Robert A. Weinstein, Section Editor

Potential Confounding in Evaluating Infection-Control Interventions in Hospital Settings: Changing Antibiotic Prescription

S. Nijssen,[1] M. Bootsma,[4] and M. Bonten[1,2,3]

[1]Department of Internal Medicine, Division of Acute Medicine and Infectious Diseases, [2]Eijkman-Winkler Institute, and [3]Julius Center for Health Research and Primary Care, University Medical Center Utrecht, and [4]Department of Mathematics, Utrecht University, Utrecht, The Netherlands

The colonization dynamics of antibiotic-resistant pathogens in hospital settings are complex, with multiple and continuously interacting variables (e.g., introduction of resistance, infection-control practices, antibiotic use). Quantification of these variables is indispensable in the evaluation of intervention studies, because these variables represent potential confounders. In this article, the complexity of colonization dynamics is described. Through a systematic review, we identified studies that evaluated the modification of antibiotic prescription to reduce antibiotic resistance in intensive care units ($n = 19$), and the extent of confounding-control was determined. Most studies evaluated antimicrobial restriction/substitution ($n = 12$) or antibiotic rotation ($n = 4$). Sixteen studies had a prospective cohort design (before-after), of which 12 were without a control group. Introduction of antibiotic resistance was determined in 10 studies. The relative importance of colonization routes and adherence to infection-control measures were not determined in any study. Therefore, it remains uncertain whether observed changes in the prevalence of antibiotic resistance after intervention were causally related to the intervention. Appropriate choices of study design, primary end point (colonization rates rather than infection rates) and statistical tests, determination of colonization routes, and control of potential confounders are needed to increase validity of intervention studies.

Nosocomial infections, especially those caused by antibiotic-resistant pathogens, are a serious complication in critically ill patients. Infections are usually preceded by colonization, and the number of colonized patients far exceeds the number of prominent infections ("tip of the iceberg" phenomenon). Subsequently, we describe the complexity of colonization dynamics in hospital settings and how different processes interact. Because, as a result of these interactions, confounding may complicate evaluation of interventions, we evaluate to what extent confounding has been controlled for in intervention trials of the modification of antibiotic use in intensive care units (ICUs).

The endemic prevalence—usually expressed as the average daily proportion of patients who are colonized with a pathogen of interest—in a hospital ward can change because of 3 processes: (1) admission and discharge of colonized and noncolonized patients, (2) de novo resistance development (or eradication of susceptible flora and selection of preexistent resistant flora), and (3) patient-to-patient transmission of resistant strains or resistance determinants [1] (figure 1). "De novo resistance development" implies processes in which susceptible bacteria become resistant to antibiotics through mutations or horizontal gene transfer (conjugation, transduction, or transformation). Subsequently, selective antibiotic pressure may facilitate overgrowth of these resistant strains.

Transmission of pathogens from patient to patient usually occurs via the hands of health care workers or through use of contaminated equipment. The success of these routes depends on colonization pressure, patients' bacterial loads, cohort levels, contact rates, staff adherence to hand hygiene measures, and the susceptibility of noncolonized patients for pathogen acquisition [1, 2]. Selective antibiotic pressure enhances the risk of transmission by increasing a patients' bacterial load (through selection of preexistent resistant flora), with a subsequent risk of hand contamination in health care workers, and by creating

Received 21 February 2006; accepted 16 May 2006; electronically published 31 July 2006.

Reprints or correspondence: Dr. M. J. M. Bonten, Dept. of Internal Medicine, Div. of Acute Medicine and Infectious Diseases, F.02.126, University Medical Center Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands (mbonten@umcutrecht.nl).

Clinical Infectious Diseases 2006; 43:616–23

1058-4838/2006/4305-0014$15.00

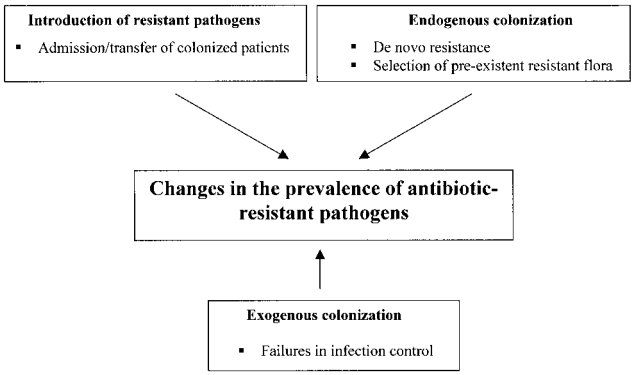

Figure 1. Processes that affect the endemic prevalence of antibiotic-resistant pathogens.

new ecological niches for resistant flora after eradication of susceptible flora in other patients [3].

Because of these different colonization processes, the relatively small numbers of patients in an ICU (typically 10–20) and the characteristic rapid patient turnover (with an average patient stay of only several days), prevalence levels of colonized patients within an ICU continuously fluctuate due to chance events [1, 4, 5]. An understanding of the dynamics of colonization is indispensable for the design of appropriate and targeted infection-control strategies. Measures to decrease the prevalence of colonization with antibiotic-resistant bacteria target the 3 processes depicted in figure 1, including barrier precautions, improvement of hand hygiene, cohorting of patients, reduction of overcrowding and understaffing, and antimicrobial prescription change (restriction, rotation, and cycling of antimicrobial agents).

