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Selection of resistance during sequential use of preferential antibiotic classes

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Abstract *Objective:* To determine the effect of antibiotic class pressure on the susceptibility of bacteria during sequential periods of antibiotic homogeneity. *Design and setting:* Prospective study in a mixed ICU with three separated subunits of eight, eight, and ten beds. *Patients and participants:* The study examined the 1,721 patients with a length of stay longer than 2 days. *Interventions:* Three different antibiotic regimens were used sequentially over 2 years as first-choice empirical treatment: cephalosporins, fluoroquinolone, or a penicillin–β-lactamase inhibitor combination. Each regimen was applied for 8 months in each subunits of the ICU, using "latin square" design. *Results:* We treated 731 infections in 546 patients (32% of patients staying more than 48 h). There were 25.5 ICU-acquired infections per 1,000 patient-days. Infecting pathogens and colonizing bacteria were found in 2,739 samples from 1,666 patients (96.8%). No significant change in global antibiotic susceptibility was observed over time. However, a decrease in the susceptibility of several species was observed for antibiotics used as the first-line therapy in the unit. Selection pressure of antibiotics and occurrence of resistance during treatment was documented within an 8-month rotation period. *Conclusions:* Antibiotic use for periods of several months induces bacterial resistance in common pathogens.

Keywords Antibiotic pressure · Bacterial resistance · Intensive care unit

Introduction

Intensive care unit (ICU) patients are unfortunately liable to develop infections because of their underlying disease(s) and numerous invasive procedures [1]. The wide use of antibiotic agents to prevent or treat infections may induce the emergence of resistance within the micro-organisms [2]. Consequently it is important to reduce the selection pressure that antibiotics exert on the microflora. Several strategies have been proposed: bitherapy instead of monotherapy, restricted use of certain broad-spectrum molecules, narrowing the activity spectrum when the pathogens are identified, automatic stop orders from the pharmacist to reduce the inappropriate duration of treatment, strict guidelines for the use of antibiotics in hospital, supervision of therapeutic decision making by a specialist in infectious disease or in "antibiology" [3, 4, 5, 6, 7, 8, 9, 10]. Recently the rotation of antibiotics or classes of antibiotics has been added to these strategies to further reduce the selection pressure [11, 12, 13, 14]. It is hoped that bacteria lose their resistance to particular molecules when exposure ends or even do not acquire resistance within the rotation cycle because the selection pressure is too short. Moreover, bacteria resistant to a particular regimen would be eradicated by the next one.

Several studies have compared "before" and "after" periods following the rotation of antibiotics [15, 16, 17, 18, 19]. These studies have not directly investigated the course of antibiotic susceptibility. A major problem is determining an appropriate control group. How can the

Table 1 Characteristics of the patients staying more than 2 days, according to the empirical regimen recommended. Number of trauma + medical + surgical patients=100% (*LOS* length of stay, *IQR* interquartile range)

	Quinolones	Penicillins	Cephalosporins
Patients with hospital LOS >2 days	572	585	564
Age (years)	61.4±16.3	62.4±15.3	60.2±17.4
ICU LOS (days)	9.8±11.9	9.5±14.9	10.0±13.7
Hospital LOS before ICU, median (IQR)	2 (0–3)	2 (0–4)	2 (1–4)
APACHE II score	15.1±6.5	15.2±6.5	16.0±6.5
Hospital mortality	18.8%	15.5%	20.7%
Trauma	64 (11.2%)	47 (8.0%)	73 (12.9%)
Medical patients	161 (28.1%)	183 (31.3%)	165 (29.2%)
Cardiology	42 (7.3%)	44 (7.5%)	51 (9.0%)
Neurology	42 (7.3%)	41 (7.0%)	35 (6.2%)
Pneumology	44 (7.7%)	56 (9.6%)	44 (7.8%)
Hematology	4 (0.7%)	3 (0.5%)	5 (0.9%)
Other medical patients	29 (5.1%)	39 (6.7%)	30 (5.3%)
Surgery patients	347 (60.7%)	355 (60.7%)	326 (57.8%)
Coronary artery bypass	146 (25.6%)	152 (26.0%)	135 (23.9%)
Cardiac valves	89 (15.6%)	98 (16.8%)	75 (13.3%)
Neurosurgery	26 (4.5%)	21 (3.6%)	20 (3.5%)
Abdominal surgery	20 (3.5%)	23 (3.9%)	28 (5.0%)
Transplants	25 (4.4%)	29 (5.0%)	26 (4.6%)
Other surgical patients	41 (7.2%)	32 (5.5%)	42 (7.4%)

