Antibiotic utilization and *Pseudomonas aeruginosa* resistance in intensive care units

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SUMMARY

Pseudomonas aeruginosa is one of the most frequent and dangerous pathogens involved in the etiology of severe nosocomial infections. A retrospective observational study was conducted at all intensive care units of the University Hospital in Olomouc, Czech Republic (155 ICU beds). Complete antibiotic utilization data of the ICUs in the period of 1999 to 2008 were processed according to ATC/DDD system and expressed in defined daily doses per 100 bed-days (DBD). Utilization of meropenem, imipenem, ciprofloxacin, ofloxacin, pefloxacin, gentamicin, amikacin, ceftazidime, cefoperazone, cefoperazone/sulbactam and piperacillin/tazobactam was measured. *Pseudomonas aeruginosa* strains were isolated from clinical material obtained from patients hospitalized in ICUs. During the ten-year period, utilization of the entire group of antibiotics monitored grew. It increased from 23.52 DBD in 1999 to 27.48 DBD in 2008 with a peak of 33.04 DBD in 2007. *P. aeruginosa* accounted for as much as 42% of pneumonias and 23% of surgical wound infections. Our results show that *P. aeruginosa* strains became gradually resistant to all antibiotics used in the treatment of the infections caused by them, with the exception of amikacin and piperacillin/tazobactam.

KEY WORDS: Pseudomonas aeruginosa, Resistance, Antibiotics, Utilization

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INTRODUCTION

Bacterial resistance to antibiotics is a burgeoning problem in the hospital setting, particularly in intensive care units. Infections caused by multidrug-resistant bacterial strains are generally associated with increased morbidity and mortality as well as with the length of hospital stay and increased hospital cost (Neu, 1992; Archibald *et al*., 1997; Shorr, 2009). *Pseudomonas aeruginosa* is one of the most frequent and dangerous pathogens involved in the etiology of severe nosocomial infections (Flamm, 2004). Infections caused by *Pseudomonas aeruginosa* are often life-threatening and difficult to treat because of its primary limited susceptibility to commonly used antimicrobial agents. Moreover, the high level of acquired resistance as well as horizontal and clonal spread of resistant *Pseudomonas aeruginosa* strains has become a serious problem and represents a limit for adequate antibiotic therapy. Zavascki *et al*. showed a higher mortality rate in nosocomial infections caused by metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* which is highly resistant to the majority of beta-lactam antibiotics.

The mortality in infections caused by MBL-positive strains reached 51%, in MBL-negative ones it was moderately lower: 32% (Zavascki *et al*., 2006). In their newer study focused on nosocomial bloodstream infections, Zavascki *et al*. documented a 62% mortality rate in MBL-positive *Pseudomonas aeruginosa* infections compared to a 44% mortality rate in MBL-negative strain infections (Zavascki *et al*., 2008).

These recent results as well as many others sup-

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port the perception of *Pseudomonas aeruginosa* as being one of the most dangerous infectious pathogens in the intensive care setting.
The aim of this study was to evaluate the clinical importance of *Pseudomonas aeruginosa* strains and the relationship between antibiotic utilization and resistance of *Pseudomonas aeruginosa* in hospital intensive care units.

MATERIAL AND METHODS

Setting
A retrospective observational study was conducted at all intensive care units of the University Hospital in Olomouc, Czech Republic (1251 standard and 155 ICU beds in 2008). The clinical importance of *Pseudomonas aeruginosa* strains was assessed during the last year of the survey (2008). Records of all infections and isolated bacterial pathogens for all patients hospitalized at an ICU were collected.

Antibiotic use
Complete antibiotic utilization data of the ICUs in the period of 1999 to 2008 were obtained from the database of the Department of Pharmacology, processed according to ATC/DDD system valid in 2009 (WHO Collaborating Centre for Drug Statistics Methodology, 2009) and expressed in defined daily doses per 100 bed-days (DBD). For the purpose of this study, consumption of meropenem, imipenem, ciprofloxacin, ofloxacin, pefloxacin, gentamicin, amikacin, ceftazidime, cefoperazone, cefoperazone/sulbactam and piperacillin/tazobactam was measured.

Microbiology testing
Pseudomonas aeruginosa strains were isolated from clinical material (tracheal secretion, bronchoalveolar lavage, sputum, blood, urine, pus, puncture samples, wound secretion, bile) obtained from hospitalized patients in ICUs in the University Hospital Olomouc over a period of 10 years (1999-2008). The isolates were selected in such a way that only one strain isolated as the first one was included from each patient. Identification was performed by standard microbiological procedures and Phoenix automated system (Becton Dickinson, USA). Susceptibility of the isolates to antibiotics was determined by the standard microdilution method meeting the CLSI standards (Clinical and Laboratory Standards Institute, 2009). *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 reference strains were used for protocol quality control.

