherlands; POWI: Post-operative wound infection; RR: Relative risk; SC: Standard care; SP: Spain; VAP: Ventilator-associated pneumonia.

Table 3. Summary of antibiotic rotation studies (cont.).

Study (year)	Location	Design/setting	Study arms (duration; months)	Intervention (indication: antibiotic schematic)	Rotation length	Reported primary outcome	Reported conclusion	Ref.
Hedrick et al. (2008; cont.)[§]							"In this study, the cycling strategy was not definitively associated with beneficial changes in unit epidemiology and in fact may have contributed to an outbreak of multidrug-resistant *P. aeruginosa*"	
Nijssen et al. (2009)	NL	Cluster randomization with crossover Single hospital, 2 ICUs	SC (8), cycling (3), prioritization (3)	Gram-negative empirical therapy: Cycling: A-B-C; Single-antibiotic prioritization: C	1 week	"Acquisition with third-generation cephalosporin-resistant Enterobacteriaceae (CRE) and fluoroquinolone-resistant CRE (FCRE)"	"Reductions in β-lactam use (Cycling and Prioritization) were not associated with reduced CRE acquisition (adjusted HRs were 1.0 [95% CI: 0.5–2.2] and 1.1 [95% CI: 0.5–2.5])" "Increased use of fluoroquinolones (Prioritization) was associated with increased acquisition of FCRE (adjusted HR 4.1 [95% CI: 1.4–11.9; $p = 0.01$])"	[41]
Rainieri et al. (2010)	IT	Before–after 2 hospitals, 2 ICUs	SC (12), cycling (12)	Treatment of VAP: A-B-C-D	3 months	"The incidence of VAP attributed to antibiotic-resistant Gram-negative bacteria"	"A nonsignificant reduction … of … antibiotic-resistant Gram-negative bacteria-related VAP (42 [45.2%] in P1 and 16 (34%) in P2 [$p = 0.21$])"	[42]
Ginn et al. (2012)	AU	Intervention-only 2 hospitals, 2 ICUs	ICU I: cycling (16); ICU II: cycling (12)	Empirical treatment of sepsis: ICU I: A-B-A-B; ICU II: B-A-B	4 months	"Acquisition … of either MRSA or *P. aeruginosa* … or of any bacteria resistant to cefepime, APP-beta, or gentamicin"	"The proportion of admissions complicated by antibiotic-resistant infection [MRSA and *P. aeruginosa*] was more than twice as high in cefepime cycles as in APP-β cycles (164.1 vs 74.2 per 1000 admissions; $p < 0.001$)"	[45]

§Continuation of Gruson 2000 study; 24 months study, 36 months routine use.
†Identical protocol to Sandiumenge 2006, different outcome pathogens compared to Sandiumenge 2006.
¶One antibiotic removed after 17 months (Warren) and 12 months (Hedrick) of cycling.
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APP-beta: Antipseudomonal penicillin beta-lactamase inhibitor combinations; AU: Austria; BE: Belgium; FR: France; GNF-GNB: Glucose nonfermenting Gram-negative bacteria; IT: Italy; MRSA: Methicillin-resistant *Staphylococcus aureus*; NL: The Netherlands; POWI: Post-operative wound infection; RR: Relative risk; SC: Standard care; SP: Spain; VAP: Ventilator-associated pneumonia.

Table 4. Methodological challenges and potential solutions for antibiotic rotation studies in intensive care units.

Characteristic	Comment	Caveats	Potential solutions for future studies
Study intervention	16 of 17 studies had different interventions†	Lack of external validity	Include interventions that have been tested in other studies†.
Heterogeneity between ICUs	ICU ecology and infection control practices affect the risk of acquiring resistant bacteria	Lack of external validity	Reduce effects of single units by including multiple ICUs and adjustment for potential confounders
Interdependence of outcome events	Cross-transmission of bacteria creates auto-correlation in results; colonization pressure influences risk of acquisition/transmission	Intervention effects might be over- or under-estimated	Account for patient dependency in study design and analysis
Differences in intervention and outcome populations	In some studies interventions are used for specific infections only and results of intervention are determined in these patients only	Overall effect on unit-wide ecology remains unknown	Perform outcome measurement in the entire ICU population
Choice of outcome measures	Colonization Infection Mortality Multiple reported outcomes	Colonization may not reflect effects on clinical outcome Infection assessment can be subjective and represents only a fraction of patients colonized Attributable mortality of ICU-acquired infections is low Multiple reported outcomes for different pathogens with multiple resistance determinants increase the risk of chance findings	For all outcomes: Use objective (and predefined) outcome measures (colonization through surveillance, or mortality). In individual patients, competing events may influence effect determination (and should be taken into account in time-to-event analyses)
Timing of rotation	Rotation period length ranged from 1 week to 8 months, and from single to multiple cycles	Short rotation periods mimic mixing, and reintroduction of an antibiotic may enhance resistance development	Consider to incorporate varying lengths of cycling periods. Use multiple cycles to identify 'rebound' effects
Choice of antibiotics	Rotated antibiotics, or antibiotic groups, have varied extensively across studies	Choice of antibiotics will depend on local ecology, which may limit external validity of results	Selection will be dictated by local bacterial ecology
Intervention adherence measurement	Not reported in most studies. Usually shown as stratified proportions or DDDs per antibiotic. Heterogeneity indices of antibiotics have been used as a single-variable proxy	Unknown or low adherence compromises assessment of intervention effects. Antibiotic consumption fractions provide incomplete insight in ecological selection pressure	Quantify adherence to protocol. Quantify effects of interventions on overall antibiotic use, for instance by using adherence indices
Confounding	Potential confounders for acquisition/transmission of bacteria: Infection control measures, nurse-to-patient ratio, case mix (e.g., reason for admission, morbidity, length of stay), changes in admission prevalence of resistant pathogens	Changes in these parameters may influence the outcome parameter, and interfere with accurate determination of the effects of antibiotic rotation	Minimizing confounding by study design (crossover) or adjustment for confounders in analysis (for which quantification of confounders is needed)

†See **Table 3**
DDD: Defined daily dose; ICU: Intensive care unit.

FOOTNOTES:

[A] Continuation of Gruson 2000 study; 24 months study, 36 months routine use

[B] Identical protocol to Sandiumenge 2006, different outcome pathogens compared to Sandiumenge 2006

[C] One antibiotic removed after 17 months of cycling

[D] Extension of Bennett 2007 study

[E] Same protocol as study by Warren 2004 under supervision of the United States Center for Disease Control and Prevention

LEGEND: 95% CI, 95% confidence interval; APP-bèta, antipseudomonal penicillin bèta-lactamase inhibitor combinations AU, Austria; BE, Belgium; FR, France; GNF-GNB, glucose non-fermenting gram-negative bacteria; IT, Italy; NL, the Netherlands; POWI, post-operative wound infection; RR, relative risk; SC, standard care; SP, Spain; US, United States; VAP, ventilator-associated pneumonia.

There are several methodological aspects underlying the heterogeneity in study design and outcomes within current research of antibiotic rotation in the ICU (Table 3). First, the wide range in reported results is probably – at least in part – due to differences in interventions. In fact, none of the 17 studies listed in Table 3 evaluated similar interventions (except for those on extensions of trials (44) or re-evaluations of data (43)). Second, there were marked differences in study settings. 15 studies were single hospital studies, and seven included more than one ICU (two multi-hospital, five single hospital but multi-ICU) (36,38–39,41–42,45). There were also considerable differences in the study populations (e.g., all ICU admissions, VAP patients and patients with (selected) ICU-acquired infections), and inevitably in the infection control measures and bacterial ecologies in these ICUs. This reduces the external validity of findings. These aspects can be mitigated – in part – by using a multicenter study design, with a crossover of interventions to control for unit-specific characteristics.

Third, in infectious disease research it is important to account for dependence of outcome events. This dependency is a characteristic of transmissible diseases, and may have especially unpredictable (and large) effects in small populations with high transmission rates and patient turnover, such as in the ICU. This can be addressed by applying a cluster design, monitoring resistance–transmission parameters (for instance; patient movements, colonization pressure, molecular typing, isolation precautions and hand-hygiene compliance). In the analysis, clustering can be accounted for through incorporating autocorrelation structures, and using mixed effects models. Of seven multi-ICU studies, multivariable analysis was performed in five, but autocorrelation in time was tested only once (42), and none of the studies described whether ICU-dependent clustering of outcomes was analyzed.

Fourth, and following from the previous point, there can be a disparity between intervention and outcome populations. Specific ICU subpopulations are sometimes targeted because infections are considered clinically relevant or because they account for a large proportion of total antibiotic consumption, which improves the impact on overall, ICU-level antibiotic use. However, the transmissibility of resistant bacteria implies that the outcome should be measured in all patients exposed to the risk of acquisition, not only to those treated with antibiotics. This was not the case in seven of 17 studies, where the outcome was measured exclusively in the intervention population (VAP: n = 5, defined infections: n = 2).

Fifth, outcome measures in studies varied extensively, and included (combinations of) colonization, infection and mortality. Each has its advantages and disad-

vantages. All three can be measured reliably, although there is considerable room for subjective interpretation of diagnostic criteria for many infections. The clinical relevance increases from colonization, to infection, to mortality, but this is inversely related to the number of endpoints potentially influenced by the intervention. Only a fraction of those colonized with a certain pathogen will develop infection, and the attributable mortality due to ICU-acquired infections is relatively low (46).

Furthermore, antibiotic rotation interventions are generally aimed at preserving ecological antibiotic susceptibility, which can not necessarily be determined in individual outcome parameters, such as acquisition. Also, it is biologically difficult to explain an effect of antibiotic rotation on infection rates. Colonization, therefore, seems the optimal primary ecological endpoint.

Sixth, the rotation period lengths ranged from weekly to 8 monthly. The optimal rotation length is unknown. Clinical studies implemented only one or two (mixing vs cycling) interventions, and variations in rotation length within one ICU, for instance with a crossover design, have not been studied. In addition, the first studies reported a 'rebound' after reintroduction of an antibiotic that had been rotated before (47). Potential explanations for such observations included, among others, induction or priming of dormant resistance genes that had remained in the population and quickly emerged after reintroduction of an antibiotic. The possibility of such a phenomenon could be incorporated in the study design, by having more than one full cycling period per strategy.

Seventh, the choice of antibiotics used for rotation depends on how often they are used in the study setting, if they infer relevant antibiotic resistance, but also whether they are safe and effective. Choices, therefore, depend on local resistance ecology. For instance, third-generation cephalosporins cannot be recommended in settings with high prevalence of ESBL-producing Enterobacteriaceae and aminoglycosides cannot be recommended for monotherapy. In this era of increasing antibiotic resistance the amount of possible antibiotic combinations and sequences (rapidly) decreases. This may well exclude this approach in the future, if only last resort antibiotics would remain available. Still, even with only two classes of antibiotics, many different combinations are possible, exemplified by the different (combinations of) antibiotics used in previous studies. A recent study suggested that 'in vitro' acquired bacterial resistance to some antibiotics was accompanied by susceptibility development to other agents, so-called 'collateral susceptibilities' (48). Further studies will be needed to determine whether this principle also occurs in vivo, and whether it might guide choices in antibiotics and sequence length for

cycling strategies. In any case, to improve the use of existing data, new studies should consider using rotation schemes that have been used in previous studies.

Eighth, monitoring of adherence to the study protocol is important, in order to assess the intervention-outcome relationship. Individual patient assessment of eligibility for empiric treatment and prescription of antibiotics would give most precise information, but requires substantial human (and thus financial) efforts. As a result, adherence is usually represented by proxy measurements, such as amounts of antibiotics used (units or defined daily dose (DDD)). An alternative is the antibiotic heterogeneity index (AHI), which describes the 'balance' between proportions of different antibiotics used during a certain period, and provides a potentially more transparent measure for selective antibiotic pressure (37). The relation between this AHI and the prevalence of antibiotic resistance has thus far only been investigated once. In that study periods with high antibiotic homogeneity (i.e., cycling) were associated with increased prevalence of carbapenem-resistant Acinetobacter spp. And ESBL-producing Enterobacteriaceae (37). Aggregate measures such as the AHI provide simplicity and can be helpful in comparing different studies, especially when comparing different rotation interventions. Yet, they could come at the cost of making incorrect inferences; having been used in only one study the effect of a high or low AHI on resistance is not yet evident and needs further investigation (37,43).

Ninth, the effects of potential confounding, for instance through differences in patient case mix, infection control measures and temporal changes in prevalence of resistant bacteria (colonization pressure) between periods must be addressed.

Because of the lack of convincing evidence for a specific strategy and the complex dynamics underlying antibiotic resistance epidemiology in ICU settings, mathematical modelling has been used to investigate 'in silico' the effects of different strategies on antibiotic resistance (49–57). Naturally, such models simplify the complex dynamics, but they can provide helpful insights for trial development and data interpretation. In most modelling studies antibiotic mixing outperformed cycling (49–51), though when antibiotics differ in the selective pressure exerted (also called asymmetry), or in settings with multidrug-resistant bacteria, cycling could be better (49–50,52,54).

Furthermore, if timing of rotation of antibiotics could be based on measured prevalence of resistance in the ICU, cycling could outperform mixing (57). In conclusion, so far, the results from the different modeling studies are almost equally ambiguous as the results from clinical trials. The next step is to integrate (new) mathematical models and clinical study results to hopefully provide guidance for clinical practice (58).

Conclusion

New – and sometimes counterintuitive – applications of existing antibiotics could potentially help to preserve antibiotic susceptibility.

Examples include selective decontamination of the oropharynx and digestive tract, topical decolonization of MRSA and antibiotic rotation. Oropharyngeal and intestinal decontamination improve ICU patients' outcome in ICUs with low prevalence of antibiotic resistance. Despite obvious concerns for antibiotic-induced selection of resistance, there is little evidence that these measures increase the prevalence of antimicrobial resistant bacteria in such settings. Yet, there is limited evidence on long-term effects and on the effects of decolonization with topical antibiotics in settings where ESBL – or carbapenemase-producing Enterobacteriaceae are endemic.

Nasal decolonization with topical mupirocin, either alone or combined with chlorhexidine body washing or addition of vancomycin to SDD/SOD, might be beneficial in reducing ICU-acquired MRSA-infection in MRSA endemic ICUs, but the ecological safety of these measures has not been studied extensively. Antibiotic rotation would be a flexible and cheap intervention, but studies with appropriate designs and analyses are needed to determine its effectiveness.

Future perspective

The prevalence of antibiotic resistance has increased in the past decades and this trend is likely to continue in the nearby future. As a result, infections with multi-drug- or pan-resistant bacteria will occur more frequently and antibiotics that are currently regarded as 'last resort' antibiotics will be used more often, with increasing resistance as a consequence.

Therefore, health providers and policy makers must take responsibility in finding means to bend the trend. Appropriate antibiotic use – limiting prescriptions to those that need antibiotics and reducing the duration of treatment at best supervised by hospital-wide antibiotic stewardship teams – and infection prevention are the cornerstones for such policies.

Furthermore, new antibiotic classes are urgently needed, but pharmaceutical industries seem to have lost interest in the development of new antibiotics, as they are unlikely to gain sufficient profit to counterbalance the required investments.

As the need for new antibiotics increases, public private partnerships, such as the New Drugs for Bad Bugs program, initiated by the Innovative Medicine Initiative, will stimulate drug discovery, fast clinical evaluation of new antimicrobial agents and explore possibilities for new (and more attractive) business models for antibiotics.

However, it will take time before these new classes of antibiotics will be widely available. As pointed out in this review, alternative, sometimes counterintuitive strategies of using established antibiotics may help us to control the emergence of antibiotic resistance.

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CHAPTER 4

Antibiotic rotation strategies to reduce antimicrobial resistance in Gram-negative bacteria in European intensive care units: study protocol for a cluster-randomized crossover controlled trial

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Abstract

Background

Intensive care units (ICU) are epicenters for the emergence of antibiotic-resistant Gram-negative bacteria (ARGNB) because of high rates of antibiotic usage, rapid patient turnover, immunological susceptibility of acutely ill patients, and frequent contact between healthcare workers and patients, facilitating cross-transmission.

Antibiotic stewardship programs are considered important to reduce antibiotic resistance, but the effectiveness of strategies such as, for instance, antibiotic rotation, have not been determined rigorously. Interpretation of available studies on antibiotic rotation is hampered by heterogeneity in implemented strategies and suboptimal study designs. In this cluster-randomized, crossover trial the effects of two antibiotic rotation strategies, antibiotic mixing and cycling, on the prevalence of ARGNB in ICUs are determined. Antibiotic mixing aims to create maximum antibiotic heterogeneity, and cycling aims to create maximum antibiotic homogeneity during consecutive periods.