CONFOUNDING IN INTERVENTION STUDIES

Strategies to reduce the nosocomial prevalence of antibiotic resistance have been evaluated in both outbreak and nonoutbreak periods. These strategies almost always include the implementation of a combination of measures that ultimately hampers evaluation of the effect of individual interventions. Moreover, such interventions are almost always evaluated in quasi-experimental study designs (i.e. before-after studies), which increase the risk of confounding. Confounders are those variables that may affect the outcome in the same manner as the variable subject to intervention. The optimal approach to evaluation of the efficacy of a single intervention is to minimize confounding either by performing a controlled, randomized trial or by quantifying potential confounders with subsequent adjustments in statistical analysis. Considering the dynamics of antibiotic resistance epidemiology, introduction of resistance, antibiotic pressure, and infection-control measures are all potential confounders.

Introduction of resistance. Introduction of resistance can be determined by obtaining samples for culture at admission. Ideally, cultures should be performed as soon as possible, because acquisition of drug-resistant pathogens may occur within several hours. Nosocomial infections have been defined as those diagnosed >48 h after hospital admission—thereby assuming that onset of disease occurred during patient hospitalization [6, 7]. In many studies, a similar time window has been used to define nosocomial acquisition of colonization. However, the optimal timing of culturing to distinguish between introduction of resistance and nosocomial acquisition has not been determined. Patient screening within 48 h of admission probably yields the majority of patients that introduce resistant pathogens. For pathogens that persist until ICU discharge, screening both at admission and at discharge would yield all information necessary to determine acquisition rates. More culture moments per patient are needed when colonization can be eradicated or when daily endemic prevalence is used as an end point.

Antibiotic use. Different units of measurement are used to express antibiotic use: proportion of patients receiving a specific (class of) antibiotic, antibiotics used as a proportion of total antibiotic use, the amount of antibiotics given (e.g., in grams or defined daily dosage), and duration of antibiotic use (in days). Integration of time, preferably in a patient-specific manner such as the number of patient-days, provides more information. To maximize comparison of patient exposure, the World Health Organization has proposed and defined the defined daily dosage methodology [8].

Cross-transmission. Dissection of the process leading to cross-transmission, typically through vectors (such as contaminated hands), identifies a multistep process with complex interaction between all determinants. The first step is for a health care worker to contact a colonized patient, which leads to contamination of the health care worker's hands for some time. Finally, the health care worker with contaminated hands must contact another, noncolonized patient, before disinfection of the hands has occurred. The success of this chain of events depends on cohorting level of nursing staff, contact rates, and adherence to hand hygiene [1].

Cohorting has been expressed as the likelihood that, after a first patient contact, the next patient contact will be with the same patient [1, 9]. Transmission is not possible with complete cohorting, as this indicates a patient/nurse ratio of 1 and that no other patients than the assigned are contacted (assuming that environmental contamination is not relevant). Thus, cohorting levels are, to a large extent, influenced by patient-staffing ratios. Understaffing has been associated with higher contact rates for nurses [10, 11], higher nursing workload, lower cohorting levels and lower hand hygiene adherence [9, 11–15]. The likelihood of hand contamination is further influenced by duration of patient care, types of body secretions handled [10],

skin diseases (such as eczema [16]), or the wearing of rings or artificial nails [17]. In fact, understaffing and/or overcrowding has been associated with increased risk of catheter-related bloodstream infections [18] and spread of methicillin-resistant *Staphylococcus aureus*, other *S. aureus* [4, 14, 15, 19], and *Enterobacter cloacae* [13].

Cohorting levels and adherence to hand hygiene are important variables in cross-transmission. Lapses in adherence to hand hygiene can be compensated for through an increase in the cohorting level, and vice versa [9]. Therefore, cohorting levels and adherence to hand hygiene, as potential confounders, should be determined in intervention studies. Cohorting levels and hand hygiene can be measured by observational studies [4, 9, 12], but these are labor-intensive and always include the risk of unintentional change in behavior of health care workers as a reaction to observation (the so-called Hawthorne effect) [20]. Instead, staffing-patient ratios and contact rates are relatively easy to record and can be used as surrogate markers for cohorting [4, 9]. Intuitively, workload assessment can be a surrogate marker for contact rates and hand hygiene adherence, but quantification of this relationship is complex. Several nursing workload measurement systems are available [21–24]; however, there is little evidence that changes in workload correlate to frequency of cross-transmission [25]. In our own experience, the Therapeutic Intervention Severity Score–28 workload assessment method is poorly associated with contact rates and adherence to hand hygiene [26].

