Impact of aminoglycoside cycling in six tertiary intensive care units: prospective longitudinal interventional study

Francetić, Igor; Kalenić, Smilja; Huić, Mirjana; Merčep, Iveta; Makar-Aušperger, Ksenija; Likić, Robert; Erdeljić, Viktorija; Tripković, Vesna; Šimić, Petra

Source / Izvornik: **Croatian Medical Journal, 2008, 49, 207 - 214**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.3325/cmj.2008.2.215

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:741076

Impact of Aminoglycoside Cycling in Six Tertiary Intensive Care Units: Prospective Longitudinal Interventional Study

Igor Francetić[1], Smilja Kalenić[2], Mirjana Huić[1], Iveta Merčep[1], Ksenija Makar-Aušperger[1], Robert Likić[1], Viktorija Erdeljić[1], Vesna Tripković[2], Petra Šimić[1]

[1]Department of Internal Medicine, Zagreb University Hospital Center, Zagreb, Croatia
[2]Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, Zagreb, Croatia

Aim To determine the effect of aminoglycoside cycling in six tertiary intensive care units (ICU) on the rates of sepsis, aminoglycoside resistance patterns, antibiotic consumption, and costs.

Methods This was a prospective longitudinal interventional study that measured the effect of change from first-line gentamicin usage (February 2002-February 2003) to amikacin usage (February 2003-February 2004) on the aminoglycoside resistance patterns, number of patients with gram-negative bacteremia, consumption of antibiotics, and the cost of antimicrobial drugs in 6 tertiary care ICUs in Zagreb, Croatia.

Results The change from first-line gentamicin to amikacin usage led to a decrease in the overall gentamicin resistance of gram-negative bacteria (GNB) from 42% to 26% ($P<0.001$; z-test of proportions) and netilmicin resistance from 33% to 20% ($P<0.001$), but amikacin resistance did not change significantly ($P=0.462$), except for *Acinetobacter baumanni* ($P=0.014$). Sepsis rate in ICUs was reduced from 3.6% to 2.2% ($P<0.001$; χ^2 test), with a decline in the number of nosocomial bloodstream infections from 55/100 patient-days to 26/100 patient-days ($P=0.001$, χ^2 test). Furthermore, amikacin use led to a 16% decrease in the overall antibiotic consumption and € 0.1/patient/d cost reduction.

Conclusion Exclusive use of amikacin significantly reduced the resistance of GNB isolates to gentamicin and netilmicin, the number of GNB nosocomial bacteremias, and the cost of total antibiotic usage in ICUs.

> **Correspondence to:**
Igor Francetić
Department of Internal Medicine
Zagreb University Hospital Center
Kišpatićeva 12
10000 Zagreb, Croatia
ifran@mef.hr

> **Received:** June 19, 2007
> **Accepted:** April 2, 2008

> **Croat Med J. 2008;49:207-14**
> doi: 10.3325/cmj.2008.2.207

Despite the introduction of newer, less toxic antimicrobial agents, aminoglycosides continue to have a role in the treatment of serious gram-negative bacillary infections. Gentamicin, because of its low cost, remains the aminoglycoside of choice in hospitals, with low levels of resistance among *Enterobacteriaceae* and *Pseudomonas aeruginosa* (1). Most gram-negative bacteria (GNB) isolated from patients in intensive care units (ICU) have become more resistant to gentamicin (2) and ICU patients are more likely to have antimicrobial-resistant organisms than other patients or outpatients (3).

Aminoglycoside resistance is mediated through three key mechanisms: a ribosomal mutation, reduced transport into the cell, and activity of plasmid-mediated aminoglycoside-modifying enzymes (4,5). These enzymes include three acetyltransferases, four adenyltransferases, and five phosphotransferases (5). Aminoglycoside-modifying enzymes are substrate-specific. Gentamicin and tobramycin are susceptible to at least five enzymes and the result is considerable cross-resistance between these two agents. Netilmicin is susceptible to four modifying enzymes, while amikacin is susceptible to aminoglycoside 6'-N-acetyltransferase, and is therefore useful against gentamicin-resistant GNB (6). No significant increase in the resistance to amikacin has been noticed during the past ten years, even with extensive and exclusive use (7-9). However, frequent use of amikacin usually results in a decreased resistance to other aminoglycosides (10).