Methods/Design

This is an open cluster-randomized crossover study of mixing and cycling of antibiotics in eight ICUs in five European countries. During cycling (9 months) third- or fourth-generation cephalosporins, piperacillin-tazobactam and carbapenems will be rotated during consecutive 6-week periods as the primary empiric treatment in patients suspected of infection caused by Gram-negative bacteria. During mixing (9 months), the same antibiotics will be rotated for each consecutive antibiotic course. Both intervention periods will be preceded by a baseline period of 4 months. ICUs will be randomized to consecutively implement either the mixing and then cycling strategy, or vice versa. The primary outcome is the ICU prevalence of ARGNB, determined through monthly point-prevalence screening of oropharynx and perineum. Secondary outcomes are rates of acquisition of ARGNB, bacteremia and appropriateness of therapy, length of stay in the ICU and ICU mortality. Results will be adjusted for intra-cluster correlation, and patient- and ICU-level variables of case-mix and infection-prevention measures using advanced regression modeling.

Discussion

This trial will determine the effects of antibiotic mixing and cycling on the unit-wide prevalence of ARGNB in ICUs.
Trial registration: ClinicalTrials.gov NCT01293071, December 2010.

Background

Infections caused by Gram-negative bacteria frequently complicate treatment of critically ill patients in the intensive care unit (ICU). Such ICU-acquired infections are associated with higher morbidity and mortality (1). The severity of illness in these patients often precludes awaiting diagnostic microbiology results. Treatment is therefore mostly empiric, covering a broad range of potential pathogens, increasing selective pressure for antibiotic-resistant bacteria.

As a response, antibiotic stewardship programs aim to optimize the rational and prudent use of antibiotics. These programs attempt to reduce selective pressure by reducing overall antibiotic consumption, but also to optimize the choice of the antibiotic, dosing and administration route. As such, antibiotic rotation has been proposed to reduce antibiotic resistance through systematically rotating antibiotics or antibiotic classes for empirical treatment.

There are two types of rotation schemes: mixing and cycling. In mixing, the treatment is changed with every new antibiotic course, and in cycling, empiric antibiotics change per time block (weeks or months). These interventions have been evaluated in ICUs, but also in neonatal-, pediatric-, oncology- and cardiothoracic-surgery departments (2-23). The methodology of these studies and the results obtained, however, vary widely. Importantly, study design, data collection and statistical analyses did not always take into account clustering of antibiotic resistance within ICUs, confounding by antibiotic use and changes in case-mix or infection prevention measures (24).

This multicenter cluster randomized crossover trial was designed to determine the effects of mixing and cycling of antibiotics in eight European ICUs, incorporating the most relevant confounders and adjusting for clustering of results in the analysis. For uniformity of reporting, we have used the CONSORT 2010 statement: extension to cluster randomized trials (25).

Study objectives

The primary objective of this trial is to compare the effects of a strategy of antibiotic mixing (rotation of empirical antibiotic treatment per next individual patient) to a strategy of antibiotic cycling (preferred empirical antibiotic treatment changes every 6 weeks) on the mean unit-wide prevalence of antibiotic-resistant Gram-negative bacteria (ARGNB). The primary hypothesis is superiority of one intervention arm over the other.

Methods/Design

Study design

The trial has a cluster-randomized, crossover design. All ICUs start with a 4-month standard care period with no interventions and are then randomized to one of two interventions of 9 months. After a wash-out period of 1 month, the ICUs cross over and perform the alternate rotation strategy for a second 9-month period (Figure 1). Inclusions will start January 2011 and the last ICU will finish February 2014.

Figure 1 All 8 intensive care units (ICUs) start with 4 months standard care and then perform both interventions: cycling then mixing or vice versa. ICUs are randomized to start with either cycling or mixing. After 9 months of intervention and a 1 month standard care wash-out period, the ICUs cross over into the second intervention for the final 9 months.

Participants

The participating ICUs are the primary object of study, individual data of all admitted patients will be used for secondary endpoints. Inclusion criteria for ICUs are listed in the "Intensive care unit inclusion criteria" section. ICUs have been selected through a tendering procedure according to EU regulations. An open tender communiqué was sent to hospitals directly and published on public websites of the European Society of Clinical Microbiology and Infectious Diseases and the European Society of Intensive Care Medicine. Interested ICUs were screened through questionnaires and on-site visits to assess eligibility. Thirty-eight ICUs showed an interest, of which eight ICUs in five countries were selected (in Belgium, France (n = 2), Germany (n = 2), Portugal and Slovenia (n = 2)). For all ICUs, IRB approval that required a waiver for individual patient written informed consent was obtained. The SATURN ICU trial was registered in the ClinicalTrials.gov (NCT01293071).

Inclusion criteria

1. At least eight beds with capacity of mechanical ventilation
2. Presence of at least one research nurse (or equivalent personnel) dedicated to the trial
3. Facilities for storage of screening swabs at -70 degrees Celsius
4. Approval of study protocol by the local Institutional Review Board (IRB)
5. Ability to obtain an informed consent waiver for individual patients
6. Ability to obtain written consent by physician and nursing staff representative to participate in the trial
7. Availability of a digital data patient management system for data extraction
8. No planned implementation of other interventions that may affect resistance prevalence during the study period
9. Not being an Intensive Care Burn Unit, Cardiothoracic Surgery Unit, Pediatric Intensive Care Unit, or Neonatal Intensive Care Unit

Interventions

During the intervention periods, the ICU-wide preferred empirical treatment for ICU-acquired infections with Gram-negative bacteria is rotated according to two protocols; mixing and cycling. The three rotated antibiotics will be 1) third- or fourth- generation cephalosporins, 2) piperacillin-tazobactam and 3) carbapenems. During mixing, the preferred choice for empiric antimicrobial treatment changes with every newly prescribed empiric antibiotic course. During cycling, empiric treat-

ment changes every 6 weeks. The order of interventions and the order of rotated antibiotics are randomized at the beginning of the trial by a person not involved in the design or execution of the study.

Treating physicians can deviate from study protocol at any point and for any reason because of patient safety or as part of de-escalation of antibiotic treatment. Combination therapy with preferred antibiotics (such as addition of an aminoglycoside or coverage of Gram-positive bacteria) is allowed. ICU-specific procedures related to standard hygienic measures, monitoring practices, outbreak management and any other infection prevention measures will not be dictated by study protocol.

Overall antibiotic use will be derived from ward-level consumption based on either individual prescription data or weekly unit level administrative antibiotic data.

As a proxy for protocol adherence, weekly point-prevalence measurements of antibiotic consumption will be taken. Antibiotic courses are then counted, regardless of dose or route of administration. The counts will then be translated to fractions of study-adherent antibiotics of all antibiotics. With optimal adherence, the three preferred antibiotics should be in equal proportions during mixing, whereas the preferred antibiotic should be dominant during cycling. Weekly graphs of ICU-specific results are then returned to each of the ICUs to provide compliance feedback.

Outcomes

The primary endpoint is the mean ICU-level point-prevalence of ARGNB, which will be obtained by combining the nine monthly point-prevalence screening results for each study period. Unit-wide monthly point-prevalence surveys will be performed by obtaining swabs from oropharynx and perineum from all patients present in the ICU during the point-prevalence survey. Swabs will be frozen directly and analyzed in a central microbiology laboratory (Appendix). Resistance is defined as extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, Piperacillin-Tazobactam resistance in Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* species, and Carbapenem resistance in *P. aeruginosa* and *Acinetobacter* species (Table 1).

Table 1 Outcome resistance per species

Species	Extended-spectrum beta-lactamase (ESBL)-producing	Piperacillin-Tazobactam resistance	Carbapenem resistance
Enterobacteriaceae	+	+	
Pseudomonas aeruginosa		+	+
Acinetobacter species		+	+

Secondary outcomes focus on individual patients and will use data on ARGNB colonization status from clinical cultures and appropriateness of empirical treatment based on blood culture isolate antibiograms. Data on other patient outcomes (length of ICU stay, and mortality) will be derived from computerized patient records.

Secondary endpoints are: 1) individual patient acquisition rates of ARGNB per admission days at risk; defined as the first positive culture for ARGNB >48 hours after admission. 2) ICU-acquired bacteremia rates with ARGNB per admission days at risk; 3) percentage of appropriate empirical treatment of ICU-acquired bacteremia; 4) mean length of stay in ICU; and 5) mean in-ICU-mortality.

For these secondary outcomes, clinical culture results will be used to determine ARGNB colonization status. Appropriateness of empirical treatment will be based on blood culture isolate antibiograms and antibiotic treatment prescribed. Length of ICU stay and in-ICU mortality will be derived from computerized patient records.

Sample size calculation

Sample size calculations for individually randomized patients assume that patient outcome is independent of other patients' outcomes, an assumption that is frequently violated when investigating the dynamics of infectious disease. Ignoring this interpatient dependency may lead to overestimation of treatment effect and underestimation of the necessary sample size.

The calculated sample size without clustering is 392 cultured patients per intervention arm, assuming a binomial distribution and based on 80% power to detect an absolute change of 10% in resistance prevalence with a 95% confidence level using a two-sided test. The sample size is based on a worst-case scenario with regard to precision; the widest distribution and thus largest needed sample size is around 50% for a binomial distribution. Therefore, the prevalence decrease was set from 55%

to 45%. As an illustration, to detect a reduction from 30% to 20%, 291 patients per intervention period are needed. To include clustering effects we used an Intra-class Correlation Coefficient (ICC) of 0.01, based on a previous cluster-randomized ICU study (26). For estimation of the average cluster sample size it was assumed that each ICU had 15 beds for every 9-month-prevalence measurement, resulting in 135 samples per ICU, per intervention arm, which yields a design effect of 2.35 (25). Multiplying the unadjusted sample size of 392 with the design effect means 921 sampled patients for one arm, and that 1,842 for both intervention arms are needed.

With an expected 2,160 sampled patients for both intervention arms (2 times 9 point-prevalence measurements in eight 15-bed ICUs), our study should therefore be adequately powered.

Data collection

Dedicated staff will collect data from (digital) patient charts and microbiology reports. Screening swabs will be collected by qualified personnel, either by the ICU-staff or study-nurse. Data of potential confounders is collected from computerized individual patient records (for example, age, gender, admission diagnosis). Ward-specific confounder data (such as hand hygiene compliance, use of indwelling devices, use of isolation measures, and staffing ratios) are obtained from monthly point-prevalence measurements.

Weekly point-prevalence measurements of antibiotic consumption will be collected either directly on the ward or from the patient data management system.

Analysis

Two types of outcome analysis will be performed: analysis of the ICU-level of resistance prevalence (primary outcome) and analyses on individual patient level, including the secondary outcome measures (Table 2).

Descriptive analysis will be performed of baseline characteristics and covariates. Where appropriate, bivariate tests will be performed. Bivariate statistical testing and advanced regression analysis will be used to model the effect of the interventions on resistance prevalence and acquisition. This will include adjusting, if necessary, for clustering of outcome data and confounding within ICUs by patient demographics, antibiotic consumption, illness severity, infection prevention measures and time trends. Results from point-prevalence measurement will be analyzed as continuous or count measurements per ICU for each intervention period, taking auto-correlation within ICUs into account in regression analysis.

Table 2 Summary of analyses for secondary outcomes

Outcome	Analysis
Acquisition rates of antibiotic resistant Gram-negative bacteria (ARGNB)	McNemar's test; Cox proportional hazard regression with random effects for ICUs.
Intensive care unit (ICU)-acquired bacteremia rates	McNemar's test; Cox proportional hazard regression with random effects for ICUs.
Percentage of appropriate empirical treatment of ICU-acquired bacteremia	McNemar's test; Generalized linear regression with random effects for ICUs.
Mean length of stay in ICU	Paired t-test; Generalized linear regression with random effects for ICUs.
In-ICU mortality	McNemar's test; Cox proportional hazard regression with random effects for ICUs.

The primary outcome measurements will be analyzed using McNemar's test for dependent pairs with all outcomes pooled for mixing or cycling. Bivariate tests, however, do not take into account differences in effects between hospitals, trends over time or possible confounding. Therefore, a stepwise mixed effects model will be constructed using the prevalence of resistance colonization as outcome, and the interventions as a factor. To assess differences in treatment effects between ICUs and time trends over study periods, random effects for the eight ICUs and the nine longitudinal measurements per ICU per study period will be added stepwise to the model. In the case of an imbalanced case-mix between interventions, confounders can be added to the model to adjust for these differences. This will facilitate adjustment for intra-cluster correlation and for imbalances in the study arm case mix (patient and ICU characteristics) not caused by the interventions themselves.

For the secondary outcomes, analyses are stated in Table 2. For acquisition rates of ARGNB, bacteremia with resistant Gram-negative bacteria, and mortality, the McNemar's test for dependent pairs will be performed first, followed by the Cox-proportional hazard regression models, accounting for differences in time-at-risk, inter-ICU intervention effect differences and possible cofounding. For the proportion of patients receiving appropriate empirical treatment, the same bivariate test and the same type regression model is used as for the primary outcome and by the same argumentation. Mean length of ICU-stay will be tested using the t-test for dependent means and if necessary with linear mixed effects regression, again with inclusion of random effects for individual ICUs and assessing possible confounding.

Because of the crossover design, period effects and carry-over effects will be assessed.

Discussion

Previous studies

The association between antibiotic use and selective pressure for ARGNB, together with the incomplete evidence base for antibiotic rotation strategies in ICU settings, warrants more research on this subject. The lack of a consistently applied methodology to study antibiotic rotation strategies, and the suboptimal designs applied in some studies, seriously hamper interpretation of available results at present (24). We have addressed these issues in our current study, attempting to maximize protocol adherence and generalizability for European ICUs (Table 3). Nonetheless, this unavoidably led to compromises with regard to interventions, study design and analyses.

Table 3 Methodological characteristics and key points

Study design feature	Advantages	Disadvantages	Remarks
Cluster allocation	Prevents allocation bias	Susceptible to case mix fluctuations in time	Prevented by adequate intervention period length
	Prevents between-intervention correlation as compared with individual randomization	Creates cluster correlation of outcomes	Will be accounted for in analysis
Open treatment design	Transparency in patient treatment	Different treatment adherence between different preferred antibiotics	Does not differ between interventions
Pre-intervention control period	Enables comparison with standard care. Enables time trend analysis for time-dependent increase in prevalence	No control group parallel in time	Comparable parallel groups/intensive care units (ICUs) not available, are expected to have higher heterogeneity in ICU characteristics than within-ICU comparisons using a crossover design
Crossover	Intervention comparison within ICUs equals out differences that influence outcome	Increases trial time-span and effect of baseline resistance increases over time	Addressed with time-trend analysis using pre-intervention control period

Selection of antibiotics, timing of interventions and outcome measures

We decided to target currently important pathogens, ARGNB, and the antibiotics mostly used for those infections. Consequently, the used antibiotic classes will be all beta-lactam antibiotics (in the case of piperacillin-tazobactam, in combinations with a beta-lactamase inhibitor). It could be hypothesized that rotation of antibiotics with different mechanisms of action would increase the effects of mixing and/or cycling. However, given the current increase in incidence of ESBL-producing bacteria and resistance to fluoroquinolones, choices of empiric antibiotics have become limited in the different regions of Europe. A too-specific choice of antibiotics would prevent inclusion of representative European ICUs, and would, therefore, reduce generalizability of findings. Indeed, the antibiotic strategies evaluated in this study may not be relevant for settings with much lower levels of ESBL-producing bacteria or in settings with endemicity of carbapenem-resistant Enterobacteriaceae.

The optimal timing of cycling interventions is as of yet unknown, and periods of weeks to months have been used in previous studies (2-18,20-22,27), and studies using mathematical modeling also did not reach definite conclusions as to which rotation timing is optimal (28-37). The studied intervention types include cycling and mixing, but dynamic and hybrid interventions have also been proposed: using prevalence data when to switch and when to use either cycling or mixing (37).

We decided to use cycling periods of 6 weeks, to evaluate reintroduction of the intervention antibiotics. Six-week periods allow two complete cycles of three antibiotics in 9 months. The aim of this reintroduction is to investigate possibly faster re-emergence of antibiotic resistance during the second cycle of antibiotics.

The monthly ICU-level, point-prevalence measurements will provide unbiased data for ARGNB prevalence. Furthermore, clinical culture results provide additional specific, though less sensitive, colonization incidence data.

Study design

The open cluster randomized design with a baseline period and crossover of interventions has methodological advantages but also limitations over the quasi-experimental before-after design or patient randomized studies.

First, the cluster design, with interventions applied unit-wide, prevents allocation bias within ICUs and reduces contamination of intervention effects. With individual patient randomization, different interventions will be executed in the same ICU at the same time. The transmissibility of infectious disease implies that colonization rates for patients in the same ward will become dependent, which may

well decrease outcome differences between interventions. A cluster-randomized study design physically separates the two intervention populations and thereby prevents transmission-based dependency in resistance outcomes. Possible associations between resistance acquisition within an ICU are now associated with only one intervention. The disadvantage is the introduction of clustering, or correlation of results within ICUs, which needs to be accounted for in sample size calculations and analysis.