Endogenous and exogenous acquisition. The relative importance of exogenous and endogenous acquisition of colonization should be optimally determined before implementation of interventions. For instance, enforcing adherence to hand hygiene might be of limited value when the vast majority of acquisitions result from endogenous selection, or when admission of colonized patients is the dominant variable that determines prevalence. Genotyping of bacterial isolates from different patients is currenly the most accurate method to quantify cross-transmission rates. However, it is a time-consuming, labor-intensive, and costly method, and the diagnostic delay inherent to conventional culturing and genotyping methods precludes real-time determination of resistance epidemiology. Rapid diagnostic and genotyping methodology and application of mathematical algorithms may facilitate real-time monitoring of the relative importance of different transmission routes in the future, which would improve the ability to design targeted infection-control strategies [27, 28].

Statistical evaluation. Finally, accurate evaluation of interventions requires the application of appropriate statistical methods. The relevance of colonization pressure in antibiotic resistance epidemiology has been unequivocally demonstrated [2, 29, 30] and results from patient-dependency, a fundamental aspect of infectious diseases. Among other variables, cross-transmission depends on the number of other colonized patients: with high endemic prevalence, risk of cross-transmission will be higher than with low prevalence, and vice versa (i.e., autocorrelation). Moreover, a period of high endemic prevalence and a high incidence of cross-transmission is likely to be followed by a period of lower endemic prevalence, because of chance events (i.e. regression to the mean). In contrast, because endogenous selection of resistance is not influenced by the colonization status of other patients, patient-dependency is irrelevant, and autocorrelation and regression to the mean is less important. As a consequence, patients cannot be considered to be independent from each other when cross-transmission is relevant; however, most statistical analyses explicitly assume independency of observations (e.g., Student's t test, Mann-Whitney U test, Fisher's exact test, and χ^2 test); as a matter of fact, the use of these statistical tests may lead to erroneous interpretations. As an example, we have performed 10,000 Monte Carlo simulations of the dynamics of a resistant pathogen in a 10-bed ICU, where 80% of acquisitions occur through cross-transmission, during a 1-year period (figure 2). If we assume that an intervention was implemented after 6 months (before-after study), without any effect on cross-transmission, $\leqslant$30% of statistical comparisons (using χ^2 or Student's t test) would reveal a statistically significant difference ($P < .05$) in antibiotic resistance acquisition and/or prevalence between both periods. When cross-transmission is considered to be a relevant transmission route, patient-dependency should not be neglected. In such circumstances, the use of classic statistical analyses will overestimate the likelihood that an intervention is effective; regression analyses or time-series analyses, adequately adjusting for relevant confounders, should be used. The likelihood of false statistical interpretation decreases with a di-

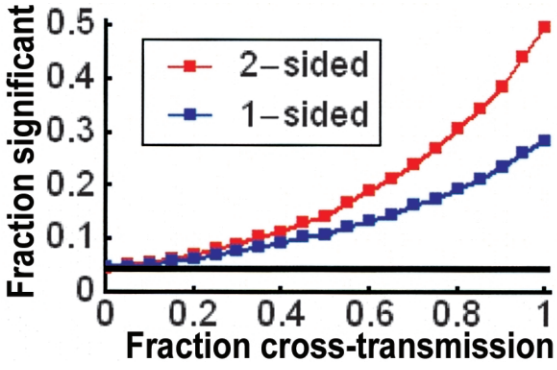

Figure 2. A fraction of the simulations with a statistically significant result (determined with the χ^2-test; $P < .05$) as a function of the relative importance of cross-transmission. The red and blue lines correspond with 2-sided and 1-sided tests, respectively.

Table 1. Studies of the effects of antibiotic prescription change, reviewed for control of confounding.

Scored item	Study					
	[32]	[33]	[42]	[34]	[36]	[37]
Study design	Before-after study	Before-after study	Retrospective and prospective data analyses	Before-after study	Prospective cross-over study	Before-after study
Pathogen of interest	GNR	GNR	GNR	GNR	GNR	GNR
Intervention	Substitution: GEN by AMK	Substitution: GEN/TOB/NET by AMK	Substitution: AMP/GEN by CXM/GEN	Restriction: CAZ	Comparison: PEN/TOB vs. AMX/CTX	Rotation: FEP, TZP, IPM, TIM
Baseline	Yes	No	Yes, although retrospective	Yes	Not applicable	Yes
End point of analysis	Colonization rates	Infection rates	Colonization and infection rates	Colonization rates	Colonization rates	Infection rates
Genotyping of isolates	Not performed	Not performed	Yes	Yes	Not performed	Not performed
Antibiotic use	Not analyzed	Percentage of patients receiving antibiotics	Not analyzed	No. of doses/month	Percentage of days of antibiotic use	Antibiotic-days
Control of confounders						
Introduction of antibiotic resistance	Analyzed	Not analyzed	Analyzed	Analyzed	Analyzed	Not analyzed
Infection-control practices	Not analyzed	Not analyzed	Not analyzed	Not analyzed	Not analyzed	Not analyzed
Conclusion	Reduction of GEN-resistant GNR; no emergence of AMK-resistant GNR	Increase in resistance to AMK/NET/TOB	Reduction in AMP- and CXM-resistant GNR	No reduction in antibiotic-resistant GNR	Reduction of resistance with PEN/TOB	Reduction of antibiotic resistance