Statistical analysis
The incidence of *Pseudomonas aeruginosa* strains resistant to selected antibiotics depending on their utilization was measured. Spearman correlation was used to determine the relationship between the use of the antibiotics and the susceptibility of pathogenic *Pseudomonas aeruginosa* strains. Statistical significance was accepted at a 5% level.

RESULTS

During the ten-year period, utilization of the entire group of antibiotics followed grew. It increased from 23.52 DBD in 1999 to 27.48 DBD in 2008 with a peak of 33.04 DBD in 2007. The utilization of all antibiotics used for the treatment of *Pseudomonas aeruginosa* infections is shown in Table 1. The antibiotics were divided into the following groups: aminoglycosides (gentamicin, amikacin), fluoroquinolones (ofloxacin, pefloxacin, ciprofloxacin), piperacillin/tazobactam, third-generation cephalosporins with activity against *Pseudomonas aeruginosa* (ceftazidime, cefoperazone, cefoperazone/sulbactam) and carbapenems (imipenem, meropenem).
During the investigated period, the number of bed-days increased from 38,541 in 1999 to 47,821 in 2008; the maximum of 49,500 bed-days was reached in 2004.
The development of *Pseudomonas aeruginosa* resistance to antibiotics is given in Table 2. There is evidence of increased resistance to gentamicin, fluoroquinolones, cefoperazone, ceftazidime and meropenem.
There was an increase in carbapenem consumption from 1.20 DBD in 1999 to 5.17 DBD in 2008. The highest growth of utilization was found in meropenem, from 0.88 DBD in 1999 to 3.56 in 2008 (Figure 1). At the same time, the resistance of *Pseudomonas aeruginosa* to meropenem increased from 14.2% to 42.1%, with a peak of 47.1% in 2004.

TABLE 1 - *Utilization of antipseudomonal antibiotics during the investigated period.*

Utilization [DBD]	*1999*	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*
Gentamicin	5.02	6.04	6.28	6.14	6.87	6.95	7.49	8.50	9.00	6.88
Amikacin	1.48	2.48	1.72	1.26	0.98	3.04	0.95	1.10	1.87	1.91
Aminoglycosides	**6.50**	**8.52**	**8.00**	**7.40**	**7.86**	**9.99**	**8.44**	**9.59**	**10.87**	**8.79**
Ciprofloxacin	4.36	2.60	4.60	4.94	4.99	4.68	6.36	6.48	5.25	3.97
Ofloxacin	4.29	1.10	0.70	1.53	1.62	2.36	3.64	2.19	2.93	2.53
Pefloxacin	1.69	1.42	2.51	2.20	3.09	3.81	1.30	3.42	3.54	0.99
Fluoroquinolones	**10.33**	**5.12**	**7.81**	**8.67**	**9.70**	**10.84**	**11.30**	**12.09**	**11.72**	**7.49**
Piperacillin/tazobactam	**3.45**	**3.10**	**2.62**	**1.41**	**2.34**	**3.25**	**3.79**	**2.88**	**4.00**	**3.17**
Ceftazidime	1.90	5.20	5.24	3.66	3.88	2.16	2.68	2.45	2.33	2.19
Cefoperazone	0.14	0.18	0.27	0.23	0.37	0.75	0.41	1.00	1.21	0.67
Cefoperazone/ sulbactam	0.00	0.00	0.00	0.00	0.00	0.02	0.16	0.46	0.21	0.00
Third-generation cephalosporins active against *P. aeruginosa*	**2.04**	**5.38**	**5.51**	**3.89**	**4.25**	**2.93**	**3.25**	**3.91**	**3.76**	**2.86**
Meropenem	0.88	0.40	0.27	0.11	0.13	0.05	0.95	0.26	1.18	3.56
Imipenem	0.33	1.51	1.72	1.84	1.67	1.41	1.89	1.65	1.51	1.60
Carbapenems	1.20	1.91	1.99	1.94	1.80	1.46	2.84	1.91	2.70	5.17

In aminoglycosides (Figure 2), the utilization of gentamicin increased from 5.01 to 6.87 DBD and that of amikacin from 1.48 to 1.91 DBD. The growth of resistance of *Pseudomonas aeruginosa* to gentamicin was significant, from 17.7 to 33.1%, but simultaneously its resistance to amikacin decreased from 6.4 to 4.8 %.