Due to the consecutive inclusions in cluster randomized studies, these are more susceptible to chance fluctuations potentially causing selection bias, which needs to be assessed and adjusted for, if present.

Second, the open design may lead to allocation bias, where certain patient groups will not be eligible for the treatment with the preferred study antibiotic. Hospital specific distributions of parameters such as treatment indication and/or comorbidities will therefore influence adherence and possibly the effect of interventions, depending on the intervention phase and preferred antibiotic. The crossover design, however, will prevent differential distribution/bias between intervention groups. Nevertheless, the different interventions could have implications for adherence still causing bias between interventions with regard to intervention effect. Assessing differences in intervention adherence is therefore part of the analysis.

Third, both baseline antibiotic use -and resistance prevalence are known to change over time, even without interventions such as antibiotic rotation. Baseline study periods allow quantification of the effects of both interventions on overall antibiotic use and resistance prevalence, and extend the possibilities for time-trend analysis, given the absence of an adequate parallel control group in time. It was decided not to include such a parallel control group because of the large differences of patient populations between ICUs, even within the same country.

Fourth, the crossover of interventions provides adjustment for differences between units that may also affect the prevalence of ARGNB.

Finally, with regard to outcome data collection, admission and discharge screening swabs are not obtained. Therefore this study is less suited to determine acquisition or cross-transmission rates of ARGNB. Also, centralized processing of samples will prevent hospital specific differences in detection of resistant bacteria.

Sample size and statistical analysis

To our knowledge, this will be the first multicenter study with eight ICUs on these interventions aiming to include approximately 10,000 patients in five different coun-

tries (38). The power calculation included adjustment for intra-cluster correlation and provides precision, accuracy and external validity.

The intraclass correlation coefficient (ICC) was used based on mortality data from a multicenter study in 13 Dutch ICUs. The ICC could therefore be different for antibiotic resistance data, which would change the power of our sample size. Nonetheless, with our current estimated sample size, the ICC could increase 60% to 0.016. In addition, this calculation does not account for the crossover design, ignoring the reduction in interclass correlation resulting from this design.

Also, the sample-size calculation assumed a similar ICC for all ICUs, did not take clustering of results obtained on the same sampling day into account, and the ICC did not include a reported standard error, as recommended in the CONSORT statement.

Outlining the analysis plan, descriptive analysis and exploration of covariates will precede final regression analysis. In general terms, the prevalence during both interventions and secondary outcomes will be compared, assessing whether any - and which of the two interventions - is superior over the other. The baseline periods will be used for comparing standard care to the intervention periods. The predefined analysis will include bivariate testing and stepwise methods adjusting for possible influences of the study design and time effects. This includes clustering of outcome data, time trends and confounding using Generalized Linear Mixed Models. Secondary outcomes will be analyzed using bivariate testing, and Cox-proportional hazard models also accounting for random effects.

Conclusion

Better understanding of the associations between antibiotic pressure and resistance emergence is needed and important. This trial will provide further insight in the use of mixing and cycling of antibiotics and guide future practice guidelines and clinical and mathematical modeling studies on the effects of antibiotic policies.

Abbreviations

ARGNB, antibiotic-resistant Gram-negative bacteria; CONSORT, Consolidated Standards of Reporting Trials; ESBL, extended-spectrum beta-lactamase; ICC, intra-class correlation coefficient; ICU, intensive care unit; IRB, institutional review board

Funding

Appendix

Screening swab protocol

Screening swabs from monthly point-prevalence measurements will be taken from oropharynx and perineum of all patients in the ICU at that time point. Swabs will be put directly in medium and frozen at -70° Celsius. The swabs will then be shipped in batches under frozen conditions to the UMCU laboratory and centrally typed in the UMCU laboratory by dedicated laboratory analysts. Swabs will be cultured on five different plates (Appendix table 1). Plates will be cultured overnight at 37 degrees Celsius, and morphologically distinct colonies will be selected and typed with the MALDI-TOF Mass Spectrometry. Resistance typing is performed with the Phoenix™ Automated Microbiology System. ESBL positive isolates will be sent to University of Antwerp (UA), Belgium for confirmation of ESBL-genes. *Acinetobacter*, *Pseudomonas* and *Stenotrophomonas* species will be sent to the Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain for pheno- and genotyping.

Appendix table 1 Selective media plates

Plates	Antibiotic (concentration mg/L)
MacConkey	None
Extended-spectrum beta-lactamase (ESBL)	Oxoid Brilliance™ ESBL Agar
MacConkey/Ceftriaxone	Ceftriaxone (0.5 mg/L)
MacConkey/Pip-Tazo	Piperacillin-Tazobactam (4 mg/L)
MacConkey/Carbapenem	Meropenem (0.125 mg/L)

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CHAPTER 5

The effects of antibiotic cycling and mixing on antibiotic resistance in Intensive Care Units: A cluster randomized crossover trial

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Summary

Background: The effects of antibiotic rotation strategies to reduce prevalence of antibiotic resistant Gram-negative bacteria (ARGNB) in intensive care units (ICU) have not been accurately determined.

Methods: In a cluster-randomized cross-over study in eight ICUs (Belgium, France, Germany, Portugal, Slovenia) one of three antibiotics(-groups) (3rd/4th-generation cephalosporins, piperacillin-tazobactam and carbapenems) were used as preferred empiric treatment during 6-week periods (cycling) or preference changed after every consecutively treated patient (mixing). Computer-based randomization of intervention and rotated antibiotic sequence was performed centrally. Cycling and mixing were applied during nine month periods. ARGNB were defined as extended spectrum-β-lactamase production or piperacillin-tazobactam resistance in Enterobacteriaceae, and piperacillin-tazobactam or carbapenem resistance in Acinetobacter species and *Pseudomonas aeruginosa*. Data was collected on all admissions during the study. Primary endpoint was average unit-wide monthly point-prevalence of ARGNB in respiratory and perineal swabs with adjustment for potential confounders. ClinicalTrials.gov (NCT01293071)

Findings: In all 4,069 and 4,707 patients were admitted during cycling and mixing. Of these, 745 and 853 patients were included for the main analysis using monthly point-prevalence surveys during cycling and mixing, respectively. Mean prevalence of the composite primary endpoint was 22.6% (168/745) during cycling and 21.6% (184/853) during mixing (p=0.64), yielding an adjusted incidence rate ratio during mixing of 1.04 (95%-CI: 0.84 to 1.29). There was no difference in all-cause in-ICU mortality between intervention periods.

Interpretation: Antibiotic cycling did not reduce the prevalence of ARGNB carriage in ICU patients. Funding source: European Union's 7th Framework Program

Introduction

Antibiotic resistance poses a risk to patient safety, as it is associated with increased morbidity and mortality, and prolonged length of stay in health care settings.[1,2] Within hospitals, antibiotic resistance is usually most prevalent in Intensive Care Units (ICUs). Here, selective antibiotic pressure is high, opportunities for cross-transmission are frequent, and patients are vulnerable to acquire carriage and subsequent infections with antibiotic-resistant bacteria. Recent studies have demonstrated in different ICU settings the clinical effectiveness of improved hand hygiene, universal chlorhexidine bathing, universal use of mupirocin nasal ointment and universal gowning in limiting acquisition of carriage and infections of Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).[3-5] Yet, none of these interventions appeared effective in controlling the emergence of antibiotic-resistant Gram-negative bacteria (ARGNB), such as Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBL) or carbapenemases.

Antibiotics are a key factor for accelerating selection of antibiotic resistance, but they are also indispensable for the treatment and protection of critically ill patients. Intravenous antibiotics have been associated with selection for antibiotic resistance within individual patients.[6,7] It has been hypothesized that alternating ecological selective antibiotic pressure at the ward level, through structured modifications in antibiotic policies, will reduce antibiotic resistance. Mathematical models predict that prolonged periods of homogeneous selective pressure create a higher selective pressure, than a strategy in which antibiotics with different selective properties are rotated. Translated to real life settings, such strategies would include the scheduled alternation of first-line empiric treatment choices to increase diversity in antibiotic use. Different approaches have been used, such as antibiotic "cycling" and "mixing". The most frequently researched strategy, dating back to the 1980s, is antibiotic cycling, a specific antibiotic is preferentially used as first-line therapy in all patients that need treatment during a pre-specified period, after which another antibiotic – with presumed different selective properties - becomes the preferred therapy for all patients needing treatment.[12] This increases homogeneity of selective pressure within each cycling period, and heterogeneity between periods. In antibiotic mixing, such antibiotics would alternate after each patient in which treatment has

been started, thereby continuously maximizing heterogeneity in antibiotic selective pressure.

In clinical studies antibiotic cycling and mixing have yielded inconclusive results.[13,14] Studies were mostly single-center (n=15), yet sometimes in multiple wards (n=5), and more frequently testing cycling interventions (n=15) than mixing strategies (n=3). In most studies (n=12) a quasi-experimental before-after design was employed. Potential confounders (such as patient characteristics and infection prevention measures) and clustering of outcome were poorly considered. This, and the lack of studies investigating mixing strategies precludes definite conclusions on the benefits of these strategies as has been expressed in international guidelines.[15-18] We, therefore, compared the effects of both strategies on the prevalence of ARGNB in ICU in an international multi-center study.

Methods

Study design

This was a cluster-randomized cross-over study. After a baseline period of 4 months, in which ICUs applied standard care treatment practices, ICUs were randomized to two 9-month intervention periods, separated by a 1-month wash-out period (Figure 1). During the intervention periods, the preferred empirical treatment choices for ICU-acquired infections in which Gram-negative bacteria were covered were 1) 3rd- or 4th-generation cephalosporins (e.g. cefotaxime, ceftriaxone, ceftazidime, cefepime), 2) piperacillin-tazobactam and 3) carbapenems (e.g. imipenem, meropenem). The order of the tested strategies (cycling or mixing) and the order of rotated antibiotics within each strategy were randomized before the start of the trial by a person not part of the study team. The intervention did not allow concealment of allocation. The protocol for this study was previously published[13]

During mixing, the preferred empiric treatment choice changed with every consecutive empiric treatment course. During cycling, preferred empiric treatment changed every six weeks, creating six cycling periods of six weeks each. Treating physicians could only deviate from the study-preferred antibiotic for reasons of patient safety (e.g. previous antibiotic use, colonization with resistant bacteria, allergies). De-escalation and the use of combination therapy that included study and

non-study antibiotics were allowed. Infection control procedures were not dictated by the study protocol and practices were monitored during the study period.
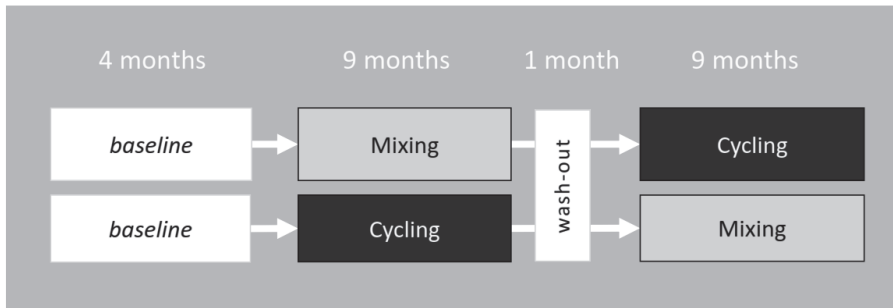

Figure 1. Study timeline

Study outcomes

The primary endpoint of this study was the change in the unit-wide prevalence of carriage with antibiotic resistant Gram-negative bacteria (ARGNB). The composite endpoint included carriage with Enterobacteriaceae harboring ESBL genes, or with phenotypical resistance to piperacillin-tazobactam (for Enterobacteriaceae, Acinetobacter species and *Pseudomonas aeruginosa*) or carbapenems (for Acinetobacter species and *Pseudomonas aeruginosa*). Unit-wide prevalence of carriage was measured through monthly point-prevalence screening cultures of oropharynx and perineum of all patients present in the ICU on a single day. This subset of patients was used for the primary analysis. Carriage with one of the indicator ARGNB in either the oropharynx or perineum was considered a primary endpoint. Of note, patients with extended ICU stay could be part of multiple monthly measurements. Secondary endpoints included length of stay and mortality in ICU.

Participants

Eligibility criteria for ICUs are listed in the Appendix (page 1, table 1). ICUs were approached and selected according to an EU-defined tender, including an assessment using questionnaires and on-site visit. Of 38 assessed ICUs, eight fulfilled all eligibility criteria (in Belgium (n=1), France (n=2), Germany (n=2), Portugal (n=1) and Slovenia (n=2), Figure 2). The study protocol was approved by each local Institutional

Review Board (IRB) and all centers obtained a waiver for individual patient written informed consent. The study was registered on ClinicalTrials.gov (NCT01293071).
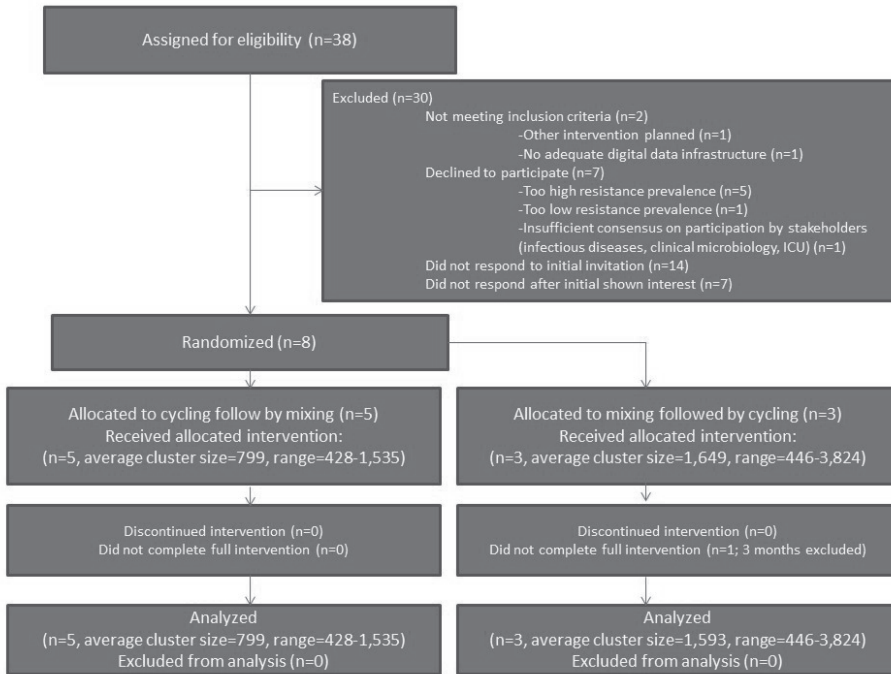

Figure 2. Flow-chart

Data collection

Data was collected on individual patient-level (e.g. age, gender, admission diagnosis, length of stay, validated illness severity scores and in-ICU mortality) for all patients admitted, or aggregated at ICU-level using monthly point-prevalence measurement (e.g., ICU bed-size, bed occupancy, isolation precautions, and staffing ratios.[19] Adherence to hand hygiene protocol was measured monthly by direct observations by trained research nurses following standardized methods (Hand Hygiene Technical Reference Manual, WHO Guidelines on Hand Hygiene in Health Care – a Summary).

Adherence to antibiotic treatment protocol

Full adherence to study protocol during cycling should result in dominance of the preferred antibiotic during the 6-week cycling periods and high variance between these periods. Mixing should yield equal use of antibiotics during the mixing period.

We, therefore, quantified the use of study antibiotics in Defined Daily Dose (DDD) per patient day for 6-week periods during cycling and mixing. To optimize protocol adherence, point-prevalence of antibiotic use of all patients in ICU were registered on a single day each week, and calculated proportions of the different study antibiotics were communicated to the ICUs. For the analysis on antibiotic consumption, overall unit-wide data were used, either based on individual courses (5 ICUs), or on ward-level administrative orders (3 ICUs).

Microbiology

Swabs obtained as part of the monthly point-prevalence studies were inoculated in Brain Heart Infusion (BHI) glycerol medium and stored at -70 degrees Celsius. From seven ICUs, swabs were processed at a central laboratory by inoculation on 5 different (selective) media plates: MacConkey agar without and with either ceftriaxone (0.5 mg/L), piperacillin-tazobactam (4 mg/L) and meropenem (0.125 mg/L), and an ESBL chromogenic agar plate (Oxoid Brilliance™ ESBL Agar). In one center the local IRB required that screening swabs were processed locally. Isolates were sent to the central laboratory for further analysis. Presence of ESBL-genes was determined by PCR (for CTX-M, SHV and TEM-genes, including subtyping) in all Enterobacteriaceae with phenotypic resistance to ceftazidime or ceftriaxone. For details of the microbiology protocol and breakpoints for non-susceptibility see Appendix material A.