potential selection pressure of antibiotics be distinguished from the natural fluctuation of microbial flora constantly modified by the admission of new patients? The present study used a "Latin square" design to separate the effect of antibiotic selection pressure from the effect of time using three 8-month regimens over 2 years in three subunits of a general ICU. All classes were used during the 2-year study, but each could be empirically administered to only one-third of patients, each class being in use in one subunit alone at a time. This design allows concentration upon sequential use effect by a time analysis and for an antibiotic pressure effect by a regimen analysis.

Material and methods

The study was conducted between January 1998 and December 1999 in the 26-bed general ICU of the University Hospital of Liège, Belgium. The ICU has three separated subunits of eight, eight, and ten beds—units A, B, and C, respectively. These subunits have their own medical and nursing teams. Type of patients or pathologies do not differ between the three subunits. Three different regimens for empirical antibiotic treatment were used on a sequential basis in the three subunits for periods of 8 months; each regimen was in use in only one subunit at a given time. Over the 2-year study period the ICUs admitted 2,746 patients (18,529 patient-days), 1,721 of whom stayed for more than 2 days (16,803 patient-days). Table 1 shows the characteristics of the patients staying for more than 2 days and classified according to the empirical regimen recommended during the period of ICU stay. No differences were observed between the groups concerning age, sex, causes of admission, mean Acute Physiology and Chronic Health Evaluation (APACHE) II score at entry, length of stay, or hospital mortality. The study design was approved by the hospital ethics committee; because the different therapeutic regimens were part of standard treatment, and because the study was essentially epidemiological, informed consent was not required from patients or their families.

Antibiotic regimens

A protocol for empirical antibiotic therapy was written which defined prospectively the type of antibiotic to use for the most frequent infections acquired in the ICU, i.e., pneumonia, urinary tract infection, catheter-related infection, intra-abdominal infection, and surgical wound infection. These infections were defined according to the Centers for Diseases Control criteria. The empirical treatments were based either on cephalosporins (primarily ceftazidime or cefepime), penicillins associated with a β-lactamase inhibitor (amoxicillin-clavulanic acid or piperacillin-tazobactam), or fluoroquinolones (ciprofloxacin or ofloxacin). Narrowing the spectrum was allowed when the susceptibility of the pathogens was known. There were no restrictions on the use of penicillin, flucloxacillin, first-generation cephalosporins, and imidazoles. Association with aminoglycosides was allowed in all regimens. Glycopeptides were used in the case of infection by methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant coagulase negative staphylococcus. Carbapenems were used in the case of resistance to the empirical regimen. In documented infections with a pathogen resistant to the recommended first-line regimen the physician could override the antibiotic recommendations of the study protocol. All antibiotic treatments were recorded, and the amounts of the prescribed and administered antibiotics were counted in terms of defined daily doses (DDD).