Several discrete strategies have been suggested to prevent or reduce microbial resistance to antimicrobials, including optimal use of agents, control, removal or restriction of antimicrobials, use of antimicrobials in combination, and rotation or cyclic use of antimicrobials (11). The latter strategy is attractive because it periodically removes certain classes or specific agents that could induce or select resistance from the institutional environment (12). The cyclic exposure prevents the development of resistance by a growth disadvantage of microorganisms when the selective antibiotic pressure is withdrawn and by eliminating the resistant microorganisms by different antibiotics (13). Studies showed that resistance to gentamicin was significantly reduced when amikacin was used (14), but it reappeared in the first gentamicin recycle. The second introduction of amikacin led to a decreased resistance to gentamicin, but the second introduction of gentamicin did not lead to reappearance of resistance (11). Trials that monitor the resistance are required to design optimal protocols and provide clinically meaningful results (15). However, the effect of empirical amikacin therapy on ICU patients with GNB in blood cultures has not been so far shown. Decreasing the number of GNB infections has both clinical and economical significance.

The aim of this study was to evaluate prospectively the effect of intensive amikacin usage on the aminoglycoside resistance patterns, number of gram-negative isolates and gram-negative bacteremias, consumption of antibiotics, and the cost of antimicrobial treatment in ICUs.

Methods

Setting

Zagreb University Hospital Center is the major hospital in Zagreb, Croatia, serving a population of 1 million. The study was conducted in 6 tertiary care ICUs at the Departments of Internal Medicine, Surgery, Cardiac Surgery, Urology, Neurology, and Neurosurgery, respectively. These ICUs have 43 beds and serve approximately 5000 patients per year. They are placed in different parts of the same building. The care in each ICU is delivered by

a team of attending physicians, residents, and nurse practitioners. Hospital infection control includes the isolation of the patient colonized with methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci*, *Enterobacteriaceae* producing extended-spectrum beta-lactamases, or any multiple resistant GNB in an individual room, whenever possible. Also, alcohol handrub dispensers are available and standard procedures of cleaning and disinfection of surfaces and equipment and hand hygiene are used.

Aminoglycoside cycling intervention

In the first year of the study period (February 28, 2002, to February 28, 2003), gentamicin was the first-line aminoglycoside used (gentamicin period) in both the combination therapy and monotherapy. At that time, amikacin was reserved for infections resistant or unresponsive to gentamicin. Other aminoglycosides have not been used. On February 28, 2003, amikacin was introduced as the sole aminoglycoside and was applied until February 29, 2004 (amikacin period). During one year, gentamicin was not used in the ICUs. The protocol was approved by the Hospital Ethics Committee.

Microbiological data and susceptibility

All blood, urine, and cerebrospinal cultures were prospectively reviewed for 4 months (November 1, 2002, to February 28, 2003) before the change from gentamicin to amikacin treatment, and for 4 months (November 1, 2003, to February 29, 2004) after 8 months of amikacin use. Microbiologic data on the presence of GNB and their resistance was recorded. Only aerobic GNB obtained are reported. Copy strains were not included in the analysis. Antimicrobial susceptibilities were determined by Kirby-Bauer disk diffusion. Aminoglycoside resistance was confirmed periodically on randomly selected isolates by minimum inhibitory concentration determination according to the National Committee for Clinical Laboratory Standards criteria (16). Gentamicin, amikacin, and netilmicin were the only aminoglycosides used in the routine antimicrobial susceptibility testing and aminoglycoside resistance could be transferable. Data on hospital length of stay from admission to bloodstream infection and the number of bloodstream infections were obtained from clinical charts by clinical pharmacologists in the previously described 4-month periods, in order to distinguish nosocomial from out-of-hospital bloodstream infections. Nosocomial infections were defined as those isolated after 48 hours of admission to ICU. During both periods, the methods of hospital infection control remained unchanged.