Sample size calculation

Calculations were performed using a parallel group comparison of two proportions, adjusted for clustering within ICUs based on an Intra-class Correlation Coefficient (ICC) of 0.01, and a design effect of 2.35, was 921 patients per intervention type (total 1,842), using 95% confidence (α) and 80% power (1-β), for an absolute unadjusted difference in carriage prevalence of 10% between cycling and mixing. The minimum number of clusters required was seven. For each participating ICU, 135 measurements were assumed to be performed per intervention period (nine monthly point-prevalence measurements multiplied by 15 estimated beds per ICU), yielding 1,080 screened patients per intervention period.

Statistical analysis

Unadjusted analysis of the ARGNB prevalence in the monthly prevalence surveys during mixing and cycling was based on an univariable chi-square test and adjust-

ed analysis was performed with a generalized linear mixed model, accounting for clustering of endpoints (proportion of patients carrying ARGNB in point-prevalence screening) within hospitals, time-trends and patient- and ICU-level confounders. The adjusted analysis uses a Poisson–distribution and a, logarithmic link, with a random intercept per ICU and random slope for intervention weeks. The resulting time-trend thus describes the change in ARGNB prevalence over time under the reference intervention. Link- and variance functions were chosen based on expert opinion by a senior statistician not involved in study design, data collection or result inference. Confounder variable selection before forward stepwise selection was based on expert opinion and visual assessment of collinearity. Pre-selected confounders were age and gender, and point-prevalence percentages of short-stay patients (with admission <48 hours), bed occupancy, ventilation rate and the staffing ratio (number of patients per qualified nurse). All variables were means over the 4 weeks preceding outcome point-prevalence measurement. A crossover design can induce carry-over effects, occurring when effects from a preceding intervention period affect outcome in a following intervention period. Before and after building the model, carry-over effects were assessed by comparing the intervention type in the first versus the second intervention period, using a statistical test for the interaction of intervention and period. Mortality was analyzed using a Cox Proportional Hazard model. Analyses and sample size calculations were performed using R software.[20]

Results

Patient and ICU characteristics

In all, 10,980 patients were admitted during the study period; 2,204, 4,069 and 4,707 patients during baseline, cycling and mixing, respectively (Table 1). Patient- and ICU-variable values, as well as hand hygiene adherence, prevalence of isolation precautions, and nurse-per-patient staffing ratios remained stable during the 23-month course of the study.

There were two major protocol deviations. One ICU failed to collect point-prevalence screening swabs during the last 3 months of the study. It was, therefore, decided to exclude all data for these months for this ICU, reducing the cycling intervention period from 9 to 6 months.

Table 1. Patient and Intensive Care Unit characteristics.

	Baseline	Cycling	Mixing
Patient characteristics			
Number of admissions N	2,204	4,069	4,707
Male gender N (%)	1,323 (60.0)	2,484 (61.0)	2,813 (59.8)
Mean age in years (SD)	61.6 (19.2)	61.1(19.1)	61.5 (18.7)
Mean length of stay in ICU (median; IQR)	6.9 (3; 5)	6.9 (3; 5)	7.1 (3; 5)
Patients discharged before day 3 N (%)	846 (38.4)	1570 (38.6)	1834 (39.0)
Mean SAPSII score (6 ICUs)	33.5	33.8	37.4
Mean SAPSIII score (2 ICUs)	47.9	48.5	46.7
Mean APACHEII score (3 ICUs)	19.4	19.8	20.3
Mean TIS28 score (3 ICUs)	22.0	21.0	22.7
Mortality (%)	11.0	10.6	11.6
Patients in point-prevalence measurements N	467	773	927
ICU characteristics*			
Bed occupancy %	82.2	77.4	80.1
Patients in contact isolation N (%)	101 (21.6)	184 (23.8)	226 (24.4)
Patients in droplet isolation N (%)	11 (2.4)	12 (1.6)	19 (2.0)
Patients in respiratory isolation N (%)	8 (1.7)	7 (0.9)	15 (1.6)
Number of nurses per patient**	0.64	0.65	0.65
Number of student nurses per patient	0.19	0.12	0.13
Hand hygiene compliance % (observed HCW hand hygiene opportunities)	69.7 (1,085)	68.8 (2,824)	72.4 (2,810)
Colonized with ARGNB on admission (%)**	14/117 (12.0)	25/201 (12.4)	18/221 (8.1)

* = based on monthly point prevalence surveys

** = registered nurses on-duty per number of patients on the ward during point-prevalence

*** = Calculated within patients from point-prevalence measurements: Number of ARGNB (study endpoint) positive patients during 1st two days of admission, divided by total patients screened during 1st two days of admission

SD = Standard Deviation

HCW= Healthcare Worker

In another ICU, an outbreak with a carbapenem-resistant *K. pneumoniae* occurred during the wash-out period. As a result of reduced treatment options full adherence to study protocol became impossible and outbreak management measures would introduce confounding. Therefore, the wash-out period was extended until the outbreak had ended, outbreak management measures had been terminated and antibiotic policy had returned to the pre-outbreak situation. The duration of interruption was five months.

Antibiotic use

The average volume of antibiotic use was 1.51 DDD/patient day during baseline and 1.59 and 1.53 DDD/patient day during cycling and mixing, respectively (Difference 0.053; 95%-CI: -0.16 to 0.15; p=0.93; Table 2), though with considerable variation in antibiotic use between ICUs (range 0.5 – 2.8 total DDD/patient day during baseline) (Appendix, page 3, figure 1a and 1b). Study antibiotics accounted for 39%, 42% and 43% of all antibiotics during baseline, cycling and mixing, respectively. Overall use of study antibiotics was comparable between the intervention periods (Table 2). Carbapenems were used most frequently (0.33 and 0.31 DDD/patient day during cycling and mixing, respectively, difference 0.02; 95%-CI: -0.02 to 0.08), followed by third- and fourth-generation cephalosporins (0.21 and 0.22 DDD/patient day during cycling and mixing, respectively, difference -0.01; 95%-CI: -0.07 to 0.014) and piperacillin-tazobactam (0.13 DDD/patient day in both study periods, difference -0.005; 95%-CI: -0.018 to 0.020).

During cycling the volume of study antibiotics varied per 6-week period. Carbapenem use was 0.49 DDD/patient day during the "carbapenem" cycles and two-fold lower (0.24-0.26 DDD/patient day) in the other cycle periods. Third and fourth generation cephalosporin use was 0.36 during the "cephalosporin" cycle and almost three-fold lower (0.14 DDD/patient day) in both other cycles. Piperacillin-tazobactam use was 0.21 DDD/patient day during the "piperacillin-tazobactam" period and almost three-fold lower (0.08 DDD/patient day) in the other cycle periods. During mixing, the volume of study antibiotics, analyzed in 6-week periods to mimic the duration of cycling periods, was stable (Appendix, page 1, table 2).

Table 2. Antibiotic use in DDD per patient day.

	Baseline DDD/patient day (range)	Cycling[1] DDD/patient day (range)	Mixing DDD/patient day (range)	p-value[2]
Total	1.51 (0.62-2.66)	1.59 (0.45-2.56)	1.53 (0.4-3.32)	0.93
3rd or 4th generation cephalosporins	0.17 (0.03-0.27)	0.21 (0.03-0.53)	0.22 (0.05-0.72)	0.21
"cephalosporin cycle"		0.36 (0.05-0.74)		
"piperacillin-tazobactam cycle"		0.14# (0.01-0.46)		
"carbapenem cycle"		0.14# (0.01-0.38)		
Piperacillin-Tazobactam	0.17 (0.05-0.25)	0.13 (0.04-0.18)	0.13 (0.04-0.20)	0.91
"cephalosporin cycle"		0.08# (0.00-0.12)		
"piperacillin-tazobactam cycle"		0.21 (0.02-0.31)		
"carbapenem cycle"		0.08# (0.01-0.19)		
Carbapenems	0.25 (0.02-0.50)	0.33 (0.04-0.61)	0.31 (0.04-0.55)	0.26
"cephalosporin cycle"		0.24# (0.01-0.39)		
"piperacillin-tazobactam cycle"		0.26# (0.00-0.59)		
"carbapenem cycle"		0.49 (0.01-0.85)		
3rd or 4th Cephalosporins				
Ceftriaxone	0.05 (0.02-0.14)	0.04 (0.01-0.17)	0.05 (0.02-0.11)	0.87
Cefotaxime	0.02 (0.00-0.13)	0.02 (0.00-0.21)	0.02 (0.00-0.18)	0.61
Ceftazidim	0.04 (0.00-0.22)	0.05 (0.00-0.09)	0.04 (0.01-0.07)	0.33
Cefepime	0.06 (0.00-0.12)	0.10 (0.00-0.24)	0.11 (0.00-0.48)	0.08
Fluoroquinolones	0.14 (0.04 0.40)	0.13 (0.02-0.29)	0.14 (0.04-0.33)	0.37
Aminoglycosides	0.07 (0.00- 0.16)	0.06 (0.00-0.10)	0.05 (0.00-0.08)	0.09
Co-trimoxazole	0.02 (0.00-0.12)	0.02 (0.00-0.08)	0.03 (0.00 0.21)	0.35
Macrolides	0.11 (0.03-0.25)	0.10 (0.03-0.22)	0.08 (0.03-0.15)	0.19
Amoxicillin-clavulanic acid	0.17 (0.06-0.41)	0.17 (0.06-0.41)	0.15 (0.05-0.34)	0.27

Values represent means per study period (ranges of individual ICUs)

[1] t-test p-value comparison of preferred antibiotic consumption rates with other 2 study antibiotics

[2] t-test p-value comparison of cycling and mixing periods

# p<0.01

Antibiotic resistance

Of all admission, 745 and 853 patients were included in the monthly point-prevalence surveys during the cycling and mixing periods, respectively. Microbiological screening results and demographic data on these patients were used for the primary analysis. The mean prevalence of ARGNB (composite primary endpoint) was 22.6% (168/745) during cycling and 21.6% (184/853) during mixing (p=0.64; Table 3). There were no relevant differences in prevalence for subgroups or specific species (Table 3, Appendix, page 2, table 3).

Table 3. Prevalence of antibiotic resistance at the patient-level.

	Baseline	Cycling	Mixing	P-value*	% difference (95%-CI)
Point-prevalence surveys	32	59#	70		
Screened patients	462	745	853		
Patients with ARGNB N (%)	129 (27.9)	168 (22.6)	184 (21.6)	*0.64*	1.0 (-3.1 to 5.1)
Enterobacteriaceae					
ESBL phenotype N (%)	97 (21.0)	128 (17.2)	127 (14.9)	*0.21*	2.3 (-1.3 to 5.9)
ESBL genotype N (%)	58 (12.6)	72 (9.7)	68 (8.0)	*0.23*	1.69 (-1.1 to 4.5)
CRE genotype N (%)	4 (0.9)	7 (0.9)	10 (1.2)	*0.65*	-0.2 (-1.2 to 0.8)
*Non-fermenters****					
Resistant to piperacillin-tazobactam or carbapenems N (%)	40 (8.7)	61 (8.2)	66 (7.7)	*0.74*	0.5 (-2.2 to 3.1)
P. aeruginosa					
Resistant to ceftazidime N (%)	5 (1.1)	5 (0.7)	2 (0.2)	*0.19*	0.4 (-0.2 to 1.1)
Resistant to piperacillin-tazobactam N (%)	20 (4.3)	37 (5.0)	25 (2.9)	*0.04*	2.0 (0.1 to 4.0)
Resistant to carbapenems N (%)	29 (6.3)	43 (5.8)	53 (6.2)	*0.71*	-0.4 (-2.8 to 1.9)
Acinetobacter species					
Resistant to piperacillin-tazobactam N (%)	4 (0.9)	7 (0.9)	6 (0.7)	*0.60*	0.2 (-0.7 to 1.1)
Resistant to carbapenems N (%)	1 (0.2)	6 (0.8)	6 (0.7)	*0.81*	0.1 (-0.8 to 1.0)

* Chi-square Mixing vs Cycling
** ARGNB is defined as carriage with Enterobacteriaceae bacteria harboring Extended Spectrum Beta-Lactamase (ESBL)-genes, or with phenotypical resistance to piperacillin-tazobactam (Enterobacteriaceae, Acinetobacter species *or Pseudomonas aeruginosa*) or carbapenems (Acinetobacter species or *Pseudomonas aeruginosa*).
*** *P. aeruginosa* and Acinetobacter species
# Number of point-prevalence surveys during cycling was lower than in mixing due to three misses surveys in one ICU and overall shorter total time period of cycling compared to mixing.
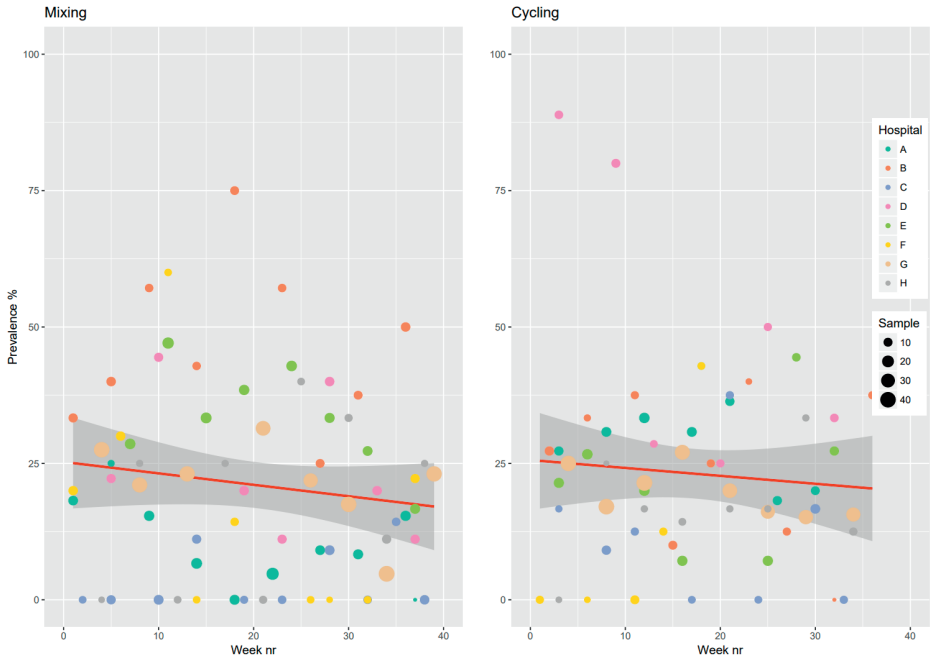
Figure 3.

The incidence rate ratio between mixing and cycling of the mixed effects analysis was 1.039 (95%-CI: 0.837 to 1.291; p=0.73), adjusted for hand hygiene compliance, gender proportion and the percentage of short-stay patients. The model was built using forward stepwise parameter selection, based on a decrease of the Akaike Information Criterion (AIC), an indicator of how well the new parameter relates to the other data of the model, including the resistance prevalence. If the AIC decreases, this new model better describes the other study confounders and the primary outcome. In the model with the lowest AIC hand hygiene compliance, gender proportion and the percentage of short-stay admissions best described the ICU-level prevalence of the primary endpoint. All variables in the final model improved model fit, thereby increasing overall model validity. However, none of the correlations between confounders and primary outcome (e.g. proportion of short-stay patients and prevalence reduction) were statistically significant.

Assessment of carry-over effects did not change the model fit significantly, with a trend towards reduced fit (AIC increase of 0.04 and 1.31 with and without adjustment for confounders). By comparison, the AIC decreased 23.7 after adding hand hygiene compliance to the model. Straightforward weighted linear regression of

point-prevalence measurements failed to demonstrate significant trends of resistance prevalence decrease during both intervention periods (Figure 3). In crude analysis there was evidence of auto-correlation of prevalence levels that extended up till 5-6 weeks, which disappeared after adjustment for potential confounders (Appendix, page 2, figure 2).

ICU mortality was 11% during baseline, 10.6% during cycling and 11.6% during mixing (p=0.38 in unadjusted Cox Proportional Hazard analysis). There were no statistically significant (p<.01) differences in subgroup endpoints (species and resistance type) (Appendix, page 3, table 3).

Discussion

In this cluster-randomized cross-over study in eight ICUs 9-month periods of antibiotic cycling and mixing did not change the unit-wide prevalence of ARGNB. Structured antibiotic rotation of antibiotic prescription policies for possible Gram-negative bacteria can therefore not be considered as a measure to reduce antibiotic resistance in ICUs.

The epidemiology of antibiotic resistance in ICUs is complex. Acquisition and prevalence of carriage is influenced by the number of colonized patients in the unit.[21] This colonization pressure may reduce the validity of a study if individual patients are randomized to interventions that may have a different effect on transmission. This is avoided by using a cluster-randomized design. Furthermore, changes in admission rates of patients carrying resistant bacteria, infection control practices, patient case mix, hand hygiene adherence and the use of non-study antibiotics may also affect acquisition rates with resistant bacteria and these variables, therefore, were carefully monitored during the study periods. Based on the observed absence of changes in these potential confounders during the study periods and the limited effects on outcome after adjustment in the statistical analysis, we conclude that it is unlikely that they affected the findings and interpretation of this study.