NOTE. AMC, amoxicillin/clavulanic acid; AMK, amikacin; AMP, ampicillin; AMX, amoxicillin; CAZ, ceftazidime; CEP, cephalosporins; CIP, ciprofloxacin; CLI, clindamycin; CPI, cefpirom; CRO, ceftriaxone; CTX, cefotaxime; CXM, cefuroxim; DDD, defined daily dose; ESBL, extended-spectrum β-lactamase; FEP, cefepime; FQ, fluoroquinolones; GEN, gentamicin; GNR, gram-negative rods; GPC, gram-positive cocci; ICU, intensive care unit; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; MRSA, methicillin-resistant *Staphylococcus aureus;* NET, netilmycin; NICU, neonatal intensive care unit; PEN, penicillin; TIM, ticarcillin/clavulanic acid; TMP-SMZ, trimethoprim/sulfamethoxazole; TOB, tobramycin; TZP, piperacillin/tazobactam; VRE, vancomycin-resistant enterococci.

[a] Surveillance team and alcohol dispensers implemented.

[b] Barrier precautions implemented because of resistance to protocolized drugs.

[c] Educational campaign to prevent VAP resulted in 73% reduction of VAP.

[d] Infection control changed because of outbreak of *Acinetobacter* infection.

minishing relative importance of endogenous acquisition (lowering the relevance of patient-dependency) [31].

EVALUATION OF POTENTIAL CONFOUNDING IN STUDIES CHANGING ANTIBIOTIC PRESCRIPTION

To evaluate to what extent confounding has been controlled for in intervention trials, we systematically reviewed studies in which the effects of changes in antibiotic prescription on colonization prevalence of antibiotic-resistant bacteria and/or infection with antibiotic-resistant bacteria in ICUs were evaluated.

We searched the PubMed database using the following search terms (first as individual terms and subsequently in a combined approach): "antibiotic" or "antimicrobial," "resistance," "ICU," "intensive care," "colonization," and "infection." Selection criteria included antibiotic-resistant bacteria of any kind, interventions targeting antibiotic use to reduce prevalence and/or incidence of antibiotic-resistant bacteria (restriction of specific classes or individual antibiotics, rotation and/or cycling), and nonoutbreak settings. Reports in languages other than English and reviews were excluded.

This search yielded 1017 articles, which were first screened by title and abstract to determine whether selection criteria were indeed met. Studies only published as abstracts or that were published without abstracts were excluded. Ultimately, only 19 studies, performed between 1984 and 2006, met the criteria mentioned above and were reviewed (table 1).

Table 1. ***(Continued.)***

Study						
[43]	[47]	[30]	[46]	[49]	[35]	[41]
Before-after study	Before-after study	Before-after study	Before-after study	Before-after study	Controlled study of 2 NICU populations	Before-after study
GNR	GNR and GPC	VRE	GNR and GPC	GNR	GNR	GNR
Substitution: CAZ by TZP	Rotation: nonprotocolized vs. protocolized empirical therapy	Substitution: CAZ by CIP	Substitution: AMC by TMP–SMZ and TZP by IPM	Cycling: IPM, TZP, CAZ, and CLI (later changed to FEP)	Rotation: GEN, TZP, and CAZ vs. unrestricted use	Restriction: third-generation CEP
Yes	Yes	Yes	Yes	No	Not applicable	Yes
Colonization rates	Infection rates	Colonization rates	Infection rates	Colonization and infection rates	Colonization rates	Infection rates
Not performed	Not performed	Not performed	Not performed	Not performed	Yes	Not performed
Grams of antibiotics used	Antibiotic courses/100 ICU admissions	Percentage of patients receiving specific antibiotic; mean duration of antibiotic therapy	Antibiotic courses/100 enrolled patients	Percentage of total antibiotic use	Antibiotic-days	Grams/month
Analyzed	Not analyzed	Analyzed	Not analyzed	Analyzed	Not analyzed	Not analyzed
Not analyzed	Not analyzed[a]	Not analyzed	Not analyzed	Not analyzed	Not analyzed	Not analyzed
Reduction in CAZ-resistant isolates and colonization and infection with ESBL-producing *Klebsiella pneumoniae/Escherichia coli*	Reduction of GNR and GPC antibiotic-resistant infections	No effect on VRE acquisition	Reduction MRSA of TZP-resistant *Pseudomonas;* increase of IPM-resistant *Pseudomonas*	No effect on resistance rates	No effect on antibiotic-resistant GNR	Reduction of resistance rates GNB to third-generation CEP

These articles were reviewed for the following items: pathogen of interest, type of intervention, study design, presence of a baseline period (if applicable), chosen end points, determination of the route of acquisition, antibiotic use, determination of potential confounders (introduction of resistance and infection-control practices), and interpretation of results (table 1).