Microbiological surveillance

Routine samples of oropharyngeal secretions, sputum or endotracheal aspirates, and urine were obtained on admission and then twice per week. In addition, when infection was suspected, full set of samples were obtained, including cultures of blood and from the suspected focus of infection. ICU-acquired bacteria were defined as those not found on admission or within the first 3 days. Every episode of infection was recorded and classified as either a community-acquired or nosocomial infection (defined as occurring at least 2 days after hospital admission). Nosocomial infections were further separated into hospital or ICU acquired infections. Infection density was expressed as number of episodes per 1,000 patient-days. If a patient remained in the ICU during the transition between two periods of different antibiotic regimens, the duration of stay was attributed to the corresponding period to obtain the correct denominator. Identification of bacteria and testing of their antibi-

Table 2 Number of infected patients and of infectious episodes. Results were classified according to the empirical treatment used in the unit at this time

	Quinolones	Penicillins	Cephalosporins
Number of infected patients	186	159	201
Community-acquired infections	43	33	51
Nosocomial infections	53	55	68
ICU-acquired infections	147	128	153
Acquired infection density[a]	26.5	23	27
ICU acquired infections			
Respiratory	92	72	95
Intra-abdominal	5	7	7
Urinary tract	9	13	8
Catheter-related	6	11	5
Other	28	20	27
Indeterminate origin	7	5	11
Bacteremia	26	31	44

[a] Infection density is expressed per thousand patient-days

otic susceptibilities were performed by the Vitek 2 automated system. A total of 2,739 positive cultures were obtained from 1,666 patients (96.8% of those staying longer than 48 h). These cultures showed 648 micro-organisms present at admission and 1,195 acquired during the ICU stay.

All colonizing and infecting ICU-acquired bacteriological species were taken into account when sensitivity testing was performed. Because the study was not designed to study the efficacy of the antibiotic treatment, no distinction was made between infecting and colonizing bacteria. The Latin square design allows two analysis. First, the antibiotic susceptibility was analyzed according to time by globalizing all the bacteria of the three subunits. The second analysis compared the antibiotic susceptibility of the bacteria according to the empirical regimen used in the unit. To further describe the possible antibiotic selection pressure the three types of Gram-negative bacteria most often encountered were scrutinized: *Escherichia coli*, *Enterobacter* spp, *Pseudomonas aeruginosa*. Sensitivity was analyzed according to the antibiotic treatment with which they were treated.

Statistical analysis

Results are expressed as means and standard deviation for quantitative variables and as counts (percentages) for categorical variables. A logistic regression model was used to test for selection pressure and longitudinal effects. Differences were considered significant at the level of $p<0.05$.

Results

Infections

We treated a total of 731 infectious episodes in 546 patients (19.9% of admitted patients, 32% of patients staying more than 48 h): 127 community-acquired, 176 hospital-acquired, and 428 ICU-acquired. Table 2 summarizes the characteristics of these infections. ICU-acquired infections occurred at a rate of 25.5 episodes per 1,000 patient-days. Antibiotic therapy was considered to be adequate within the first day of treatment if bacteria were sensitive to at least one antibiotic in use. This was observed in 82.1% of the cases with no differences between regimens.

Global antibiotic susceptibilities

No significant change in antibiotic susceptibility was observed over time (Table 3) except the following: (a) the proportion of ICU-acquired MRSA increased in the three subunits during the second period, the same phenomenon being observed for MRSA on admission, and (b) expanded-spectrum β-lactamase producing *Klebsiella pneumoniae* were isolated in five patients in subunit A during the third period. Specifically, no changes were seen in the susceptibility of inducible Enterobacteriaceae and *P. aeruginosa*. Table 3 also shows the susceptibilities of micro-organisms on admission. They usually were equal or higher than the susceptibilities of ICU-acquired micro-organisms except for noninducible Enterobacteriaceae, which were less susceptible to amoxycillin–clavulanic acid combination.

When the micro-organisms were analyzed according to the empirical treatment in use in the unit where they were isolated, some significant differences appeared. As shown in Table 4, a statistically significant reduction in ciprofloxacin susceptibility was observed in *P. aeruginosa* when fluoroquinolones were used as empirical treatment. Table 4 also highlights the significant reduction in susceptibility to cefotaxime in inducible Enterobacteriaceae when cephalosporins were used. The same phenomenon was observed for piperacillin-tazobactam in inducible Enterobacteriaceae when penicillins were used empirically. A reduction in imipenem susceptibility was also observed in *P. aeruginosa* when fluoroquinolones were the first-line therapy. These observations were valid only for ICU-acquired micro-organisms.