Some aspects of the study design deserve explanation. First, the rationale of the study was based on the observed emergence of ARGNB and decline in invasive infections caused by MRSA in Europe, which warranted an intervention targeting antibiotics that influence the epidemiology of ARGNB. The choice of antibiotics

eligible for rotation at the time of study design (2010) already excluded the use of amoxicillin-clavulanic acid, 2nd generation cephalosporins or fluoroquinolones as suitable options for empiric treatment of presumed Gram-negative infections in many European ICUs. In the absence of endemic carbapenem-resistant Entero-bacteriaceae; 3rd- or 4th-generation cephalosporins, piperacillin-tazobactam and carbapenems were considered equally acceptable for empiric treatment. There was (and is) no evidence base to define the optimal duration of cycling periods. Previous studies have used cycling periods ranging from one to eight months. Our decision for two nine-month study periods was, at least partly, guided by the available funding. Within the 9-month cycling period, it was decided to use two 6-week periods for each preferred antibiotic, instead of one 3-month period per antibiotic without reintroduction. This decision was guided by the available theoretical evidence and discussions with experts in mathematical modeling.[10,11, 22] Determination of the primary outcome was based on monthly point-prevalence studies. For feasibility reasons these surveys were fixed on a standard day each month, and the point-prevalence days, therefore, did not coincide with the end of the 6-week cycling periods. Our aim was to determine the effects of changing antibiotic exposures during a total of nine months, and not to determine immediate effects per six weeks. The study was underpowered for subgroup analyses of individual resistance- and species types. This should be taken into account when interpreting results. In addition we did not achieve the sample size initially calculated for the main outcome, though by not taking into account the crossover design we likely overestimated the needed sample size. Patient follow-up was restricted to the ICU period, and mortality was not a primary study outcome as it was not expected to be majorly influenced by the intervention with the planned population size. Eight ICUs in five European countries may not be fully representative for all European ICUs. Although participating ICUs did not have extraordinary features, their characteristics should be taken into account when extrapolating results to individual ICUs. In addition, there were two major protocol deviations in two ICUs, one of which missed 3 point-prevalence measurements. However, sensitivity analyses excluding these ICUs did not change interpretation of results (data not shown). Finally, as individual level prescription data were not available from three ICUs, our analyses of antibiotic use were restricted to aggregated data.

Based on observed antibiotic use during baseline, implementation of the study protocol neither significantly changed overall antibiotic use nor the overall use of study

antibiotics. The three study antibiotics accounted for about 40% of all antibiotics used and the total volume of antibiotics and the amounts of the study antibiotics were very similar in both study periods. So, the intervention studied, actually was – as pursued - the variance of the use of the three study antibiotics, without a change in the volume of these antibiotics in time. Considerable differences in exposure of the three study antibiotics during the study periods, though, were achieved. During the cycling periods antibiotic use for the non-preferred agents declined 2 to 3-fold, whereas use of study antibiotics was remarkably stable during mixing. The achieved differences in antibiotic exposure failed to establish differences in the unit-wide prevalence of antibiotic resistance, confirming the findings of a recent theoretical study that cycling and mixing in real-life circumstances are unlikely to achieve large effects on antibiotic resistance.[11]

Controlling the emergence of ARGNB in ICUs is important, but universally useful and successful strategies remain to be identified. Previously, the combined intervention of improved hand hygiene adherence and universal chlorhexidine body washing followed by on admission screening for carriage followed by isolation of carriers, failed to reduce the acquisition of ARGNB carriage in 13 European ICUs.[3] In settings with low levels of antibiotic resistance topical application of non-absorbable prophylactic antibiotics in the respiratory and gastro-intestinal tract, has been successful in preventing infections and improving patient outcome, and at the same time maintaining low prevalence of ARGNB.[23,24] Yet, whether that approach is equally successful and safe in settings with higher levels of ARGNB remains to be determined. Reductions of the total volume of antibiotics, though, will probably contribute to controlling the emergence of ARGNB through a reduction in antibiotic selective pressure. This can be achieved by better diagnostics, distinguishing which patients do and do not need antibiotics, as was demonstrated with invasive diagnostics for patients with a clinical suspicion of ventilator-associated pneumonia.[25] Furthermore, selective pressure can be reduced by biomarker-guided reductions of the duration of antibiotic treatment.[26,27]

Authors' contributions

Role of the funding source

Supplementary table 1. Inclusion criteria

Inclusions criteria:
1) Approval of the study protocol by the local Institutional Review Board (IRB)
2) Consent by ICU physician- and nursing staff to participate in the trial
3) ICUs with more than 8 beds with capacity of mechanical ventilation
4) Presence of at least one research nurse (or equivalent personnel) dedicated to the trial
5) Facilities for storage of screening swabs at -70 degrees Celsius
6) Availability of a digital data patient management system for data extraction
7) No planned implementation of other interventions, which may affect resistance prevalence during the study period
8) Not being an Intensive Care Burn Unit, Cardiothoracic Surgery Unit, Paediatric Intensive Care Unit, or Neonatal Intensive Care Unit

Supplementary table 2. Antibiotic use in DDD per patient day, 6-week period specific

	Cycling	Mixing[1]	p-value[2]
3rd or 4th generation cephalosporins			
Overall cephalosporin use	0.21	0.22	
"cephalosporin cycle"	0.36	0.22	*Comparator*
"piperacillin-tazobactam cycle"	0.14	0.25	*.57*
"carbapenem cycle"	0.14	0.21	*.73*
Piperacillin-Tazobactam			
Overall piperacillin-tazobactam use	0.13	0.13	
"cephalosporin cycle"	0.08	0.15	*.03*
"piperacillin-tazobactam cycle"	0.21	0.11	*Comparator*
"carbapenem cycle"	0.08	0.13	*.39*
Carbapenems			
Overall carbapenem use	0.33	0.31	
"cephalosporin cycle"	0.24	0.31	*.78*
"piperacillin-tazobactam cycle"	0.26	0.30	*.92*
"carbapenem cycle"	0.49	0.31	*Comparator*

[1] For mixing dummy 6-week periods were assumed corresponding with 6-week cycling periods

[2] T-test p-value is a comparison of preferred antibiotic consumption rates with other 2 study antibiotics

**Supplementary table 3.** Endpoints and subgroup-endpoints.

	Baseline	Cycling	Mixing	p-value (cycling vs mixing)
Total PPs (N)	32	59	70	
Total screening events	462	745	853	
Composite endpoint N (%)	129 (27.9)	168 (22.6)	184 (21.6)	0.637
ESBL-producing enterobacteriaceae				
Phenotype only N (%)*	97 (21.0)	128 (17.2)	127 (14.9)	0.212
Phenotype and PCR confirmed N (%)**	58 (12.6)	72 (9.7)	68 (8.0)	0.233
Species subgroups				
Escherichia coli **N (%)**	25 (5.4)	26 (3.5)	33 (3.9)	0.689
Klebsiella species N (%)	29 (6.3)	35 (4.7)	35 (4.1)	0.562
Klebsiella pneumoniae **N (%)**	29 (6.3)	35 (4.7)	28 (3.3)	0.147
Enterobacter cloacae **N (%)**	4 (0.9)	9 (1.2)	3 (0.4)	0.048
CRE phenotype and PCR confirmed N (%)***	4 (0.9)	7 (0.9)	10 (1.2)	0.651
Non-fermenter****				
Ceftazidim resistant N (%)	8 (1.7)	5 (0.7)	4 (0.5)	0.590
Ceftazidim or carbapenem resistant N (%)	40 (8.7)	61 (8.2)	66 (7.7)	0.740
P. aeruginosa				
Ceftazidim resistant N (%)	5 (1.1)	5 (0.7)	2 (0.2)	0.187
Piperacillin-tazobactam resistant N (%)	20 (4.3)	37 (5.0)	25 (2.9)	0.036
Carbapenem resistant N (%)	29 (6.3)	43 (5.8)	53 (6.2)	0.711
Acinetobacter species				
Ceftazidim resistant N (%)	4 (0.9)	0 (0.0)	2 (0.2)	0.186
Piperacillin-tazobactam resistant N (%)	4 (0.9)	7 (0.9)	6 (0.7)	0.600
Carbapenem resistant N (%)	1 (0.2)	6 (0.8)	6 (0.7)	0.814

* Cefotaxime or ceftazidime resistant Enterobacteriaceae
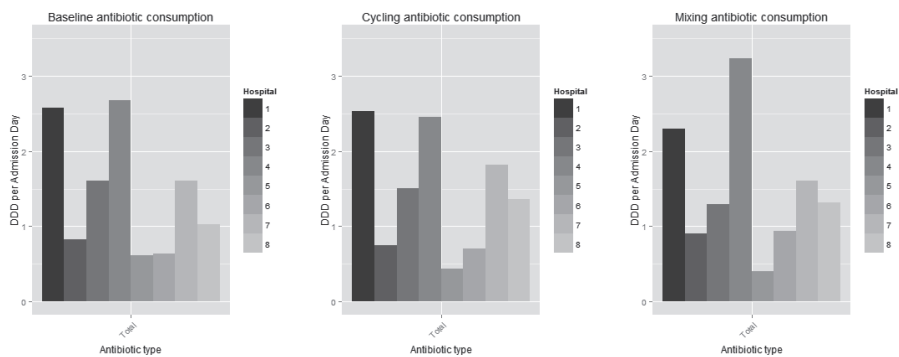
** ESBL phenotype and presence of CTX-M, SHV, TEM ESBL-gene

*** Meropenem or imipenem resistant and presence of KPC, VIM, NDM or OXA Carbapenemase-gene
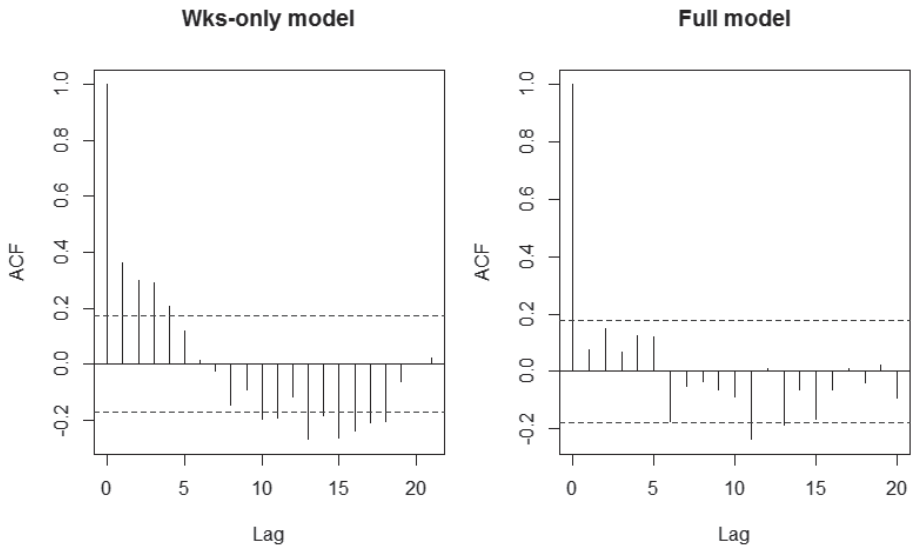
**** *P. aeruginosa* or Acinetobacter species
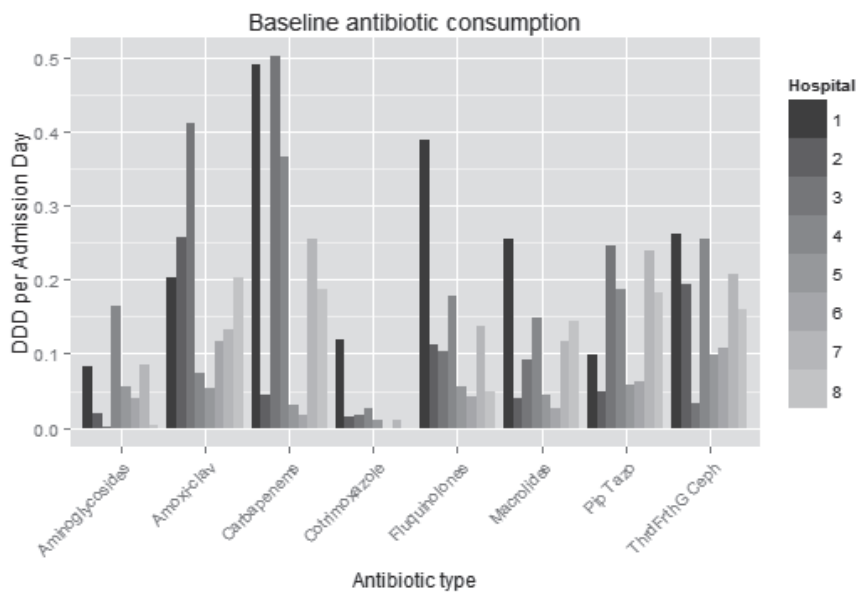
Supplementary figure 1a. Baseline consumption of specific antibiotics.

Supplementary figure 1b. Overall antibiotic consumption in DDDs per admission day

Supplementary figure 2. Auto-correlation of endpoint prevalence between study-weeks

Supplementary text 1

It's too soon to pull the plug on antibiotic cycling

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In their recent study, Pleun Joppe van Duijn and colleagues evaluated the relative merits of antibiotic mixing and antibiotic cycling using a cluster-randomised crossover study in eight intensive care units, ICUs, finding that 9 month periods of cycling and mixing did not change the unit wide prevalence of antibiotic-resistant, Gram-negative bacteria (1). We commend the design of this study, both for its scale and grounding in evolutionary theory that was drawn from the work of both experimentalists and mathematical modellers. However, we find the conclusion of this study, namely that "structured rotation of antibiotic prescription policies for possible Gram-negative bacteria cannot be considered as a measure to reduce antibiotic resistance in ICUs", may be too strong.

Previous theoretical studies of antibiotic resistance indicate that any efficacious antibiotic cycling strategy will likely depend upon evolutionary trade-offs, wherein mechanisms of resistance arising under exposure to a first antibiotic will be costly, and thus lost through evolution under exposure to a subsequent antibiotic (2,3). This pattern of evolutionary tradeoffs need not be a universal property of sequences of antibiotics drugs or antibiotic drug classes. In modelling efforts conducted in our labs, we found that randomly chosen sequences of drugs are predicted to make no difference, or to promote resistance, in greater than 70% of cases (4). However, a small number of sequences from the same pool of drugs were predicted to mitigate

drug resistance. As such, the failure of a specific antibiotic cycling strategy, or of a cycling strategy wherein antibiotics are chosen arbitrarily within drug classes, does not preclude the existence of an alternative efficacious cycling strategy. There is not yet sufficient empirical evidence to justify the assertion that antibiotic cycling cannot be considered as a measure to reduce antibiotic resistance.

Any future clinical assessment of antibiotic cycling must aim to evaluate specifically designed cycling strategies, for which there is sufficient preclinical evidence to indicate potential efficacy. To generate this preclinical evidence will likely require the efforts of experimentalists, mathematical modellers and clinicians working in close collaboration. We hope that the results presented by van Duijn et al will not deter others from this potentially critical avenue of research.

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Supplementary text 2

It's too soon to pull the plug on antibiotic cycling–Authors' reply

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Nichol et al. express concern that the negative findings from our study will discourage future investigators from attempting to further study the effects of antibiotic rotation. Indeed, we concluded that antibiotic rotation (both mixing or cycling) currently is not justified as clinical practice, but we do not intend to halt further research, and sincerely hope it will not deter others from exploring antibiotic rotation strategies to reduce antibiotic resistance.

Yet, we feel our study results illustrate the challenges in identifying an optimal rotation strategy in real-life clinical settings. We fully agree that mathematical studies and in vitro experiments can assist in identifying scenarios with potential efficacy. However, these approaches will need assumptions for important real-life variables and cannot capture all within-host complexities. Therefore, empirical testing through clinical trials will always be necessary.

We concur that "there is not yet sufficient empirical evidence" to either exclude that antibiotic rotation can work, nor that we can justify implementation of this approach in clinical practice. We hope this inspires, and does not deter, others to initiate research on this topic.

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CHAPTER 6

The effects of antibiotic cycling and mixing on acquisition of antibiotic resistant bacteria in the ICU: A post-hoc individual patient analysis of a prospective cluster-randomized crossover study

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Abstract

Background: Repeated rotation of empiric antibiotic treatment strategies is hypothesized to reduce antibiotic resistance. Clinical rotation studies failed to change unit-wide prevalence of antibiotic resistant bacteria (ARB) carriage, including an international cluster-randomized crossover study. Unit-wide effects may differ from individual effects due to "ecological fallacy". This post-hoc analysis of a cluster-randomized crossover study assesses differences between cycling and mixing rotation strategies in acquisition of carriage with Gram-negative ARB in individual patients.