Study design, baseline period, and intervention period. Fourteen studies focussed on gram-negative bacteria [32–44], 2 studies addressed vancomycin-resistant enterococci [30, 45], and 3 studies evaluated all types of antibiotic-resistant pathogens [46–48]. The earliest studies addressed aminoglycoside resistance and evaluated the effects of using another aminoglycoside for which resistance did not exist [32, 33].

The interventions tested were antimicrobial cycling with recurrence of the initial regimen (A-B-A) ($n = 2$) [39, 49], antimicrobial rotation with different antibiotic regimens (A-B-C-D) ($n = 4$) [35, 37, 38, 47], antimicrobial rotation per month versus per consecutive patient ($n = 1$) [44], and antimicrobial restriction or substitution ($n = 12$) [30, 32–34, 36, 40–43, 45, 46, 48].

Sixteen studies had a prospective cohort design executed within a single unit, 2 had a prospective crossover design in 2 wards, and 1 had a controlled design in 2 neonatal ICU populations. In 12 of 16 studies that did not have a simultaneously studied control group, end points and potential confounders were first determined during a baseline period and then, with identical methodology, during the intervention period (before-after studies). Cohort studies with different antibiotic policies (B-C-D) compared with a standard policy (A) were considered to be before-after studies if separate analyses were not performed between cycles B, C, and D.

End points of analysis. Colonization rate was the primary end point in 10 studies [30, 32, 34–36, 39, 40, 42–44], 6 studies used antimicrobial susceptibility rates of clinical isolates (and, therefore, determined infection rates) [33, 37, 41, 46–48], and both colonization and infection rates were used as end points in 4 studies [30, 38, 44, 49].

Relative importance of acquisition routes. The relative importance of different acquisition routes was not determined in any study, although some information about transmission dynamics was provided by genotyping of selected isolates in 4 studies. This revealed the polyclonal presence of cefuroxime-resistant gram-negative bacteria ($n = 1$) [42], monoclonal epidemics of ceftazidime-resistant gram-negative bacteria ($n =$

Table 1. ***(Continued.)***

Study					
[48]	[40]	[39]	[38]	[45]	[44]
Before-after study	Before-after study	Prospective cohort study	Prospective cohort study	Before-after study	Prospective cross-over study
MRSA + GNR	GNR	GNR	GNR	VRE	GNR
Substitution: nonprotocolized vs. protocolized antibiotics	Substitution: nonprotocolized antibiotics vs. FEP	Cycling: LVX, CPI, LVX, and TZP	Rotation: FEP, FQ, IPM, and TZP	Substitution: TIM by TZP	Rotation per month vs. rotation per consecutive patient of FEP/CAZ, CIP, MEM/IPM, and TZP
Yes	Yes	No	Yes	Yes	Not applicable
Infection rates	Colonization rates	Colonization rates	Colonization and infection rates	Colonization and infection rates	Colonization and infection rates
Not performed	Not performed	Yes	Not performed	Not performed	Not performed
Antibiotic-days/1000 days ICU presence	Antibiotic-days	DDD/1000 patient-days	Percentage of patients receiving specific antibiotic	DDD/1000 patient-days	Percentage of patients receiving specific antibiotic; DDD/100 patient-days
Not analyzed	Analyzed	Analyzed	Analyzed	Not analyzed	Analyzed
Not analyzed	Not analyzed	Not analyzed[b]	Not analyzed[c]	Not analyzed	Not analyzed[d]
Reduction of MRSA and CRO-resistant GNR	Reduction in antibiotic-resistant GNR	Increase resistance during LVX and TZP	No effect on resistance rates	Reduction in VRE acquisition	Reduction of FEP-resistant *P. aeruginosa* during cycling

1) [34], low cross-transmission rates of ceftazidime-resistant gram-negative bacteria ($n = 1$) [35] and the monoclonal spread of fluoroquinolone-resistant pathogens in combination with the monoclonal and polyclonal spread of β-lactam–resistant pathogens ($n = 1$) [39].

Antibiotic use. Antibiotic use—the subject of intervention in each study—was analyzed in 16 of 18 studies. There is, however, no uniform measure of antibiotic use, and time components were also variably used. Six of the reviewed studies expressed use of a specific antibiotic as a percentage of total antibiotic use ($n = 1$) [49], as a percentage of patients receiving antibiotics ($n = 2$) [30, 33, 38], as the number of courses per 100 enrolled patients or ICU admissions ($n = 2$) [46, 47], or as the total grams of antibiotics used ($n = 1$) [43]—thus, none used time in the denominator. Eleven studies integrated time in the expression of antibiotic use, either as number of doses or grams/month ($n = 2$) [34,41]), as antibiotic-days ($n = 4$) [30, 35, 37, 40], as percentages of ICU-days ($n = 2$) [36,48], or as defined daily doses/1000 patient-days ($n = 3$) [39, 44, 45]. Two studies expressed antibiotic use with and without time components [30, 44].