Antibiotic consumption

During their first-choice use period the consumption of third- or fourth-generation cephalosporins reached a mean of 11.8 DDD/100 patient-days in the three subunits, that of β-lactamines–βlactamases inhibitor combination 20.9

Table 3 Antibiotic susceptibility (%) of specified groups of bacteria classified according to time (*Adm.* at admission, *ICU* ICU-acquired micro-organisms)

	Period 1		Period 2		Period 3	
	Adm.	ICU	Adm.	ICU	Adm.	ICU
Noninducible Enterobacteriaceae (*E. coli*, *Klebsiella* spp, *Proteus* spp)						
Number of strains	41	55	20	70	43	52
Amikacin	85.4	100	100	98.6	97.7	100
Ciprofloxacin	97.6	90.9	95	92.9	93	94.2
Cefotaxime	100	98	90	98.6	100	90.7
Imipenem	100	100	100	98.6	100	98
Piperacillin-tazobactam	82.9	90	90	88.6	83.7	82.7
Amoxicillin-clavulanic acid	61	83.6	55	77.1	55.8	81.1
Inducible Enterobacteriaceae (*Enterobacter* spp, *Serratia* spp, *Citrobacter* spp, *Morganella* spp)						
Number of strains	19	48	17	45	27	41
Amikacin	84.2	89.1	100	91.1	96.3	92.5
Ciprofloxacin	73.7	71.7	88.2	84.4	81.5	70.7
Cefepime	94.7	97.7	100	95.3	100	95.1
Imipenem	100	97.9	100	97.8	100	97.6
Cefotaxime	78.9	78.3	100	73.3	100	64.3*
Piperacillin-tazobactam	52.6	58.7	76.5	57.1	66.6	51.3
Pseudomonas aeruginosa						
Number of strains	3	56	0	57	13	43
Amikacin	66.6	78.2	–	93	84.6	97.8
Ciprofloxacin	100	49.1	–	52.8	46.1	48.8
Ceftazidime	100	71.4	–	73.7	92.3	79.1
Imipenem	66.6	71.4	–	63.1	100	65.1
Piperacillin-tazobactam	33.3	87.5	–	84.2	69.2	86
Staphylococcus aureus						
Number of strains	25	23	41	35	15	26
Oxaciclin	82.1	91.3	48.8	62.9*	86.7	84.6

*$p \leq 0.05$ between the level of sensitivity marked by an asterisk and the level without asterisk for a specified antibiotic for ICU-acquired micro-organisms (logistic regression)

DDD/100 patient-days, and that of fluoroquinolones 14.0 DDD/100 patient-days. Their uses when they were not the first-choice empirical treatment were, respectively, 3.2 DDD, 10.4 DDD, and 3.0 DDD/100 patient-days. During the 2-year study period the consumption of other antibiotics were: 19.4 DDD/100 patient-days for aminoglycosides, 4.2 for carbapenems, and 8.6 for glycopeptides. Table 5 gives the antibiotic consumption according to period of treatment in the three subunits.

To further document the antibiotic selection pressure the susceptibilities of the 172 *P. aeruginosa* cultured from 123 patients were analyzed. Among the 123 patients 25 did not receive any antibiotics, 21 received a therapy with no activity against *P. aeruginosa*, 47 were treated for *Pseudomonas* infection, and 30 others were treated for other bugs but with antibiotic covering *P. aeruginosa* (most often before the occurrence of *P. aeruginosa* colonization). Table 6 reports the resistance of *P. aeruginosa* in patients with no therapies and in patients receiving active therapies.