Methods

This was a controlled cluster-randomized crossover study in 7 ICUs in 5 European countries. Clinical cultures taken as routine care were used for endpoint assessment. Patients with a first negative culture and at least one culture collected in total were included. Community acquisitions (2 days of admission or less) were excluded. Primary outcome was ICU-acquisition of Enterobacterales species with reduced susceptibility to: third- or fourth generation cephalosporins or piperacillin-tazobactam, and Acinetobacter species and *Pseudomonas aeruginosa* with reduced susceptibility for piperacillin-tazobactam or carbapenems.

Cycling (altering first-line empiric therapy for Gram-negative bacteria, every other 6-weeks), to mixing (changing antibiotic type every empiric antibiotic course). Rotated antibiotics were third- or fourth generation cephalosporins, piperacillin-tazobactam and carbapenems.

Results

For this analysis 1,613 admissions were eligible (855 and 758 during cycling and mixing, respectively), with 16,437 microbiological cultures obtained. Incidences of acquisition with ARB during ICU-stay were 7.3% (n=62) and 5.1% (n=39) during cycling and mixing, respectively (p-value 0.13), after a mean of 17.7 (median 15) and 20.8 (median 13) days. Adjusted odds ratio for acquisition of ARB carriage during mixing was 0.62 (95% CI 0.38 to 1.00). Acquired carriage with ARB were Enterobacterales species (n=61), *Pseudomonas aeruginosa* (n=38) and Acinetobacter species (n=20), with no statistically significant differences between interventions.

Conclusions

There was no statistically significant difference in individual patients' risk of acquiring carriage with Gram-negative ARB during cycling and mixing. These findings substantiate the absence of difference between cycling and mixing on the epidemiology of Gram-negative ARB in ICU.

Trial registration: This trial is registered with ClinicalTrials.gov, registered 10 January 2011, NCT01293071, https://clinicaltrials.gov/ct2/show/NCT01293071

Introduction

Treatment of critically ill patients admitted in Intensive Care Units (ICU) is frequently complicated by infections caused by antibiotic resistant Gram-negative bacteria. Patients in ICU often receive broad spectrum antibiotics which increases antibiotic resistance selective pressure and the chance of acquiring colonization with Gram-negative antibiotic resistant bacteria (ARB). To reduce this selective pressure, unit-wide antibiotic stewardship programs (ASP) have been advocated, sometimes advocating but also discouraging antibiotic rotation strategies. (1–3) These strategies aim to modulate the diversity of antibiotic exposure in a ward, rather than reducing overall antibiotic use. The increased heterogeneity of antibiotic exposure, hypothetically, reduces antibiotic resistance selection pressure and occurrence of antibiotic resistant bacteria. (4–12)

Previous observational and quasi-experimental studies, however, yielded non-conclusive results for different pathogens, rotation schedules and outcomes. (13-26) In a multi-center cluster crossover study, two antibiotic rotation interventions, cycling and mixing, we found similar effects on the unit-wide ecology of Gram-negative ARB. (27) In that study two different antibiotic rotation strategies for empiric treatment of patients with presumed Gram-negative infections were compared: During cycling the preferred antibiotic treatment changed every six weeks and during mixing it changed after every single patient. Effectiveness of the intervention was determined by measuring the prevalence of carriage with antibiotic resistant Gram-negative bacteria at the unit level, through monthly point-prevalence surveys. However, group-ecological and individual risks can differ, even within the same experimental study, due to what is called the "ecological fallacy". (28) We, therefore, performed a post-hoc analysis on study data of this previously performed

study to investigate whether two antibiotic rotation schemes, cycling and mixing, yielded differences in the individual risk of acquiring Gram-negative ARB during ICU admission.

Methods

Study design

This was a post-hoc nested cohort analysis of a cluster-randomized cross-over study.(29) Four months of standard care treatment preceded ICU randomization to two 9-month intervention periods. A one-month wash-out period separated the intervention periods. ICUs were cluster-allocated by randomization to perform either the cycling strategy followed by mixing or vice versa. Computer randomization of the allocation to interventions (in a 1:1 ratio) and randomization of the order of consecutively rotated antibiotics (in a random sequence per ICU) was performed by a person not involved in designing or performing the study. First-line empirical therapy for patients with assumed infections that required treatment of Gram-negative bacteria was rotated in 6-week periods between 3rd or 4th-generation cephalosporins, piperacillin-tazobactam and carbapenems (cycling). During mixing, the preferred empiric therapy was rotated for every new patient needing treatment. Patients could be treated with different antibiotic courses during admission. Readmissions were included. To safeguard optimal patient care, protocol allowed for physicians to change patient therapy on an individual basis at any time (e.g., de-escalation, combination therapy, allergic reactions or patient safety). There was no blinding of intervention allocation for physicians during admission, for those responsible for data collection or the patient. For the current analysis we had access to individual microbiological culture data from seven of eight participating European ICUs from Belgium, France, Germany, Portugal, and Slovenia. These were ICUs with mixed, medical or surgical patient populations.

Patients were included if a first culture was negative for Gram-negative ARB in cultures from the respiratory- or gastrointestinal tract (e.g. feces, rectum, perineum or gastric contents).

Enrolment of patients was preceded by approvement of the study and a waiver for individual informed consent, by all local Interne Review Boards of each participating center. This trial is registered with ClinicalTrials.gov, number NCT01293071. There were no changes to the study design after trial commencement.

The primary endpoint was the first clinical culture with Gram-negative ARB, defined as non-susceptibility to 3rd- or 4th generation cephalosporins and piperacillin-tazobactam in Enterobacterales species, and piperacillin-tazobactam or- meropenem resistance in *Pseudomonas aeruginosa* and Acinetobacter species. For the current analysis patients were eligible if microbiological cultures had been obtained from either the respiratory tract or gastrointestinal tract. Admissions with detected Gram-negative ARB carriage in the first two days of admission were excluded, as were admissions with positive endpoints on the same day as a first negative culture. Acquired carriage with Gram-negative ARB after day 2 of admission was assumed to be permanent for the duration of the study, i.e., readmissions of these patients were excluded from the analysis.

Data collection

All clinical cultures taken during the study periods were included for endpoint analysis. If the primary endpoint was reached in an individual patient, subsequent culture results were excluded. Microbiological procedures were performed according to local laboratory practices, including local Minimum Inhibitory Concentration cutoff values. Participating microbiological laboratories did not change protocols for antibiotic susceptibility testing during the study. Likewise, infection prevention- and control measures did not change during the study period.

Systemic antibiotic use was collected at the individual or aggregate level. Consumed quantities of antibiotics were converted to WHO Defined Daily Dose (DDD). Use of different antibiotic groups were represented as subdivided 6-week periods for cycling, and divided over the 9-month intervention period for mixing.

Ethics approval and consent to participate

The study protocol was approved by each local Institutional Review Board (IRB) and all centers obtained a waiver for individual patient written informed consent.

Statistical analysis

Demographic- and infection prevention variables comparison between interventions were performed using bivariate tests. Dichotomous endpoints were tested using Pearsons' chi-square test and for continuous outcomes using Students' t-test. The primary outcome analysis was performed using chi-square test for binary endpoints. Odds ratios for acquisition between the two intervention periods (cycling and mixing) were calculated using mixed effects logistic regression modelling, with

adjustment for clustering within each hospital and for confounders age, gender, length of stay, previous admission, origin of transfer to ICU and survival at ICU-discharge). Independent variables for this model were chosen based on potential correlation with the intervention and endpoint and being reasonably objectifiable.

Additionally, detection bias between intervention periods from clinical cultures was assessed by modeling the probability of having a culture taken during admission using a mixed effects model correcting for clustering within individual ICU. This analysis was performed on all admissions during the intervention period, including patients without clinical cultures obtained. To assess competing events bias, mean and median length of stay was calculated and linear regression modelling of the effect of intervention type on length of stay.

Carryover effects between first and second intervention period were assessed using the mixed effects model with an additional interaction term between intervention type and the sequence of performing mixing-then-cycling or vice-versa.

Post-hoc power calculations were performed using the *pwr* package, an effect size of 0.1 based on an arbitrary 'small' effect, significance level of 5% and assumed a two-sided alternative outcome. The calculated power to find a relevant difference was 88%. Analyses were performed using R *software.*(30)

Results

During the cycling and mixing intervention periods, there were 8,267 admissions overall in 7 ICUs in 5 countries (figure 1). For this nested cohort study, 1,613 (19.5%) admissions were eligible. Data was collected from June 27, 2011, to February 16, 2014. Baseline demographics and ICU characteristics of interventions were comparable (table 1, 2).

In these patients 16,437 microbiological cultures were collected (mean of 10.27 cultures per admission); upper- or lower respiratory materials (%), blood- or intravascular cultures (34.8%), enteric cultures (e.g. gastric fluid, bile, feces, rectum swabs, 29.9%) and urine (12.2%) (appendix table 1). In total 8.3% of the cultures was taken as part of surveillance for carriage, not for diagnosis and treatment of infection (missings 12.7%). In cultures that grew *Enterobacterales*, most were *Escherichia coli* (22.5%)*,* Pseudomonas species (21.2%), Klebsiella species (18.8%) and Enterobacter species (11.2%, appendix table 2).
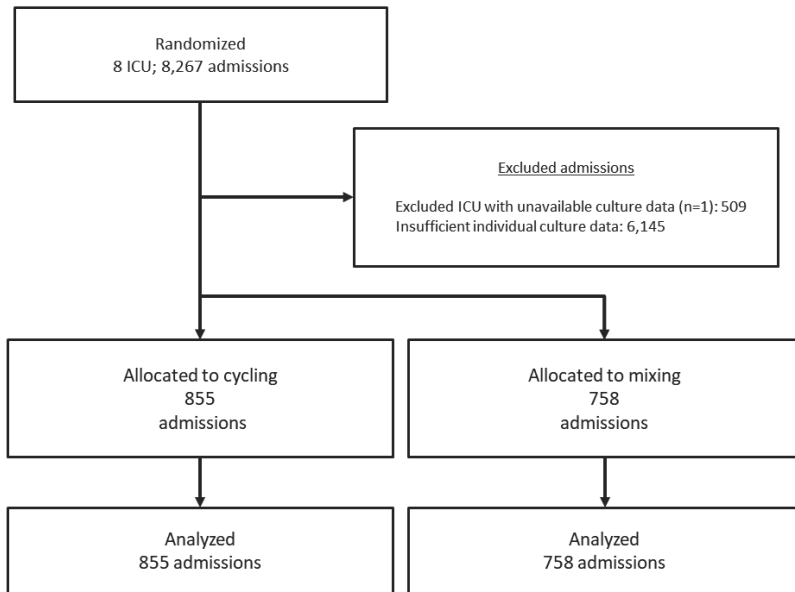
Figure 1 Flow chart

Table 1 Demographic data

Demographic variables	Total
Total Admissions	1,613
Male % (N)	60.8 (980)
Age mean (median)	61.2 (63.8)
Length of stay mean (median)	11.5 (6)
Short-stay patients (<=2days)[a] % (N)	15.4 (248)
Mortality % (N)	12.3 (198)
APACHEII mean (median, N) (31)	19.9 (19 ; 408)
APACHEII (N hospitals)	3
SAPSII mean (median, N)	36.6 (33 ; 544)
SAPSII (N hospitals)	5
SAPSIII mean (median, N)	55.6 (54 ; 278)
SAPSIII (N hospitals)	2
TIS28 mean (median, N)	24.3 (24 ; 160)
TIS28 (N hospitals)	2

[a] Patients with a LOS of 2 days or less

Table 2 Overall ICU characteristics, all admissions during study periods

ICU characteristic, mean	Cycling	Mixing	p-value
Bed occupancy % (beds taken/available)	77.3 (734/949)	80.4 (867/1,079)	0.59[a]
Mechanically ventilated patients % (N)	48.8 (358)	42.2 (366)	0.24[a]
CVVH % (N)[b]	4.6 (34)	4.8 (42)	0.94[a]
ECMO % (N)[c]	3.0 (22)	4.8 (42)	0.09[a]
Thoracic drains % (N)	16.8 (123)	16.5 (143)	0.96[a]
Abdominal drains % (N)	12.3 (90)	8.2 (71)	0.02[a]
Intra-cranial pressure monitors % (N)	1.9 (14)	1.9 (16)	1.00[a]
Contact isolation % (N)	24.9 (183)	24.8 (215)	1.00[a]
Droplet isolation % (N)	1.6 (12)	2.2 (19)	0.54[a]
Airborne isolation % (N)	1.0 (7)	1.7 (15)	0.27[a]
Staffing ratio (registered nurses/1 patient)	0.66	0.64	0.67[d]
Staffing ratio (student nurse/1 patient)	0.11	0.10	0.74[d]

[a] Pearson Chi square
[b] Continuous Veno-Venous Hemofiltration Dialysis
[c] Extra-Corporeal Membrane Oxygenation
[d] Student t-test, two-sided

The odds ratio (OR) of having a culture taken was lower during the mixing intervention (OR 0.83, 95% confidence interval (CI) 0.76 to 0.91, p-value <0.01) (appendix table 3), which did not change after correction for confounders (age, gender, length of stay, previous admission and origin of transfer/referral): Adjusted odds ratio (aOR) 0.84 (95% CI 0.76 to 0.93).

In all, there were 855 and 758 admissions eligible for analysis during cycling and mixing, respectively. The primary endpoint was reached in 62 (7.3%) and in 39 (5.1%) patients during cycling and mixing, respectively, (p-value: 0.13), after a mean of 17.7 (median 15) and 20.8 (median 13) days, in cycling and mixing respectively (p-value: 0.43). Distributions of endpoint defined micro-organisms were: Enterobacterales species (n=61), *Pseudomonas aeruginosa* (n=38) and Acinetobacter species (n=20), without statistically significant differences between interventions (table 3).

Antibiotic use per interventions were similar, as expected by the protocol of equal use of each antibiotic (group) type over time (appendix table 4).

In mixed effect logistic regression modelling the unadjusted odds ratio for acquiring Gram-negative ARB was 0.72 (CI 95% 0.47 to 1.10) during mixing compared to cycling (table 4).

Table 3 Acquisition of ARB (primary endpoint)

Acquisition variables		Cycling	Mixing	p-value[a]
Included clinical cultures N[b]		9,236	7,201	
Included admissions N (%)		855 (21.9)	758 (17.4)	<0.01
Number of cultures per patient	mean (SE)	10.6 (0.50)	9.2 (0.43)	0.03
	median (range)	5 (1-129)	5 (1-117)	
Admissions with ≥1 ARB endpoints N (%)[c]		62 (7.3)	39 (5.1)	0.13
Enterobacterales species resistance endpoint N (%)		38 (4.4)	23 (3.0)	0.18
***Pseudomonas aeruginosa* resistance endpoint N (%)**		25 (2.9)	13 (1.7)	0.15
Acinetobacter species resistance endpoint N (%)		10 (1.2)	10 (1.3)	0.96
Days from admission till first negative culture	mean (SE)	3.1 (0.09)	2.7 (0.05)	<0.01
	median (range)	2 (2-28)	2 (2-14)	
Days from first negative culture till endpoint	mean (SE)	12.1 (1.4)	15.1 (3.1)	0.39
	median (range)	9 (1-49)	8 (1-108)	

[a] Chi-square test for binary variables and T-test for continuous variables.

[b] Cultures of patients with >1 culture, of which the first was negative, excluding endpoints <=2 days admission

[c] Aggregated endpoint of Enterobacterales species, *Pseudomonas aeruginosa* and/or Acinetobacter species endpoints

SE = Standard error

Table 4 Mixed effects logistic regression odds ratios

Analysis type	Model type	Mixing:cycling odds ratio	Confidence interval (2.5%-97.5%)	p-value
Primary analysis[a]	**Unadjusted model[b]**	0.72	0.47 till 1.11	0.13
	Adjusted model[c]	0.62	0.38 till 1.00	0.05

[a] Patients with >1 clinical culture taken with the first culture negative

[b] Random effect: Hospital

[c]Adjusted variables: Age, Gender, LOS, Previous admission, Community of hospital referral, random effect: Hospital

After adjustment for age, gender, length of stay, previous admission and origin of referral (community or hospital), adjusted odds ratio was 0.62, 95% CI 0.38 to 1.00). Adding an interaction term for intervention strategy with the sequence of strategies to test for a carryover effect did not change results (appendix table 5).

The culture probability OR was used for a sensitivity analysis. Here we assume the mixing population was indeed cultured less with missed endpoints. We assumed the size of this hypothetical mixing population was equal to the 'real' cultured cycling population. With an OR of 0.83, the mixing hypothetical population is roughly 120% of the real mixing population. To meet statistical signifi-

cance in a comparison of the hypothetical mixing population and the real cycling population, the incidence in this missed group would need to be 17%. This is more than two times the prevalence found in the real mixing study population.