Control of potential confounders. Introduction of resistance, defined as carriage of drug-resistant bacteria within the first 48 h of hospital admission, was measured in 10 studies [30, 32, 34, 36, 38–40, 43, 44, 49]. Neither adherence to hand disinfection nor cohorting levels, staffing levels, or workload were determined in any of these 18 studies. In 5 studies, infection control strategies were implemented or enhanced while interventions were ongoing [38–40, 44, 47].

Interpretation of efficacy. In 12 studies, authors concluded that infection-control intervention was effective in reducing the prevalence of resistance, either with or without increased resistance in other pathogens [32, 36, 37, 40–48]. Others concluded that their studied intervention had no effect on antibiotic resistance ($n = 6$) [30, 34, 35, 38, 39, 49] and/or had an opposite effect on antibiotic resistance ($n = 1$) [33]. All studies used standard statistics (i.e., Student's t test, χ^2 test, or Fisher's exact test) for data analysis.

DISCUSSION

Colonization dynamics of drug-resistant pathogens in small hospital settings, such as ICUs, are complex, with multiple relevant and interacting variables. Therefore, there is a high risk

Table 2. Points to reduce confounding in intervention trials addressing antibiotic use in intensive care units.

Optimal study design
Cluster-randomized
Quasi experimental (see [50])
Optimal end point
Colonization rates
Infection rates
Determination of relative importance of different acquisition routes (cross-transmission, endogenous selection, and/or de novo resistance development)
Quantification (or control) of confounders, such as:
Patient characteristics
Introduction of antibiotic resistance
Infection control variables (hand hygiene, contact rates, cohorting levels, patient-staff ratios)
Antibiotic use

of confounding in intervention studies that may seriously hamper the interpretation of study results. For example, 19 studies of antibiotic interventions (selected using specified search criteria) were systematically reviewed to determine to what extent potential confounding had been controlled for.

In all studies, potential confounders (introduction of resistance, infection control practices) either were not measured or changed during the study period. Therefore, it remains uncertain whether observed changes in antibiotic resistance prevalence after intervention were causally related to the intervention. Moreover, even absence of efficacy could have resulted from opposing effects due to confounding, despite an effective intervention.

We, therefore, propose the following 4 points, to reduce confounding in intervention trials addressing antibiotic resistance in ICUs (table 2): first, the optimal study design would be a randomized, controlled trial, with each participating ward as a unit of study (i.e., cluster-randomized trial). Needless to say, such a trial will be expensive. A quasi-experimental design (such as a before-after study) might be an alternative, as long as results are interpreted carefully; such a design inherently increases the likelihood of confounding, regression to the mean, and maturation effects [50]. To provide a greater internal validity, and potential causation between intervention and outcome, Harris et al. [51] proposed a hierarchy in quasi-experimental designs, reflecting designs that do or do not include control groups. Second, colonization rates are preferred over infection rates, because the latter only represent the tip of the iceberg. Third, determination of the relative importance of acquisition routes is indispensable to optimally target interventions and to choose the appropriate statistical methods for analysis. Genotyping of colonizing isolates, in combination with epidemiological linkage, is still the standard method to distinguish between exogenous and endogenous colonization events. As mentioned, these methods are labor-intensive and, therefore, hardly feasible in daily practice outside dedicated research settings. Recently, mathematical models have been proposed as alternatives for determining transmission parameters on the basis of longitudinal prevalence data [4, 27, 52]. With these so-called Markov chain models, the relative importance of either cross-transmission or the endogenous route can be determined upon observation of fluctuations in prevalence. In addition, rapid molecular tests will be available in the near future, which will also enhance abilities to better understand the epidemiology of antibiotic resistance. Importantly, standard statistical tests—all assuming independency of observations—are only valid when patient-dependency is not relevant. When cross-transmission is important, these tests should be interpreted with care, because inflated rates of type I errors are likely to occur [28]. Again, recent developments in biostatistical analyses may offer better alternatives for the future [27, 52]. Finally, potential confounders should be quantified and included in the final analyses.

Antibiotic resistance will remain a relevant problem in ICUs in the coming decades; with no new antibiotic classes on the horizon, the minimization of further emergence of resistance is of utmost importance. Characteristics of ICU populations and of colonization dynamics enhance the risk of confounding when analyzing control measures. The measures proposed in this review will reduce the risk of false interpretation of study results.

Acknowledgments

Potential conflicts of interest. All authors: no conflicts.

References

1. Austin DJ, Bonten MJ, Weinstein RA, Slaughter S, Anderson RM. Vancomycin-resistant enterococci in intensive-care hospital settings: transmission dynamics, persistence, and the impact of infection control programs. Proc Natl Acad Sci U S A **1999**; 96:6908–13.
2. Bonten MJ, Slaughter S, Ambergen AW, et al. The role of "colonization pressure" in the spread of vancomycin-resistant enterococci: an important infection control variable. Arch Intern Med **1998**; 158:1127–32.
3. Lipsitch M, Samore MH. Antimicrobial use and antimicrobial resistance: a population perspective. Emerg Infect Dis **2002**; 8:347–54.
4. Grundmann H, Hori S, Winter B, Tami A, Austin DJ. Risk factors for the transmission of methicillin-resistant *Staphylococcus aureus* in an adult intensive care unit: fitting a model to the data. J Infect Dis **2002**; 185:481–8.
5. Bonten MJ, Bergmans DC, Speijer H, Stobberingh EE. Characteristics of polyclonal endemicity of *Pseudomonas aeruginosa* colonization in intensive care units: implications for infection control. Am J Respir Crit Care Med **1999**; 160:1212–9.
6. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control **1988**; 16: 128–40.
7. The Society for Hospital Epidemiology of America, the Association for Practitioners in Infection Control, the Centers for Disease Control, the Surgical Infection Society. Consensus paper on the surveillance of surgical wound infections. Infect Control Hosp Epidemiol **1992**; 13: 599–605.