Antibiotic consumption

Consumption of antibiotics was recorded as the total weight of the drug in grams and then converted to the defined daily dose (DDD) per 100 patient-days, in accordance with the World Health Organization recommendation (17). The number of admissions and the number of days spent in the ICU were recorded for each ICU, both by the clinical pharmacologist visiting the ICU and from the hospital administrative data. Admission and discharge days were counted as one day.

Measurement of costs

The daily antibiotic costs per patient were calculated in Euros, based on the prices of antimicrobial agents provided by the hospital pharmacy. The daily antibiotic cost per infected patient was calculated by the multiplication of price per package and the number of daily doses that was used for the treatment of infection. Because the major benefit of the primary amikacin usage was a reduction in the need for antimicrobial treatment, the primary end-

point of the cost-saving analysis was the rise in the cost of antibiotic usage avoided by amikacin treatment compared with gentamicin treatment. This cost-effectiveness ratio was calculated by dividing the difference in mean daily amikacin and gentamicin costs per patient in the ICU by the difference in the total daily antibiotic costs per patient in the ICU in the two treatment periods.

Statistical analysis

Susceptibilities and frequencies of isolated bacteria obtained during the gentamicin period were compared with the data from the amikacin period by z-test of proportions. Number of patients admitted to ICUs, sepsis rate, number of hospital acquired and out-hospital infections and antibiotic consumption were compared by χ^2 test. Average number of patient-days spent in ICU was compared by *t*-test. Software used for the analysis was the Statistical Package for the Social Sciences, version 10 (SPSS Inc., Chicago, IL, USA). The level of significance was set at $P<0.05$.

Results

Resistance to aminoglycosides

We tested 676 g-negative isolates from blood, urine, and cerebrospinal fluid of patients in ICUs against aminoglycosides in gentamicin and amikacin period. The overall gentamicin resistance of GNB decreased from 42% to 26% ($P<0.001$), netilmicin resistance decreased from 33% to 20% ($P<0.010$), whereas amikacin resistance did not significantly change (from 20% to 19%; $P=0.462$) in the amikacin period (Table 1). However, *Escherichia coli* and *Pseudomonas aeruginosa* showed a significant reduction and *Acinetobacter baumannii* a significant increase of amikacin resistance in the amikacin period (Table 1). All GNB tested showed significant reduction in gentamicin and netilmicin resistance during amikacin period, except for *Klebsiella pneumoniae* and *Enterobacter*, which showed a non-significant reduction (Table 1).

Incidence of bacteremia

P. aeruginosa was most frequently isolated GNB in both periods, followed by *K. pneumoniae* and *A. baumannii* in gentamicin period and *K. pneumoniae*, *Enterobacter spp*., and *E coli* in amikacin period (Table 2). The overall

Table 2. Gram-negative bacteria (GNB) in blood cultures taken from patients in intensive care units

GNB	No (%) of isolated bacteria in blood cultures in gentamicin period (n = 142)†	amikacin period (n = 155)†	*P**
Pseudomonas aeruginosa	9 (6.4)	9 (5.8)	>0.950
Klebsiella pneumoniae	5 (3.5)	3 (1.9)	0.157
Acinetobacter spp.	5 (3.5)	2 (1.3)	0.083
Escherichia coli	3 (2.1)	3 (1.9)	>0.950
Proteus mirabilis	2 (1.4)	0	0.157
Enterobacter spp.	0	3 (1.9)	0.083
Other	8 (5.6)	4 (2.6)	0.046
All GNB	32 (22.5)	24 (15.5)	0.005

*Gentamicin vs amikacin period, z-test of proportions.
†n – number of blood cultures.