In third-generation cephalosporins with antipseudomonal activity, an increase from 2.04 DBD in 1999 to 2.86 DBD in 2008 was noted, going together with an increase of *Pseudomonas aeruginosa* resistance to ceftazidime from 16.9% in 1999 to 30.3% in 2008 (Figure 3).

The most widely used antibiotic of this group was ceftazidime.

The utilization of quinolones (Figure 4) decreased from 10.33 DBD in 1999 to 7.49 DBD in 2008; the highest utilization of 12.09 DBD was noted in 2006. Despite a decrease in the use of quinolones, we observed an increasing trend in the resistance

TABLE 2 - *Resistance of Pseudomonas aeruginosa to antipseudomonal antibiotics.*

Resistance (%)	*1999*	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*
Gentamicin	17.9	16.5	11.9	12.8	17.4	15.1	12.0	14.7	20.1	33.1
Amikacin	6.4	10.7	13.7	6.4	7.0	3.3	8.1	3.1	4.6	4.8
Ciprofloxacin	16.0	18.5	16.1	39.5	39.3	47.8	31.2	34.3	40.5	44.1
Ofloxacin	28.2	25.2	23.0	43.8	43.2	51.3	34.0	36.8	45.0	46.3
Piperacillin/ tazobactam	11.5	5.8	7.5	11.6	17.6	17.5	5.0	11.3	13.0	8.0
Cefoperazone	17.5	15.6	23.1	41.2	36.3	37.8	23.6	29.4	27.8	36.8
Ceftazidime	16.9	16.1	25.1	31.2	19.6	22.8	24.7	19.3	18.5	30.3
Meropenem	14.2	23.7	24.6	26.2	37.6	47.1	26.8	28.0	35.0	42.1

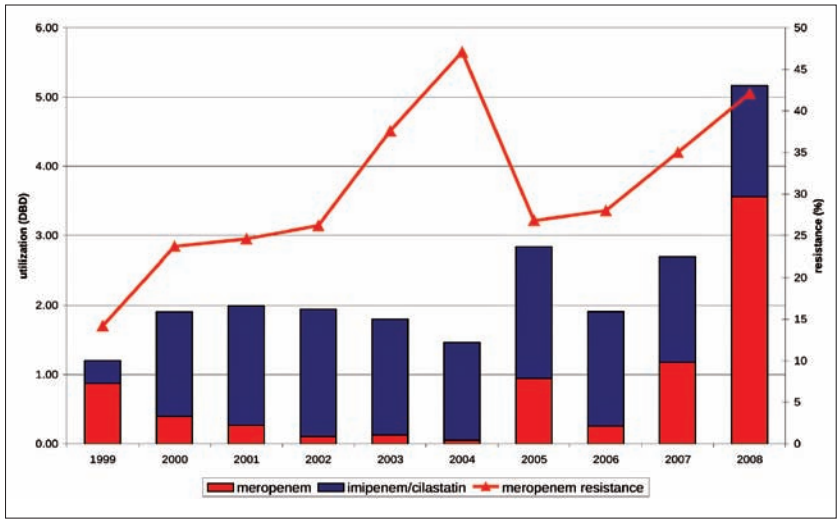

FIGURE 1 - *Utilization of carbapenems and resistance of Pseudomonas aeruginosa to meropenem.*

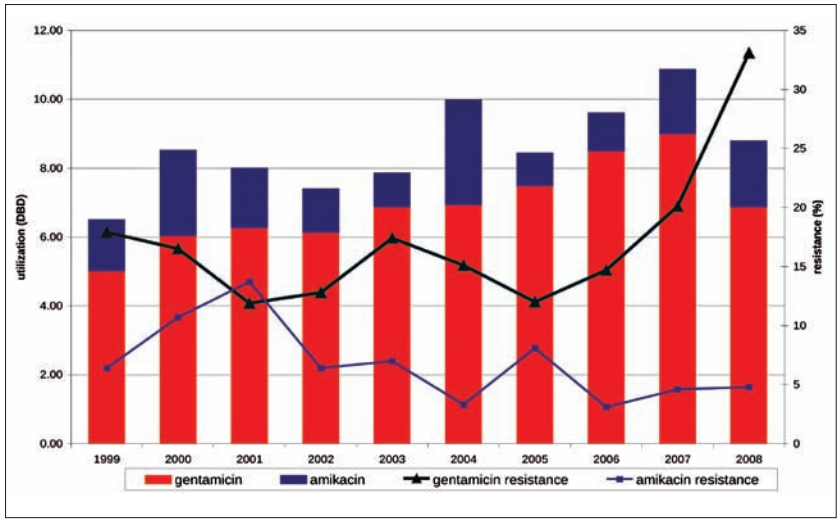

FIGURE 2 - *Utilization of aminoglycosides and resistance of Pseudomonas aeruginosa.*