Discussion

In this patient-level analysis of a randomized cluster design multi-center study, we found no difference in effect of two antibiotic rotation strategies on the individual risk of acquiring colonization with Gram-negative ARB. These findings are in line with the results of the previously reported ecological analysis of this trial. (29) Cycling and mixing are strategies that create opposite extremes of antibiotic diversity: Cycling maximizes homogeneity during a 6-week period, and mixing maximizes heterogeneity.

The cluster randomized, crossover design allows comparison of unit-wide interventions in ICUs. As compared to cohort studies it reduces the likelihood of differences in clinical practice between ICUs, and other differences such as case-mix that may affect endpoint risk estimates. Compared to individual randomization, cluster randomization prevents non-adherence to protocol in individual patients, but provides an estimate of intervention effect at the ward level.

With respect to patient characteristics there were statistically significant differences in mortality and illness severity scores. For mortality this was assumed to be a chance finding, as both intervention arms did not aim to influence mortality, and both arms received the same amounts and types of antibiotics. This difference is also reflected in the mortality scores APACHE II, SAPS II, TIS-28, which do not represent relevant differences: 2, 4.1 and 4.8 respectively points on these scores with maximum scores being from 55, 78 to 163.

It was not possible to compare the interventions to a non-intervention period due to high baseline variability of antibiotic use between ICUs. More specific, there is no parameter to measure antibiotic diversity, therefore it is not possible to quantitatively compare baseline periods, whether these are alike -or different- to their corresponding mixing or cycling periods.

This post-hoc analysis was motivated by previous findings of different results from ecological- and individual-based analyses. (28) This study analyzed individual risks ratio of acquisition of ARB, in contrast to the previous ecological study that evaluated aggregated monthly point-prevalence surveys. Though ecological

analyses offer useful information on group-wise interventions, such analyses can omit patient-level causal relations between exposure and outcome. This can lead to a form of bias called the ecological fallacy. Consequently, it has been advised to use both ecological as well as individual-level analyses for studies in antibiotic resistance when possible.(28)

Ecological fallacy occurs when group-averaged risks lead to incorrect associations between exposure and outcome. For instance, when the exposed patients are not the same patients that acquire the endpoint. Or, when group analyses overlook that the outcome might have occurred before the exposure, distorting causal inference. Or when individual baseline and longitudinal risks differ over time, creating different subgroup risk profiles. For instance, when in time, enrolled patients have incrementally higher baseline-risk for adverse outcome at admission, (due to, for instance, stricter admission criteria), but individual treatment outcomes during admission improve over time. A mechanism that has been termed Simpsons Paradox. (32) The first two mechanisms were prevented by the cluster design of the study, in which all patients underwent the same cluster-intervention from the time of admission, precluding a distorted exposure-outcome relation. Yet, the third mechanism for ecological fallacy – differences in individual baseline and longitudinal risks - could have occurred.

For this analysis, available clinical cultures were used as endpoint determinant. The inclusion criterion of having a first negative enteric or respiratory culture ascertained acquisition of colonization with Gram-negative antibiotic resistant bacteria. The intervention is still ecological, but the analysis aims to provide individual risk estimates for colonization in a ward where antibiotic rotation is applied.

The restricted availability of these cultures prompted us to assess the presence and size of detection bias, which could have resulted from differential inclusion between intervention arms, due to the absence of individual randomization. We found no indication that baseline demographics or confounders were different between interventions, and adjustment for this in regression analysis did not change effect estimates. Alternatively, there could be a direct effect of the intervention on diagnostic culture practices. We used the source population (all included patients with and without cultures) to perform two additional analyses to assess inclusion bias or an intervention effect on culture rates: 1) Assessing the differences between interventions of the probability of a culture being taken by regression analysis, and 2) a sensitivity analysis to contextualize the size of the potential intervention-effect on detection bias and outcome.

The probability of having a culture taken was lower during the mixing period, but there was no evidence that this was due to differences in patient characteristics, nor were there differences in intervention effects after adjusting for confounders in the primary analysis. Sensitivity analysis yielded that detection bias would have needed to have caused a relative decrease in incidence of ARB acquisition with 29.2% (absolute 2.8%) to achieve statistical significance of the primary analysis. Or, in other words, in the uncultured patients in the mixing population that should have been cultured, based on our model, endpoint incidence would have to have been 16.6%, compared to 7.3% in the included mixing population, to achieve statistical significance. We consider this unlikely and therefore conclude that detection bias did not substantially reduce the validity of our analysis.

Naturally, generalizability of our findings is shifted towards patients with a clinical culture taken, and thus patients with a relatively long stay in ICU. In fact, despite generally a low threshold for collecting clinical cultures in ICU patients in the participating ICUs, excluded patients were mostly short-stay patients, discharged after a median 2 days of admission. Furthermore, results would not be generalizable to ICUs with higher endemic prevalence of antibiotic resistant bacteria, where the antibiotics that were used in this study cannot be used for empiric therapy. These results however, are generalizable to most European ICUs, and any non-European ICU with similar technical capacities, staffing and resistance prevalence.

Conclusions

Our findings do not support superiority of effects of cycling over mixing or vice versa on acquisition of ARB in the participating ICUs. Based on current scientific evidence, including our study, antibiotic rotation should, therefore, not be recommended as standard care. (13-27) This study, however, rotated beta-lactam antibiotics exclusively, with fixed and pre-defined rotation schedules of per-patent rotation and 6-week periods. There are many variations of antibiotic rotation and effectiveness of some scheme is not excluded. (33) Resistance acquisition under antibiotic rotation strategies has layered complexity from the microbiome, infection control measures, and collateral sensitivity. (34) Further research is needed, and future clinical studies will benefit from a multi-disciplinary approach by including basic sciences and mathematical modeling. Ultimately the goal should be to provide tai-

lor-made algorithms to guide ICU antibiotic policies, in order to optimize resistance selective pressure and patient safety.

Abbreviations

ARB   Antibiotic resistant bacteria
ICU   Intensive Care Unit
ASP   Antibiotic stewardship programs
DDD   Defined Daily Dose
aOR   Adjusted odds-ratio

Funding

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Supporting information

S2 Appendix, appendix tables 1-5

Appendix table 1 Material categories of all clinical cultures

Material type	Cycling N (%)	Mixing N (%)
Total	9,245	7,209
Respiratory	3,060 (33.1)	2,660 (36.9)
Blood or intravascular	2,805 (30.3)	2,112(29.3)
Enteric	1,310 (14.2)	701 (9.7)
Urine	979 (10.6)	999 (13.9)
Wound	183 (1.98)	125 (1.73)
Abdominal	88 (0.95)	51 (0.71)
Intracerebral	69 (0.75)	128 (1.78)
Other	751 (8.1)	433 (6.0)

Appendix table 2 Identification of Gram-negative endpoint species*.

Microorganism	Cycling N (%)	Mixing N (%)
Total	669	631
Escherichia coli	199 (28.6)	172 (27.3)
Pseudomonas aeruginosa	170 (25.4)	106 (16.8)
Klebsiella species	111 (16.6)	133 (21.1)
Enterobacter spp.	61 (9.1)	84 (13.3)
Proteus spp.	47 (7.0)	27 (4.3)
Serratia spp.	36 (5.4)	32 (5.1)
Acinetobacter species	15 (2.2)	28 (4.4)
Citrobacter spp.	12 (1.8)	21 (3.3)
Morganella spp.	10 (1.5)	21 (3.3)
Hafnia alvei	7 (1.0)	1 (0.2)
Providencia spp.	4 (0.6)	2 (0.3)
Salmonella spp.	4 (0.6)	1 (0.2)
Raoultella spp.	1 (0.1)	3 (0.5)

* Enterobacterales species, *Pseudomonas aeruginosa*, Acinetobacter species

Appendix table 3 Modelling carryover effects

Variable	Adjusted model[a]	p-value	Adjusted model with carryover interaction term[a]	p-value
Model fit change ΔAIC[b]			-4 (59.69 – 596.36)	0.85
Carry over model estimates	Hazard *ratio[c]* (95% CI)		Adjusted hazard *ratio[c]* (95% CI)	
Intervention[d]	0.62 (0.38 – 1.00)	0.05	0.66 (0.35-1.28)	0.22
Intervention *sequence[e]*			0.86 (0.40-1.85)	0.69
Interaction term (Intervention*sequence)[f]			0.91 (0.33-2.50)	0.85
Age	1.01 (0.996 – 1.022)	0.17	1.01 (0.996 - 1.022)	0.18
Gender	1.40 (0.86 – 2.28)	0.18	1.39 (0.85-2.26)	0.19
Previous admission	0.66 (0.41 – 1.07)	0.09	0.66 (0.40-1.07)	0.09
Referral origin[g]	1.24 (0.76 – 2.03)	0.40	1.21 (0.74-2.00)	0.45
Length of stay	1.07 (1.06 – 1.08)	<0.01	1.07 (1.06-1.08)	<0.01
Deceased during admission	2.10 (1.21 – 3.63)	<0.01	2.10 (1.21-3.65)	<0.01

[a] Interaction term of intervention and sequence of intervention (cycling-mixing or mixing-cycling)

[b] Difference after adding interaction term to model, tested with ANOVA

[c] Exponent of model coefficient

[d] Cycling as reference

[e] Cycling then mixing as reference

[f] Interaction term between intervention and sequence

[g] Community versus hospital

Appendix table 4 Antibiotic consumption per 100 admission days

	Cycling	Mixing	p-value
Carbapenems	21.98	19.25	0.07
3rd/4th generation cephalosporins	14.48	17.18	0.84
Broad-spectrum penicillins w/ betalactamase-inhibitor	13.89	13.11	0.15
Piperacillin-tazobactam	9.61	9.45	0.17
Fluoroquinolones	9.06	10.37	0.89
Aminoglycosides	2.55	2.50	0.15
Cotrimoxazole	1.77	2.67	0.53

Appendix table 5 Mixed effects logistic regression odds ratios for chance of having a clinical culture taken

Analysis type	Variable	Mixing:cycling odds ratio	Confidence interval (2.5%-97.5%)	p-value
Unadjusted *model* [a,b]	**Intervention [c]**	0.83	0.76 till 0.91	<0.01
Adjusted *model* [d]	**Intervention**	0.84	0.76 till 0.93	<0.01
	Age	1.00	1.00 till 1.01	<0.01
	Gender	0.97	0.88 till 1.08	0.59
	Length of stay	1.27	1.25 till 1.30	<0.01
	Previous admission	2.10	1.88 till 2.34	<0.01
	Origin of referral	0.99	0.88 till 1.11	0.82

[a] All admissions during cycling and mixing interventions

[b] Adjusted variables: Random effect: Hospital

[c] Mixing is the reference

[d] Adjusted variables: Age, gender, length of stay, previous admission, community or hospital referral, random effect: Hospital

CHAPTER 7

Discussion

Interventions to prevent antibiotic resistance

Patients admitted to hospital are at risk of acquiring colonization and infections caused by antibiotic resistant Gram-negative bacteria. Such infections are difficult to treat, especially when the causing pathogens have reduced susceptibility to antibiotics, and, therefore, jeopardize patient safety. In the Intensive Care Unit (ICU) both selection of bacteria with resistance to some antibiotics and transmission of bacteria between patients is amplified by increased selective pressure exerted by broad-spectrum antibiotic use and the need of frequent contacts between healthcare workers and patients. (1) There are multiple approaches to reducing unwanted consequences of infections caused by antibiotic resistant bacteria: developing new antibiotics or other treatment options, preventing transmission of bacteria, optimizing antibiotic use to minimize antibiotic selective pressure, and preventing infections.

Development of new antibiotics has declined in the last three decades, mainly because of economic reasons. For pharmaceutical companies, antibiotic development is hardly cost-effective and return of investments is low; the clinical development phase (from phase 1 to phase 3 studies) may take many years, and is very expensive. If successful, newly approved antibiotics will be used cautiously; because of a limited therapeutic range if developed for extremely resistant pathogens, and also to limit the risk of selecting bacteria resistant to the agent. Furthermore, effective antibiotics are used in short courses. Finally, although antibiotic resistance is widely considered a realistic threat for human health, the vast majority of infections can still be treated by very effective, very cheap and very safe antibiotics, hampering the introduction of new, more expensive alternatives with a yet unknown safety profile. (2)

The unmet medical need of new antibiotics is widely recognized, and large-scale public-private partnerships have been funded to integrate skills and knowledge, in order to develop and evaluate new antibiotics, such as the New Drugs for Bad Bugs program of the Innovative Health Initiative in Europe and CARB-X in the United States. Yet, it may take years before new antibiotics will be available for patients. (3,4)

Until then (but also with new antibiotics being available) patients need to be protected by measures that prevent transmission of pathogens and development of infections, such as the classic infection control measures hand hygiene, patient

isolation and environmental disinfection and optimizing antibiotic use. (5) The latter, prudently prescribing antibiotics, also called 'antibiotic stewardship', aims to minimize the evolutionary selection of antibiotic resistant bacteria that is exerted under antibiotic pressure, while maintaining optimal treatment of infections. (6,7)

Antibiotic Stewardship

Antibiotic stewardship is the systematic effort to use evidence-based prescribing of antimicrobials, to treat patients with infections optimally and – at the same time - to minimize antimicrobial overuse and selection of antimicrobial resistance. In many instances it attempts to reduce the quantity of (broad-spectrum) antibiotic consumption. However, there are also more unorthodox modalities, such as high-dose topical antibiotics to eradicate or suppress (antibiotic-resistant) bacteria in the nose or in the gut. Practical examples are eradication of carriage with methicillin-resistant Staphylococcus aureus (MRSA), pre-operative eradication of *S. aureus* carriage, and Selective Digestive tract Decontamination (SDD) in critically ill and mechanically ventilated patients. All three interventions are currently used in standard of care in Dutch hospital healthcare. (8–10) In each of these interventions, the use of antibiotics for infection prevention contradicts the dogma that all antibiotic use leads to selection of antibiotic resistance and should thus be minimized. These interventions have, therefore, been subject to considerable scientific debate. Nonetheless, based on well-designed clinical studies the benefits for patients have been considered to outweigh the risk of selection for resistance. Moreover, up till now, widespread selection of antibiotic resistant bacteria has not materialized, not even after prolonged periods of time. (11–15)

Antibiotic rotation

Scheduled rotational schemes of different antibiotics, or antibiotic rotation, has also been proposed to reduce antibiotic resistance. In theory, the consecutive use of different antibiotics (antibiotic rotation) could decrease overall antibiotic selective pressure. The first clinical studies of antibiotic rotation described convenience driven cyclic changes reactive to a rising prevalence of antibiotic resistance. In one – widely cited – study, cycling was initiated because of shortage of antibiotics, and

a fluctuating pattern emerged with gradually declining resistance, which was interpreted as resulting from the rotating pattern of empiric therapy in the hospital. (16) From this, the concept of antibiotic rotation originated; certain changes in the diversity of antibiotic exposure could modulate selective pressure and reduce antibiotic resistance.

Core to this thesis is the international multi-center cluster-randomized crossover trial comparing mixing to cycling of beta-lactam antibiotics in ICUs. The study data was analyzed from two different perspectives; for differences in prevalence from a ward-level (ecological) perspective (**chapter 5**), and for differences in *acquisition* of antibiotic resistant Gram-negative bacteria at the individual patient level (**chapter 6**). Such a "dual analysis" approach of individual and ecological outcomes has been advocated when there is a possibility of bias due to data structure (e.g., when data are aggregated), design choices (e.g. cluster allocation) and statistical analyses (e.g. omitting subgroup- or time-parameters). (17,18) It can provide deeper insight in exposure-outcome confounding and potential trade-offs between ecological and individual effects of an intervention. Both approaches failed to demonstrate clinically relevant or statistically significant differences between both strategies.

The compiled results of previous clinical studies evaluating antibiotic rotation effectiveness, including the study in **chapter 5** and **6** of this thesis (19–40), and two more recent studies (41,42), fail to demonstrate benefits of antibiotic rotation strategies in reducing antibiotic resistance. It is, therefore, highly unlikely that current international guidelines that do not recommend antibiotic rotation will be adapted (43–46), or that antibiotic rotation will be implemented in healthcare settings in the foreseeable future.

Yet, although there currently is absence of evidence, that may not imply 100% evidence of absence. Multiple variations of rotation strategies have been tested and it is, therefore, difficult to compare studies. Variations include, amongst others, the number and types of rotated antibiotics, duration of cycle periods, and targeted Gram-negative micro-organisms. Other caveats for study comparison include incomplete or inaccurate compliance measurement, not correcting for confounders such as infection prevention- and control measures, cluster effects in multi-center studies, and not including the entire ward population but only patients who received intervention (rotation) antibiotics.