8. World Health Organization. WHO drug information. Geneva: World Health Organization, **2001**.
9. Nijssen S, Bonten MJ, Franklin C, Verhoef J, Hoepelman AI, Weinstein RA. Relative risk of physicians and nurses to transmit pathogens in a medical intensive care unit. Arch Intern Med **2003**; 163:2785–6.
10. Pittet D, Mourouga P, Perneger TV. Compliance with handwashing in a teaching hospital: infection control program. Ann Intern Med **1999**; 130:126–30.
11. Gould D. Systematic observation of hand decontamination. Nurs Stand **2004**; 18:39–44.
12. Pittet D, Dharan S, Touveneau S, Sauvan V, Perneger TV. Bacterial contamination of the hands of hospital staff during routine patient care. Arch Intern Med **1999**; 159:821–6.
13. Harbarth S, Sudre P, Dharan S, Cadenas M, Pittet D. Outbreak of *Enterobacter cloacae* related to understaffing, overcrowding, and poor hygiene practices. Infect Control Hosp Epidemiol **1999**; 20:598–603.
14. Haley RW, Cushion NB, Tenover FC, et al. Eradication of endemic methicillin-resistant *Staphylococcus aureus* infections from a neonatal intensive care unit. J Infect Dis **1995**; 171:614–24.
15. Vicca AF. Nursing staff workload as a determinant of methicillin-resistant *Staphylococcus aureus* spread in an adult intensive therapy unit. J Hosp Infect **1999**; 43:109–13.
16. Larson EL, Hughes CA, Pyrek JD, Sparks SM, Cagatay EU, Bartkus JM. Changes in bacterial flora associated with skin damage on hands of health care personnel. Am J Infect Control **1998**; 26:513–21.
17. Trick WE, Vernon MO, Hayes RA, et al. Impact of ring wearing on hand contamination and comparison of hand hygiene agents in a hospital. Clin Infect Dis **2003**; 36:1383–90.
18. Fridkin SK, Pear SM, Williamson TH, Galgiani JN, Jarvis WR. The role of understaffing in central venous catheter-associated bloodstream infections. Infect Control Hosp Epidemiol **1996**; 17:150–8.
19. Andersen BM, Lindemann R, Bergh K, et al. Spread of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. J Hosp Infect **2002**; 50:18–24.
20. Roethlisberger FH, Dickson WJ. Management and the worker. Cambridge, MA: Harvard University Press, **1939**.
21. Reis, Miranda D, Moreno R, Iapichino G. Nine equivalents of nursing manpower use score (NEMS). Intensive Care Med **1997**; 23:760–5.
22. Miranda DR, de Rijk A, Schaufeli W. Simplified therapeutic intervention scoring system: the TISS-28 items—results from a multicenter study. Crit Care Med **1996**; 24:64–73.
23. Urbanowicz JA. An evaluation of an acuity system as it applies to a cardiac catheterization laboratory. Comput Nurs **1999**; 17:129–34.
24. Trundle CM, Farrington M, Anderson L, Redpath CK. GRASPing infection: a workload measurement tool for infection control nurses. J Hosp Infect **2001**; 49:215–21.
25. Soulier A, Barbut F, Ollivier JM, Petit JC, Lienhart A. Decreased transmission of *Enterobacteriaceae* with extended-spectrum beta-lactamases in an intensive care unit by nursing reorganization. J Hosp Infect **1995**; 31:89–97.
26. Nijssen S, Bonten M, Hoepelman IM. Can the therapeutic intervention severity score_28 (TISS_28) be used as a tool to determine contact rates between healthcare workers and ICU-patients [abstract K-1357]? In: Program and abstracts of the 42nd International Conference on Antimicrobial Agents and Chemotherapy (San Diego). **2002**.
27. Pelupessy, Bonten, Diekmann. How to assess the relative importance of different colonization routes of pathogens within hospital settings. PNAS **2002**; 99:5601–6.
28. Cooper BS, Stone SP, Kibbler CC, et al. Isolation measures in the hospital management of methicillin resistant *Staphylococcus aureus* (MRSA): systematic review of the literature. BMJ **2004**; 329:533.
29. Merrer J, Santoli F, Appere de Vecchi C, Tran B, De Jonghe B, Outin H. "Colonization pressure" and risk of aquisition of methicillin-resistant *Staphylococcus aureus* in a medical intensive care unit. Infect Control Hosp Epidemiol **2000**; 21:718–23.
30. Puzniak LA, Mayfield J, Leet T, Kollef M, Mundy LM. Acquisition of vancomycin-resistant enterococci during scheduled antimicrobial rotation in an intensive care unit. Clin Infect Dis **2001**; 33:151–7.