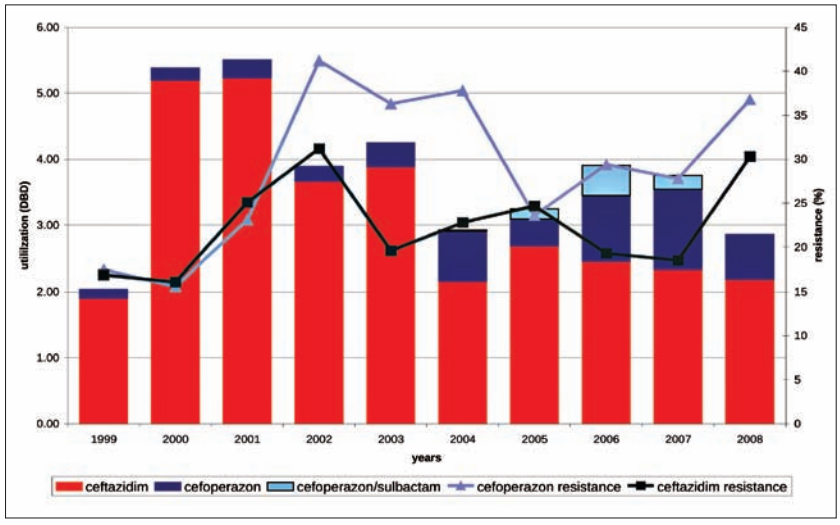

FIGURE 3 - *Utilization and resistance of third-generation cephalosporins with activity against Pseudomonas aeruginosa.*

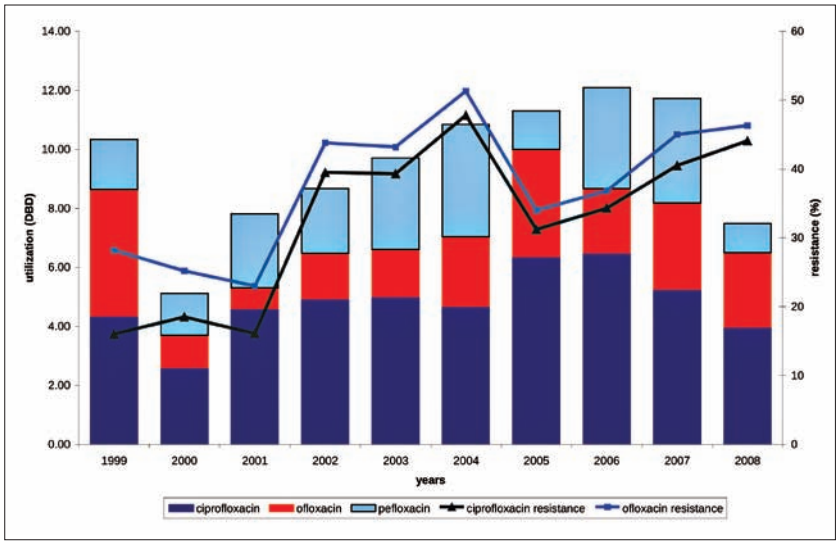

FIGURE 4 - *Utilization of quinolones and resistance of Pseudomonas aeruginosa.*

of *Pseudomonas aeruginosa* to ciprofloxacin from 16% in 1999 to 44.1% in 2008 and to ofloxacin from 28.2% in 1999 to 46.3% in 2008. The peak value of 51.3% for ofloxacin in 2004 was also the highest level of *Pseudomonas aeruginosa* resistance to any antibiotic tested in this study.

The combination of piperacillin/tazobactam (Figure 5) exhibited a stable consumption as well as level of resistance, which was even lower at the end of the study (8.0%) than at the beginning (11.5%).

The relationship between antibiotic utilization and *Pseudomonas aeruginosa* resistance was tested by Spearman's rank correlation. For all the groups of antibiotics tested, the Spearman's Rank coefficient was positive with the highest value for fluoroquinolones and resistance to ofloxacin (r=0.2485). In individual antibiotics, amikacin, piperacillin/tazobactam and meropenem showed a negative correlation with the resistance of *Pseudomonas aeruginosa* to them, but none of these correlations was statistically significant.