Table 1. Gram-negative bacteria (GNB) resistant to aminoglycosides (gentamicin, amikacin, and netilmicin) in intensive care units in gentamicin and amikacin period

	No. (%) of resistant isolates in										
	gentamicin period				amikacin period; *P**						
GNB*	n†	gentamicin	amikacin	netilmicin	n†	gentamicin	*P*	amikacin	*P*	netilmicin	*P*
Proteus mirabilis	36	10 (28)	1 (2)	8 (22)	18	4 (22)	0.030	1 (6)	>0.950	2 (11)	0.014
Enterobacter spp	31	13 (42)	6 (19)	10 (32)	26	8 (31)	0.157	3 (12)	0.317	7 (27)	0.083
Escherichia coli	123	37 (30)	32 (26)	31 (25)	75	10 (13)	<0.001	5 (7)	<0.001	4 (5)	<0.001
Acinetobacter baumannii	34	25 (73)	6 (17)	16 (48)	24	14 (58)	0.001	14 (58)	0.014	10 (42)	0.014
Pseudomonas spp	122	59 (48)	45 (37)	51 (42)	62	23 (37)	<0.001	11 (18)	<0.001	22 (35)	<0.001
Klebsiella spp	78	24 (31)	17 (22)	20 (26)	47	9 (19)	0.083	5 (11)	0.157	6 (13)	<0.001
All GNB	424	178 (42)	85 (20)	140 (33)	252	66 (26)	<0.001	48 (19)	0.456	50 (20)	<0.001

*Gentamicin vs amikacin period, z-test of proportions.
†n – number of isolates.

Table 3. Number of patients, infections, antibiotic consumption, and cost in intensive care units (ICU)

Parameters	Gentamicin period	Amikacin period	*P*
Number of patients admitted to ICUs	5706	6001	0.006*
Average number of patient-days spent in ICU (mean±standard deviation)	2.3 ± 0.5	2.5 ± 0.6	0.756†
Sepsis rate (%)	3.6	2.2	0.001*
Number of hospital acquired infections/100 patient-days	55	26	0.001*
Number of out-of-hospital infections/100 patient-days	99	89	0.466*
Antibiotic consumption (defined daily dose/100 patient-days)	92	79	0.300*

*χ^2 test.
†*t* test.

number of patients having GNB bloodstream infection was significantly reduced from 15.4/1000 patient-days to 11.5/1000 patient-days ($P<0.001$; z-test of proportions) during amikacin period, with the reduction of sepsis rate from 3.6% to 2.2% ($P<0.001$; χ^2 test). The percentage of GNB in blood cultures taken from ICU patients was significantly reduced from 23% to 16% ($P=0.005$) in the amikacin period. Hospital-acquired GNB bloodstream infections were significantly reduced from 55/100 patient-days to 26/100 patient-days in the amikacin period ($P=0.001$; χ^2 test), while out-hospital bloodstream infections in ICUs remained unchanged, ie, dropped from 99/100 patient-days to 89/100 patient-days. Frequencies of *P. aeruginosa* and *E. coli* did not show significant difference in these two periods. *Enterobacter spp*. was the only GNB that was isolated more frequently during amikacin period. The remaining GNB were reduced with exclusive use of amikacin (Table 2).