31. Bootsma MCJ, Diekmann O, Nijssen S, Bonten MJM. Estimating transmission parameters by direct likelihood computation [PhD thesis]. Utrecht, The Netherlands: University of Utrecht, **2005**.
32. Raz R, Sharir R, Shmilowitz L, Kenes I, Ephros M. The elimination of gentamicin-resistant gram-negative bacteria in a newborn intensive care unit. Infection **1987**; 15:32–4.
33. Hammond JM, Potgieter PD, Forder AA, Plumb H. Influence of amikacin as the primary aminoglycoside on bacterial isolates in the intensive care unit. Crit Care Med **1990**; 18:607–10.
34. Toltzis P, Yamashita T, Vilt L, et al. Antibiotic restriction does not alter endemic colonization with resistant gram-negative rods in a pediatric intensive care unit. Crit Care Med **1998**; 26:1893–9.
35. Toltzis P, Dul MJ, Hoyen C, et al. The effect of antibiotic rotation on colonization with antibiotic-resistant bacilli in a neonatal intensive care unit. Pediatrics **2002**; 110:707–11.
36. de Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. Lancet **2000**; 355:973–8.
37. Gruson D, Hilbert G, Vargas F, et al. Rotation and restricted use of antibiotics in a medical intensive care unit: impact on the incidence of ventilator-associated pneumonia caused by antibiotic-resistant gram-negative bacteria. Am J Respir Crit Care Med **2000**; 162:837–43.
38. Warren DK, Hill HA, Merz LR, et al. Cycling empirical antimicrobial agents to prevent emergence of antimicrobial-resistant gram-negative bacteria among intensive care unit patients. Crit Care Med **2004**; 32: 2450–6.
39. van Loon HJ, Vriens MR, Fluit AC, et al. Antibiotic rotation and development of gram-negative antibiotic resistance. Am J Respir Crit Care Med **2004**; 171:480–7.
40. Toltzis P, Dul M, O'Riordan MA, et al. Cefepime use in a pediatric intensive care unit reduces colonization with resistant bacilli. Pediatr Infect Dis J **2003**; 22:109–14.
41. Du B, Chen D, Liu D, et al. Restriction of third-generation cephalosporin use decreases infection-related mortality. Crit Care Med **2003**;31: 1088–93.
42. Kalenic S, Francetic I, Polak J, Zele-Starcevic L, Bencic Z. Impact of ampicillin and cefuroxime on bacterial colonization and infection in patients on a neonatal intensive care unit. J Hosp Infect **1993**; 23:35–41.
43. Lan CK, Hsueh PR, Wong WW, et al. Association of antibiotic utilization measures and reduced incidence of infections with extended-spectrum beta-lactamase-producing organisms. J Microbiol Immunol Infect **2003**; 36:182–6.
44. Martinez JA, Nicolas JM, Marco F, et al. Comparison of antimicrobial cycling and mixing strategies in two medical intensive care units. Crit Care Med **2006**; 34:329–36.
45. Winston LG, Charlebois ED, Pang S, Bangsberg DR, Perdreau-Remington F, Chambers HF. Impact of a formulary switch from ticarcillin-clavulanate to piperacillin-tazobactam on colonization with vancomycin-resistant enterococci. Am J Infect Control **2004**; 32:462–9.
46. Allegranzi B, Luzzati R, Luzzani A, et al. Impact of antibiotic changes in empirical therapy on antimicrobial resistance in intensive care unit-acquired infections. J Hosp Infect **2002**; 52:136–40.
47. Raymond DP, Pelletier SJ, Crabtree TD, et al. Impact of a rotating empiric antibiotic schedule on infectious mortality in an intensive care unit. Crit Care Med **2001**; 29:1101–8.
48. Geissler A, Gerbeaux P, Granier I, Blanc P, Facon K, Durand-Gasselin J. Rational use of antibiotics in the intensive care unit: impact on microbial resistance and costs. Intensive Care Med **2003**; 29:49–54.
49. Moss WJ, Beers MC, Johnson E, et al. Pilot study of antibiotic cycling in a pediatric intensive care unit. Crit Care Med **2002**; 30:1877–82.
50. Harris AD, Lautenbach E, Perencevich E. A systematic review of quasi-experimental study designs in the fields of infection control and antibiotic resistance. Clin Infect Dis **2005**; 41:77–82.
51. Harris AD, Bradham DD, Baumgarten M, Zuckerman IH, Fink JC, Perencevich EN. The use and interpretation of quasi-experimental studies in infectious diseases. Clin Infect Dis **2004**; 38:1586–91.
52. Cooper B, Lipsitch M. The analysis of hospital infection data using hidden Markov models. Biostatistics **2004**; 5:223–37.