Second, among the 47 patients treated for *P. aeruginosa* infection 13 received piperacillin with or without tazobactam, 11 ceftazidime or cefepime, 13 a fluoroquinolone, and 10 a carbapenem as initial therapy. Regardless of the presence of aminoglycoside in the treatment there were 12 instances of inappropriate therapy; resistance was observed in one patient receiving piperacillin, three receiving cephalosporin, six receiving fluoroquinolones, and two receiving carbapenem. Moreover, during treatment after appropriate change in therapy resistance to the drug in use occurred in 3 of the 18 piperacillin treatments (16.7%), 3 of the 13 cephalosporin treatments (23.1%), 5 of the 7 ciprofloxacin treatments (71.4%), and 8 of the 13 carbapenem treatments (61.5%).

In contrast, 149 cultures were positive for *E. coli* in 139 patients, most of them during the first week of ICU stay (72.5%). There were only 8 rods resistant to ciprofloxacin and one to cefotaxime. Patients with these bacteria had not received the corresponding antibiotic during their ICU stay. Sixteen *E. coli* were resistant to piperacillin-tazobactam combination. Two instances of resistance were acquired during or after treatment in the ICU. Concerning *Enterobacter* spp 102 patients were colonized or infected by 119 strains (55 *E. cloacae*, and 64 *E. aerogenes*). No resistance occurred during treatment by fluoroquinolones (n=8) or by piperacillin tazobactam (n=15). By contrast, 4 *Enterobacter* (2 *E. cloacae*, and 2 *E. aerogenes*) became resistant during 13 treatments by cephalosporin.

Table 4 Antibiotic susceptibility (%) of specified groups of bacteria classified according to the empirical treatment used at this time

	Quinolones	Penicillins	Cephalosporins
Noninducible Enterobacteriaceae (*E. coli*, *Klebsiella* spp, *Proteus* spp)			
Number of strains	52	63	62
Amikacin	98.0	100	100
Ciprofloxacin	88.5	90.5	98.4
Cefotaxime	98.0	90.7	100
Imipenem	100	100	90.8
Piperacillin-tazobactam	94.2	78.1*	91.4
Amoxicillin-clavulanic acid	86.5	73.0	82.5
Inducible Enterobacteriaceae (*Enterobacter* spp, *Serratia* spp, *Citrobacter* spp, *Morganella* spp)			
Number of strains	42	45	47
Amikacin	95.2	88.1	89.3
Ciprofloxacin	75.6	80.0	71.7
Cefepime	97.6	95.5	95.7
Imipenem	100	97.8	95.7
Cefotaxime	83.3	80.0	55.3*
Piperacillin-tazobactam	76.2	42.5*	51.1*
Pseudomonas aeruginosa			
Number of strains	55	53	48
Amikacin	96.3	96.2	95.8
Ciprofloxacin	35.2*	50.0*	66.7
Ceftazidime	80.0	69.8	72.9
Imipenem	50.0*	73.6	77.1
Piperacillin-tazobactam	90.9	81.1	85.1
Staphylococcus aureus			
Number of strains	32	25	n=27
Oxacillin	78.1	84.0	70.3

*$p \leq 0.05$ between the level(s) of sensitivity marked by an asterisk and the level(s) without asterisk for a specified antibiotic (logistic regression)

Table 5 Table 5 Consumption of antibiotics by class and according to the period and subunit: defined daily dose (DDD)/100 patient-days. Values of DDD: cefotaxime 6 g, ceftazidime 6 g, cefepime 4 g, ciprofloxacine 500 mg, ofloxacine 400 mg, amoxy-clavulanic acid 4 g, piperacillin-tazobactam 16 g, imipenem 2 g; amikacin 1 g, gentamicin 240 mg, vancomycin 2 g, teicoplanine 400 mg

	Period 1			Period 2			Period 3		
	Unit A	Unit B	Unit C	Unit A	Unit B	Unit C	Unit A	Unit B	Unit C
Cephalosporin	1	9.5[a]	6.2	3.2	1.1	16.9[a]	9.3[a]	2.3	6.5
Quinolones	17.1[a]	0.4	2.9	6.2	14.3[a]	1.5	5	0.4	10.2[a]
Penicillins	12.3	8.2	26[a]	17.2[a]	2.3	13.7	6.8	20.7[a]	10.4
Imipenem	4.9	3.9	2.5	0.4	2.6	6.3	6.3	4.7	6.6
Aminosides	20.6	16.9	22.1	21.4	16.1	16.9	27.1	12.7	16.6
Glycopeptides	8.6	8.9	10.6	8.9	9.1	1.7	7.9	6.3	5.8