Antibiotic consumption

Overall antibiotic consumption was reduced from 92 DDD/100 patient-days in gentamicin period to 79 DDD/100 patient-days in amikacin period ($P=0.300$) (Table 3). Amikacin and gentamicin consumption showed significant changes between these two periods, while the consumption of other antimicrobials remained unchanged. There were no differences in the length of stay in the ICU between amikacin (average 2.364 days/patient) and gentamicin period (average 2.466 days/patient; $P=0.757$; *t* test)

Cost analysis

The mean increase in the initial treatment costs for amikacin instead of gentamicin was €0.14 per ICU patient daily, while resource utilization and related health care costs remained unchanged. Use of amikacin was associated with 0.8% reduction in the total antibiotic costs, from €12.72/patient/d in gentamicin to €12.62/patient/d in amikacin period. In particular, total antibiotic costs were reduced by €0.1 per patient daily in amikacin period, as compared with gentamicin period. The incremental cost-effectiveness ratio for amikacin period was 1.4 (€0.14/patient/d/€0.1/patient/d).

Discussion

We showed that exclusive usage of amikacin significantly reduced the resistance of GNB isolates to gentamicin and netilmicin, the number of GNB nosocomial bacteremias, and the cost of total antibiotic usage in the ICUs. The primary mechanism of aminoglycoside resistance is through the activity of plasmid-mediated modifying enzymes (5). There are no effective clinical ways to inhibit these resistance enzymes (18) as there are ways to inhibit beta-lactamases. Since there is no effective strategy to directly interact with the mechanism of aminoglycoside resistance, rational use of aminoglycosides is of utmost importance (19). Rotational usage practices are likely to be most appropriate for drugs acting against GNB, because of

the wide choice of drugs available for rotation (11). Antibiotic cycling is not associated with significant changes in the receipt of appropriate empirical antimicrobial therapy for the treatment of ICU infections (20). In critically ill medical patients, a monthly rotation of anti-Pseudomonas beta-lactams and ciprofloxacin is better than the strategy of mixing in the acquisition of *P. aeruginosa* resistant to selected beta-lactams (21). The optimal cost-effective rotational regimen for aminoglycosides is yet to be explored (22). We have shown that a year of primary use of amikacin significantly reduced the resistance of GNB to gentamicin and netilmicin, without affecting amikacin susceptibility. Although significant changes occurred over time, the overall rate of GNB resistant to aminoglycosides in Croatia remained high (23). Baršić et al (24) reported slightly higher resistance rates among GNB isolates in ICU in 2001, but the study was performed in the ICU of a hospital for infectious diseases. In a similar study, but with four antimicrobial agents and a focus on rectal swab cultures, cycling did not result in a significant change in enteric acquisition of resistant GNB among ICU patients (25). However, for cycling of two other antibiotic classes, quinolones and beta-lactams, there was no control of the emergence of gram-negative antimicrobial resistance in ICUs (26).

More importantly, we have shown that amikacin treatment significantly reduced the number of GNB hospital infections in the ICU. By using amikacin rather than gentamicin for treatment of infections, more bacteria were susceptible to antibiotic treatment and the ICU ecology was altered in such a way that fewer patients acquired nosocomial GNB, reducing the overall number of infections in ICUs. The overall drop in the infection rates resulted also in a reduced sepsis rate. Results of this study are supported by other studies demonstrating improved susceptibility profiles of selected clinically important gram-negative ICU isolates after the initiation of the cycling protocol. We previously showed that ampicillin plus gentamicin, as empirical therapy of infection in a neonatal ICU, significantly reduce the number of cases of *K. pneumoniae* from the bacteremia and meningitis, as compared with ampicillin plus cefuroxime (27). We have also shown earlier that replacement of cefuroxime and gentamicin with ceftazidime and amikacin as empirical therapy lead to a significant reduction in the number of positive blood cultures and the number of bacteriologically verified septic episodes in neurosurgical ICU (28). In this study, the same trend of reduction of GNB from bactremia is observed when gentamicin is replaced by amikacin. The 2-year duration of the study minimizes the potential impact of short-term confounding variables, such as concurrent outbreaks and limited clinical practice changes. During both periods, methods of the hospital infection control remained unchanged. Although we are unaware of any other concurrent changes in the practice that would preferentially affect the bacteremia, confounding variables, such as changes in the severity of illness and other antimicrobial usage patterns, cannot be excluded and could be possible limitations of the study. Variables like catheters, disinfection policies, or surgical techniques were not changed. However, we did not observe major changes in other infections in those ICU patients (data not shown) that would presumably be influenced by the same confounding variables.