From a clinical perspective one may, therefore, consider that "the book on antibiotic rotation strategies in ICU to control antibiotic resistance" has been closed. Yet, there are three research areas that may reopen the book in the future.

Mathematical modeling

Execution of antibiotic rotation clinical trials is resource- and time-consuming. Mathematical modelling is a versatile alternative to enhance insight into specific processes of complex selection and transmission dynamics. Yet, modelling always remains a simplification of reality. Or, as stated by Marc Lipsitch: "Mathematical models simplify some aspects of transmission dynamics to enhance understanding of other aspects". (47) Nevertheless, there is a growing body of in silico analyses that has increased our understanding of the complex relationship between groupwise antibiotic consumption and individual resistance emergence and transmission in hospital wards. (48–58)

An important unknown variable is how long it takes for rotation strategies to affect resistance prevalence in the ICU. Likewise, it is extremely difficult to determine the exact duration between resistance acquisition and detection of acquired antibiotic resistant bacteria in a patient through culture or molecular methods. These unknowns pose a risk of obtaining incorrect associations between antibiotic rotation and subsequent changes in resistance prevalence.

Acquisition of bacterial carriage may result from cross-transmission or from endogenous selection. The latter questions the accuracy of the term "acquisition" as this reflects the fact that a bacterium has become detectable with the detection method applied. The incidence of cross-transmission of resistant bacteria primarily depends on colonization pressure, the prevalence of carriage of such bacteria among other patients in the ward. Furthermore, cross-transmission rates are determined by the frequency of physical contacts between healthcare workers and other patients, the adherence to infection prevention measures that reduce transmission and the relevance of environmental sources for contamination.

For each of these mechanisms the time between a change in antibiotic exposure and a detectable difference in ward epidemiology may differ. As a result, effects of

antibiotic rotation may occur directly after starting a new rotation period, but may also occur later, and even in the subsequent rotation period. Therefore, effects of scheduled changes in antibiotic pressure should be measured longitudinally across entire intervention periods, and preferably with a follow-up period. If a crossover design is used, carryover effects should be assessed.

Mathematical models have demonstrated that antibiotic rotation can lead to oscillating patterns of antibiotic resistance prevalence, that become stable in time. Even when patterns seem stable with non-changing prevalence, there is the possibility, depending on model characteristics, parameterization and most importantly the investigated time window, that concurrent short-term and long-term equilibria can exist. Resistance prevalence can, therefore, seem fixed while in the long-term, prevalence, for instance, decreases. (59) This apparent paradox may invite modelers to further elucidate the short- and long-term effects in order to better inform clinical trial design and analysis.

Mathematical models have also illustrated that if selection of resistance differs between rotated antibiotics (asymmetry), cycling performs better than mixing. (52) If confirmed in vivo, this would have consequences for choosing combinations of antibiotics for rotation and bacteria and phenotypes as targeted endpoints.

Also, interventions that modulate heterogeneity in antibiotic use should be compared to the baseline heterogeneity of antibiotic use (i.e., before intervention), which has been done in few clinical studies, so far. (21,50) This is amongst others, hampered by the lack of a parameter for antibiotic diversity.

Baseline heterogeneity in antibiotic exposure may be influenced by antibiotic resistance prevalence, which may limit options for modulation of antibiotic use. For instance, heterogeneity can be higher if more different antibiotics are eligible for an intervention, but options usually decline if the prevalence of antibiotic resistance increases. In fact, high levels of antibiotic resistance in an ICU may exclude options for rotation and may create homogeneous antibiotic exposure. Hence, antibiotic rotation and its effects, antimicrobial resistance prevalence and intervention compliance are all connected, underlining the need for interventional studies and precise confounder measurement.

Moreover, difference in antibiotic heterogeneity may disappear based on duration of cycling periods. Mixing is defined as rotation per antibiotic course or per admitted patient. Yet, the heterogeneity in overall antibiotic exposure with mixing might be comparable to that of relatively short rotation periods, say 1 or 2 weeks. Indeed, modelling studies illustrated that cycling and mixing strategies can yield the same effects on antibiotic resistance prevalence, obscuring a clear definition of what is cycling and what is mixing. (51)

In summary, mathematical models have demonstrated multiple aspects that may affect the clinical effects of antibiotic rotation strategies. An important science gap is a parameter that quantifies antibiotic diversity, even though diversity parameters are now being incorporated in mathematical studies. (21,50) Clear guidance for a testable intervention based on convincing outcomes of mathematical studies might reopen "the book on antibiotic rotation strategies in ICU to control antibiotic resistance".

Antibiotic homogeneity index

There is no parameter that quantifies the relationship between diversity in antibiotic exposure and selection of antibiotic resistance. There is, however, a measure to quantify the diversity of antibiotic use; the antibiotic homogeneity index. It computes the variance of different groups of antibiotics into one numeric value. (21) The index is based on the differences in proportions compared to when all antibiotic proportions would be equally divided. There are, however, several problems with this index that prohibit its use in research and practice.

The equation for this index contains no variable for time or number of antibiotic courses. This makes the antibiotic homogeneity index vulnerable to bias when the types of antibiotics, number of antibiotic courses, and the period over which the index is calculated are not controlled for.

According to the principles of rotation, antibiotic homogeneity index values should be low during mixing (i.e., reflecting a continuously high diversity) and should be high *within* cycling periods (i.e., reflecting homogeneity). Between cycling periods, the homogeneity index may not differ, even though the antibiotics used are

completely different. The antibiotic homogeneity index, therefore, must be used carefully but can be informative if data standardized for similar antibiotics, time periods and sample sizes.

Second, this index defines diversity as the deviance from an equilibrium of equal use of different antibiotics. This hampers comparisons of the index between settings. The proposed index, therefore, has not yet been used extensively in clinical studies. Though we chose not to use the homogeneity index for said reasons, we did, likewise, standardize our data in the descriptive analyses in **chapters 5** and **6**. Sandiumenge and coworkers used only descriptive homogeneity indices to indicate protocol adherence. (21) And Abel zur Wiesch et al. corrected for baseline antibiotic homogeneity index values in a multi-variate regression meta-analysis, but found no effect on antibiotic resistance incidence. (50)

It is nonetheless important to use a parameter for antibiotic use diversity to assess effects of antibiotic rotation strategies. Identification (and validation) of such a parameter might play a role in reopening "the book on antibiotic rotation strategies in ICU to control antibiotic resistance".

Collateral sensitivity

Relatively recent and mainly in-vitro research has provided evidence for the concept of collateral sensitivity; Antibiotic exposure selects for resistant mutants, but in the same bacterium can select for susceptibility to other antibiotics. (60–62) As such, targeted antibiotic exposure could be used to select for susceptible bacteria, for which treatment options exist. For instance, CTX-M-15 extended spectrum beta-lactamase producing *Escherichia coli* gained cefotaxime susceptibility after mecillinam exposure (63), in *Staphylococcus aureus* alternating paired combinations of trimethoprim, neomycin and ciprofloxacin, slowed resistance evolution for trimethoprim and ciprofloxacin. (64) *S. aureus* exposed to fusidic acid had relevant increases in erythromycin susceptibility. (65), *Burkholderia multivorans* had increased susceptibility after meropenem or trimethoprim-sulfamethoxazole exposure (66) and *Pseudomonas aeruginosa* had increased susceptibility for, amongst others, aminoglycosides after exposure to trimethoprim-sulfamethoxazole, a drug to which *P. aeruginosa* is intrinsically not susceptible. (67,68) This increased sus-

ceptibility was also observed in consecutive clinical isolates of *P. aeruginosa* from cystic fibrosis patients after antibiotic therapy. And in *Mycobacterium smegmatis* susceptibility increased to isoniazid and other hydrophilic antibiotics, such as ethionamide and aminoglycosides, after exposure to rifampicin. (69,70) Based on such phenotypic and molecular data, statistical models have been used to predict single-isolate collateral sensitivity after antibiotic exposure. (71) Exposure of bacteria to consecutive antibiotics to increase susceptibility has been called directed evolution. (72) Multiple routes may lead to the same target phenotype, together forming so-called *susceptibility landscapes.* (60,72–74)

More studies will be needed to determine whether this concept has practical implications for antibiotic stewardship practices. Currently, there is no clinical implementation of collateral sensitivity or directed evolution, mainly for its lack of robust data in *in vivo* settings. So far studies have investigated single isolates, and not yet microbiota of large groups of patients. Nonetheless, this approach may augment our understanding of antibiotic resistance emergence, and may offer counter-measures through applying antibiotic rotation. Identification (and validation) of clinically relevant path(s) of directed evolution might be the next step to reopen "the book on antibiotic rotation strategies in ICU to control antibiotic resistance".

Future Prospects

The science of antibiotic rotation has made large progress in the last 25 years. It followed a countercurrent direction from conception in daily routine practice, but later proved to be without clear empirical evidence for clinical benefits. Investigations then moved down to more fundamental research in mathematical modelling and microbiology. In these fields, considerable headway is required before moving back to clinical trials, and if positive, implementation. Nevertheless, there is still considerable perspective for new insights and breakthroughs. Through collaboration of different research fields (such as microbiology, genomics and mathematical modelling) the aim should be to identify basic principles for rotation strategies and, importantly, explicit rotation protocols that can then be validated in real-life settings. These potential interventions should include - at least - the number and types of antibiotics, the rotation sequences, parameters of colonization pressure, rotation duration and conditions of switching, colonization status on admission,

the specific diagnostic conditions to assess colonization (phenotypic and genotypic testing on enteral-, respiratory- and wound colonization), and if possible whole genome and metagenomic analyses.

With all these factors to be included, what could possibly be feasible study design? Different antibiotic combinations could be selected based on molecular and phenotypic susceptibility landscapes and applied in varying factorial combinations in an adaptive trial. Each potential antibiotic sequence would be predicted in vitro and intermittently analyzed for its probability of successfully reducing resistance prevalence in the individual patient and at the ward level. Unsuccessful arms will be terminated, successful sequences will continue, and data obtained on the synergy of certain resistance traits or genes can be used to modify candidate antibiotic combinations, thereby constructing continuously changing sequences. The same could be done for rotation periods: Starting with randomly varying rotation periods to identify which period lengths perform best, maybe even for specific antibiotic combinations. Antibiotic choices can be informed by real-time ecological epidemiology, leading to dynamically changing antibiotic rotations that constantly move over the spectrum between mixing and cycling with regard to cycling period length. With new rapid diagnostics, it is even thinkable that in the future, patients' microbiota is screened at admission to pre-emptively define empirical therapy for infection. Yet, this scenario would require considerable efforts of combined commercial and public expertise and funding.

Nonetheless, given the potential benefits of antibiotic rotation strategies, it would be worthwhile not closing "the book on antibiotic rotation strategies in ICU to control antibiotic resistance", but adding a new chapter.

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Nederlandse samenvatting

Wereldwijd is antibioticaresistentie een groot en toenemend probleem (hoofdstuk 2). Het voorkomt adequate behandeling van infecties en leid zo tot meer ziekte en zorgkosten.

Er zijn verschillende interventies mogelijk om resistentie te voorkomen of infecties met antibioticaresistente bacteriën te voorkomen of tegen te gaan. Daar waar deze interventies als doel hebben om antibiotica gebruik te verbeteren wordt dit antibiotic stewardship genoemd. Dit houdt in dat er niet alleen gekeken wordt wat er op dat moment goed is voor de patiënt, maar ook dat rekening gehouden wordt met de effecten op langere termijn voor de gemeenschap.

Er zijn verschillende soorten bacteriën-, resistentie uitingen en behandelstrategieen (hoofdstuk 3). Vaak zijn die erop gericht om minder- of zelfs geen antibiotica te gebruiken. Niet in alle gevallen is het mogelijk om minder antibiotica te gebruiken. Het gebruik ervan is namelijk vooral ook gebonden aan de gunstige effecten van antibiotica, het behandelen van infecties. In dit proefschrift worden antibiotica interventies beschreven die niet minder maar juist meer of andere antibiotica gebruiken om resistentie tegen te gaan. De strategie die daarbij de meeste aandacht krijgt is antibiotica rotatie.

Antibiotica rotatie is het systematisch variëren van het soort antibioticum. Door elke patiënt of elke andere tijdsperiode een ander antibioticum te geven voor dezelfde infecties zorg je voor een ander diversiteitspatroon van gebruik van antibiotica. Door de antibiotica steeds te veranderen, worden bacteriën doorlopend aan verschillende antibiotica blootgesteld. De hypothese is dat dit het moeilijker maakt voor resistentie bacteriën om zich langdurig te vestigen -en verspreiden, op een ziekenhuisafdeling. Het meest is dit onderzocht in ziekenhuizen en voor meer ernstige infecties bij Gram-negatieve infecties. Dit proefschrift onderzoekt hoe antibiotica rotatie zou kunnen helpen om bij ernstig zieke patiënten op de Intensive Care afdeling, het voorkomen van resistente bacteriën te verminderen. Er dus wordt niet minder of meer antibiotica gebruikt maar dezelfde antibiotica wordt op een andere manier toegepast. Er is nog relatief weinig wetenschappelijk onderzoek gedaan naar dit soort interventies. Het onderzoeken van dit soort interventies is onderhevig aan

verschillende uitdagingen op methodologisch- en statistisch-analytisch gebied. In hoofdstuk 4 wordt een voorstel beschreven om de effecten van twee van de meest gebruikte antibiotica rotatie interventies te kwantificeren. Deze twee interventies zijn *antibiotic cycling* en -*mixing*. Zij vormen twee uitersten van hoe je gebruik van antibiotica kunt variëren.

In dit hoofdstuk wordt het gebruik geroteerd van 3 (groepen van) antibiotica voor de empirische behandeling van infecties waarbij vermoedt wordt dat dit door Gram-negatieve bacteriën veroorzaakt wordt. Bij *antibiotic cycling* wordt elke 6 weken een ander middel gebruikt voor alle patiënten, bij *antibiotic mixing* wordt elke patiënt een ander middel gebruikt. Dit leidt tot contrasterende diversiteitspatronen van antibioticagebruik. In hoofdstuk 5 wordt de uitvoering van deze studie beschreven. Hieruit kan geconcludeerd worden dat er geen verschil is in waargenomen effectiviteit tussen de twee vergeleken interventies. Tevens werd er geen verschil gevonden over tijd op de verschillende Intensive Care afdelingen.

Deze waarnemingen zijn gedaan op de maandelijkse puntprevalentie metingen van Europese Intensive Care afdelingen. Hierbij zijn het aantal antibioticaresistentie bacteriën gemeten op de op dat moment opgenomen patiënten. De analyse is daarom op een groep uitgevoerd en niet op individuele patiënten. Dit wordt ook wel een ecologische analyse genoemd.

In hoofdstuk 6 wordt op dezelfde groep patiënten een analyse gedaan die onderzoekt wat de individuele kans is om een antibioticaresistente bacterie op te doen. Ook hier werd geen verschil gevonden.

Het is belangrijk om vanuit zowel een ecologische- als individuele perspectief een statistische analyse te doen. Omdat er verschillen kunnen zijn in wie belang heeft bij (kennis over) de interventie: groepsbelangen en individuele belangen. Deze kunnen, maar hoeven niet overeen te komen. Tevens kunnen hierdoor vormen van bias ontstaan die kunnen leiden tot foute (interpretaties van) de uitkomsten.

In hoofdstuk 4 worden verschillende methodologische en statistische voorzorgsmaatregelen genomen om vormen van bias tegen te gaan. Er zijn echter nog veel meer factoren die veel moeilijker zijn te meten dan die in deze studie opgenomen konden worden en is er dus nog veel aanvullend onderzoek nodig om echt tot spe-

cifieke antibiotica rotatie adviezen te kunnen komen. In hoofdstuk 7 worden hier voorstellen voor gedaan. Het zou uiteindelijk mogelijk moeten zijn om op zowel afdelingsniveau als patiënt-niveau de antibiotica te kunnen kiezen om tot de best mogelijk balans tussen preventie en behandeling te komen. En hiermee de patiëntenzorg op beide niveaus te verbeteren.

Curriculum vitae

Joppe van Duijn was born on November 12 in 1979. In 1999 he graduated from de Meergronden in Almere and after a year of Nursing School at the Hogeschool van Amsterdam he started his medical study at the University of Amsterdam. In 2008 he obtained his medical degree and worked at the emergency department of the then Westfriesgasthuis in Hoorn and Onze Lieve Vrouwe Gasthuis in Amsterdam.

In 2010 he started his research at the Julius Centre for Life Sciences and Primary Care. In 2015 he began his residency in Medical Microbiology at the University Medical Centre Groningen. Since 2021 he is a Clinical Microbiologist at the division of Medical Microbiology at Stichting Certe Medische Diagnostiek en Advies in Groningen.

Joppe lives in Groningen with his family Wietske Lambers, Mels (2017) and Nyne (2013).