[a] First empirical choice

Table 6 Resistance (%) of *Pseudomonas aeruginosa* to antibiotics in patients having received active therapy against *P. aeruginosa* or not

	Therapy active on *P. aeruginosa*	
	No (n=50)	Yes (n=112)
Ceftazidime*	5.70	26.20
Piperacillin-tazobactam*	4.70	17
Imipenem*	16	37.50
Ciprofloxacin*	25	54.50
Amikacin	4	4.50
Tobramycin	11.80	15.20

*$p<0.05$ (Fisher exact test)

Discussion

Antibiotic resistance in the intensive care unit is a growing problem and one that can affect patient outcome [20]. It is clear that prevention of antibiotic resistance is based on the appropriate use of antibiotics to treat infections and on increased adherence to infection control practices by the entire medical and nursing team [21]. In an attempt to reduce selection pressure two types of strategy have been proposed. First, restriction in the use of specific antibiotics or antibiotic classes has been advocated to reduce the occurrence of resistance. However, a clear demonstration of the effectiveness of this strategy does not exist [11]. The heterogeneous use of antibiotics

is the second option. This has led to the concept of antibiotic class cycling, thought to limit antibiotic pressures from the cycled antibiotics as a stimulus for antimicrobial resistance as recently discussed by Fridkin [22]. The present study questioned the validity of this strategy and tested the effect of sequential use of three antibiotic classes (broad spectrum penicillins with β-lactamase inhibitors, third- or fourth-generation cephalosporins, and fluoroquinolones) for 8-month periods over 2 years.

Use of the Latin square design distinguishes the present study from other previously published studies on antibiotic rotation in ICU [15, 16, 17, 18, 19]. Those compared consecutive periods of different antibiotic treatments with periods called "before" and "after" modification of antibiotic policy. Comparing consecutive periods always entails a possible bias in the interpretation. The occurrence of outbreaks during one period, especially during "before" period or the natural course of bacterial flora may completely invalidate the observation of a beneficial effect of antibiotic rotation. The present study observed the increase in the occurrence of MRSA during the second 8-month period in all three subunits and an outbreak of extended-spectrum β-lactamase resistant *Klebsiella pneumoniae* in one subunit during penicillin empirical treatment. The increase in ICU-acquired MRSA was related to a larger increase in MRSA coming from outside the ICU, as shown by Table 3. This fact was evidenced by the use of the Latin square design. Without it this increase in MRSA may have been falsely attributed to a particular regimen.

During the study period 1,721 patients stayed for more than 2 days in the ICU. As shown in Table 1, distribution across treatment regimens was homogeneous. Interestingly, no differences appeared between the three groups regarding the number of positive bacterial cultures, the number of patients colonized or infected by the different types of bacteria (Table 4), or the number and type of infections (Table 2). This indicates that no specific antibiotic class led to colonization by certain types of pathogen such as MRSA or *P. aeruginosa*.

The incidence of ICU-acquired infections was 25.5/1,000 patient-days. This figure corresponds to those from the literature which reported values between 11.5 and 57.1 infections/1,000 patient-days in mixed or surgical ICU [1]. No differences were observed between groups. In this study, unlike the previous studies concerning rotation, we did not observe a change in infection rate nor in patient outcome. The previous studies were designed to determine the impact of rotation on the incidence of ventilator associated pneumonia [15, 16, 19], nosocomial bacteremia [15], the incidence of resistant bacterial infections and mortality due to infection [18], or the occurrence of inadequate antimicrobial treatment [17]. All of these events decreased, but, curiously, no change in ICU stay or in overall mortality was observed.