The reduction in the overall number of infections in ICUs in amikacin period subsequently led to a reduced antibiotic consumption. Though daily expenses per ICU patient were increased by €0.14 for amikacin instead of gentamicin use, total antibiotic costs were decreased by €0.1 per ICU patient daily. Cost

is an important factor which determines the physician's choice of medication to treat patients in specific situations (29). Although the true cost of GNB infection is disputed, daily cost of antimicrobial treatment has been reported to be a significant extra cost attributable to infections (30).

The results collectively show that exclusive usage of amikacin significantly reduces the resistance of GNB isolates to gentamicin and netilmicin and, more importantly, reduces the number of patients with GNB nosocomial infections and overall cost of antimicrobial treatment in ICUs.

Acknowledgment

This study was funded by Ministry of Science, Education, and Sports, grant No. 0108085.

References

1 Edson RS, Terrell CL. The aminoglycosides. Mayo Clin Proc. 1999;74:519-28. Medline:10319086

2 Flournoy DJ, Reinert RL, Bell-Dixon C, Gentry CA. Increasing antimicrobial resistance in gram-negative bacilli isolated from patients in intensive care units. Am J Infect Control. 2000;28:244-50. Medline:10840345 doi:10.1067/mic.2000.103836

3 Archibald L, Phillips L, Monnet D, McGowan JE Jr, Tenover F, Gaynes R. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis. 1997;24:211-5. Medline:9114149

4 Bryan LE. General mechanisms of resistance to antibiotics. J Antimicrob Chemother. 1988;22 Suppl A:1-15. Medline:3062000

5 Wright GD. Mechanisms of resistance to antibiotics. Curr Opin Chem Biol. 2003;7:563-9. Medline:14580559 doi:10.1016/j.cbpa.2003.08.004

6 Larson TA, Garrett CR, Gerding DN. Frequency of aminoglycoside 6'-N-acetyltransferase among Serratia species during increased use of amikacin in the hospital. Antimicrob Agents Chemother. 1986;30:176-8. Medline:3752978

7 Betts RF, Valenti WM, Chapman SW, Chonmaitree T, Mowrer G, Pincus P, et al. Five-year surveillance of aminoglycoside usage in a university hospital. Ann Intern Med. 1984;100:219-22. Medline:6691664

8 Lee JT Jr. Three-year experience with amikacin sulfate as an exclusive surgical aminoglycoside in a large acute-care hospital. Am J Med. 1985;79:37-42. Medline:3849259 doi:10.1016/0002-9343(85)90189-5

9 Peetermans WE, Bobbaers HJ. Amikacin as first-choice aminoglycoside in a medical intensive care unit: a one-year bacteriological surveillance study. J Chemother. 1996;8:17-24. Medline:8835103

10 Friedland IR, Funk E, Khoosal M, Klugman KP. Increased resistance to amikacin in a neonatal unit following intensive amikacin usage. Antimicrob Agents Chemother. 1992;36:1596-600. Medline:1416839

11 Gerding DN. Antimicrobial cycling: lessons learned from the aminoglycoside experience. Infect Control Hosp Epidemiol. 2000;21(1 Suppl):S12-7. Medline:10654630 doi:10.1086/503168

12 Brown EM, Nathwani D. Antibiotic cycling or rotation: a systematic review of the evidence of efficacy. J Antimicrob Chemother. 2005;55:6-9. Medline:15531594 doi:10.1093/jac/dkh482

13 Bennett KM, Scarborough JE, Sharpe M, Dodds-Ashley E, Kaye KS, Hayward TZ III, et al. Implementation of antibiotic rotation protocol improves antibiotic susceptibility profile in a surgical intensive care unit. J Trauma. 2007;63:307-11. Medline:17693828

14 Berk SL, Alvarez S, Ortega G, Verghese A, Holtsclaw-Berk SA. Clinical and microbiologic consequences of amikacin use during a 42-month period. Arch Intern Med. 1986;146:538-41. Medline:3954526 doi:10.1001/archinte.146.3.538

15 Dubberke ER, Fraser VJ. Cycling and other strategies to slow and reverse antibiotic resistance. Infect Med. 2004;21:544-56.

16 National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. In: National Committee for Clinical Laboratory Standards. Vol 10. 2nd ed. Villanova (PA): NCCLS publication 1990. M7-A2.

17 Collaborating WHO. Centre for Drug Statistics Methodology. ATC Index with DDDs. Oslo: WHO; 2003.

18 Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. Clin Microbiol Rev. 2003;16:430-50. Medline:12857776 doi:10.1128/CMR.16.3.430-450.2003

19 Guven GS, Uzun O. Principles of good use of antibiotics in hospitals. J Hosp Infect. 2003;53:91-6. Medline:12586566 doi:10.1053/jhin.2002.1353

20 Merz LR, Warren DK, Kollef MH, Fridkin SK, Fraser VJ. The impact of an antibiotic cycling program on empirical therapy for gram-negative infections. Chest. 2006;130:1672-8. Medline:17166981 doi:10.1378/chest.130.6.1672

21 Martinez JA, Nicolas JM, Marco F, Horcajada JP, Garcia-Segarra G, Trilla A, et al. Comparison of antimicrobial cycling and mixing strategies in two medical intensive care units. Crit Care Med. 2006;34:329-36. Medline:16424711 doi:10.1097/01.CCM.0000195010.63855.45

22 Sommers BD. Economics of antibiotic administration. Crit Care Nurs Clin North Am. 2003;15:89-96. Medline:12597044 doi:10.1016/S0899-5885(02)00029-1

23 Tambic Andrasevic A, Tambic T, Kalenic S, Jankovic V; Working Group of the Croatian Committee for Antibiotic Resistance Surveillance. Surveillance for antimicrobial resistance in Croatia. Emerg Infect Dis. 2002;8:14-8. Medline:11749742

24 Barsic B, Tambic A, Santini M, Klinar I, Kutlesa M, Krajinovic V. Antibiotic resistance among nosocomial isolates in a Croatian intensive care unit – results of a twelve-year focal surveillance of nosocomial infections. J Chemother. 2004;16:273-81. Medline:15330325

25 Warren DK, Hill HA, Merz LR, Kollef MH, Hayden MK, Fraser VJ, 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. Medline:15599150 doi:10.1097/01.CCM.0000147685.79487.28

26 van Loon HJ, Vriens MR, Fluit AC, Troelstra A, van der Werken C, Verhoef J, et al. Antibiotic rotation and development of gram-negative antibiotic resistance. Am J Respir Crit Care Med. 2005;171:480-7. Medline:15516540 doi:10.1164/rccm.200401-070OC

27 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. Medline:8095946 doi:10.1016/0195-6701(93)90128-M

28 Kalenic S, Sekulic A, Francetic I, Scap M, Justinic Z. Empirical therapy of sepsis based on local resistance at neurosurgical intensive care unit. Neurol Croat. 1993;42:47-56.

29 Pirson M, Dramaix M, Struelens M, Riley TV, Leclercq P. Costs associated with hospital-acquired bacteraemia in a Belgian hospital. J Hosp Infect. 2005;59:33-40. Medline:15571851 doi:10.1016/j.jhin.2004.07.006

30 Inan D, Saba R, Gunseren F, Ongut G, Turhan O, Yalcin AN, et al. Daily antibiotic cost of nosocomial infections in a Turkish university hospital. BMC Infect Dis. 2005;5:5. Medline:15679899 doi:10.1186/1471-2334-5-5