The aim of the present study was to examine changes in microbial susceptibilities to antibiotics, in the hope that antibiotic rotation would decrease the occurrence of antimicrobial resistance. We believe that this is a necessary first step to demonstrate the usefulness of the procedure. The duration of rotation was somewhat longer than the 6-month period in the studies of Kollef et al. [15, 17], the 4-month of Raymond et al. [18], and much longer than the 1 month period in studies by Gruson et al. [16, 19]. Unfortunately, details concerning changes in bacterial sensitivities are not reported in most studies—except for certain resistant pathogens in one of the Gruson et al. study [16]. However, our study allowed us to observe the effects of selection pressure. Although there was no difference in global bacterial resistances over time (Table 3), only ICU-acquired bacteria showed a clear trend to reduced sensitivity to the first choice empirical treatment used in the subunit (Table 4).

These differences in susceptibilities correspond to what is already known (or feared) for the molecules under consideration: inducible Enterobacteriaceae were less susceptible to third-generation cephalosporins when cephalosporins were used, noninducible Enterobacteriaceae were less sensitive to broad-spectrum penicillin, and *P. aeruginosa* to ciprofloxacin when βlactam–βlactamase inhibitor combination or fluoroquinolones were used. In addition, *P. aeruginosa* was less sensitive to imipenem when fluoroquinolones were used. This latter observation may constitute a clinical confirmation of the role of fluoroquinolones in the change in membrane porins in *P. aeruginosa* described in vitro [23, 24].

Differences in susceptibilities occurred with moderate antibiotic pressures differing by a factor of only three to four: cephalosporins (DDD/100 patient-days) were used at a rate of 11.8 (first-line therapy) vs. 3.2 (non-first-line therapy) and fluoroquinolones at a rate of 14.0 vs. 3.0. Protocol compliance was less strict with βlactam–β-lactamase inhibitors. Only a factor of two differentiated first-line from non-first-line periods. This is explained by the fact that this class of antibiotics is the first choice in our hospital; therefore many patients admitted to the ICU were already receiving this type of drug. In fact, the allocated antibiotic regimen was administered in every case except when it was not suitable for an already identified pathogen, or when another antibiotic was started prior to the ICU admission. This is a clear weakness of the present study, but the differences between periods in terms of antibiotic consumption was of the same magnitude as in the study of Raymond et al. [18].

The increase in resistance rate may be due to three types of events occurring within the ICU: the disappearance of sensitive germs due to treatment or to discharge of patient, the cross-contamination of patients by resistant strains, and the selection of resistance during treatment. The latter event was rarely seen with *E. coli*, sometimes with *Enterobacter*, and often with *Pseudomonas aerugi-*

nosa. All classes of antibiotics were concerned. As previously shown by Harbarth et al. [25], the individual patient level analysis may provide more relevant data than group level analysis. The selection of resistance within a few weeks may also indicate that rotation for period of 1 month would already be too long. This type of data may be relevant to develop mathematical models as tools for controlling the spread of antibiotic resistance in hospitals, as discussed recently by Bonten et al. [26].

Thus resistance occurred during treatment and the exact role of change in antibiotic classes may be questioned. It was not the change which kept the susceptibilities of bacteria stable over time, but the use of the three classes at the same time and the replacement of patients. This study is in complete agreement with that by van Loon et al. [27] which very recently observed the same development of antibiotic resistance during antibiotic rotation.

Theoretical considerations and mathematical modeling suggest that cycling is not the correct approach to preventing antibiotic resistance [28, 29]. Creating constant heterogeneity with simultaneous use of all classes of antibiotics but the right one for particular pathogens [30], together with a correct diagnosis and optimization of hygiene practices is the possible alternative. The individual effects of the antibiotics would remain, but their ecological effect, if any, would be reduced to a minimum. It is hoped that increased antibiotic susceptibilities will be observed over time. This should be worth being investigated.

In conclusion, the succession of antibiotic homogeneity periods of several months does not appear to reduce resistance levels in common pathogens. Moreover, selection of resistance can easily be documented with all classes of antibiotics.

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