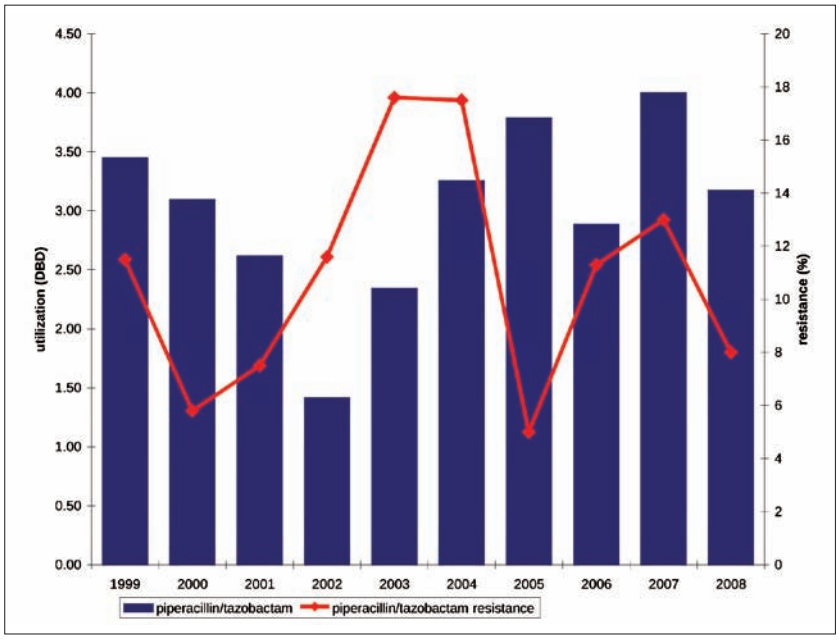

FIGURE 5 - *Utilization of piperacillin/tazobactam and resistance of Pseudomonas aeruginosa.*

Tables 3 and 4 show the clinical importance of *Pseudomonas aeruginosa*. During the year monitored 81 ICU patients suffering from a bacterial infection were included in the survey. The most common pathogens were *Enterobacteriacae* while the most frequent individual species was *Pseudomonas aeruginosa* (28%), most frequently causing pneumonia (42%) and post-surgery infections (23%).

DISCUSSION

Our results show the clinical importance of *Pseudomonas aeruginosa* and its influence on antibiotic consumption at ICUs. The data from the US National Nosocomial Infections Surveillance System (NNIS) document that in 2003 *Pseudomonas aeruginosa* accounted for 18% of pneumonias, 16% of urinary tract infections and 10% of surgical wound infections among ICU patients (Gaynes *et al.*, 2009). In our hospital ICU survey, it accounted for as much as 42% of pneumonias and 23% of surgical wound infections.
Negative clinical consequences, associated with a high level of morbidity and mortality, occur together with *Pseudomonas aeruginosa* strains resistant to anti-pseudomonal antibiotics (Carmeli *et al.*, 1999; Aloush *et al.*, 2006; Johnson *et al.*, 2009). The problem of *Pseudomonas spp*. resist-

TABLE 3 - *Statistical values of tested data.*

Variable 1 *Antibiotic utilization [DBD]*	*Variable 2* *Resistant P. aeruginosa strains to an antibiotic [%]*	ρ	*p-value*
Aminoglycosides	amikacin	-0.5714	0.0865
Third-generation cephalosporins with activity against *Pseudomonas aeruginosa*	cefoperazone	-0.2364	0.4783
Amikacin	amikacin	-0.1702	0.6096
Piperacillin/tazobactam	piperacillin/tazobactam	-0.1515	0.6494
Meropenem	meropenem	-0.1152	0.7297
Third-generation cephalosporins active against *P. aeruginosa*	ceftazidim	-0.0545	0.8700
Gentamicin	gentamicin	0.0303	0.9276
Ceftazidime	ceftazidime	0.1394	0.6758
Fluoroquinolones	ciprofloxacin	0.1515	0.6494
Ciprofloxacin	ciprofloxacin	0.1636	0.6235
Carbapenems	meropenem	0.1763	0.5969
Aminoglycosides	gentamicin	0.2364	0.4783
Fluoroquinolones	ofloxacin	0.2485	0.4560
Ofloxacin	ofloxacin	0.3091	0.3538
Cefoperazone	cefoperazone	0.4182	0.2096

ρ = Spearman Correlation.

TABLE 4 - *Proportion of bacterial and fungal pathogens in ICU patients in 2008.*

Pathogen	*No. of strains*	*Proportion [%]*
Pseudomonas aeruginosa	90	28.4
Klebsiella pneumoniae	61	19.2
Escherichia coli	28	8.8
Staphylococcus sp.	28	8.8
Enterococcus sp.	19	6.0
Candida sp.	17	5.4
Burkholderia cepacia	11	3.5
Staphylococcus aureus	10	3.2
Enterobacter cloacae	10	3.2
Stenotrophomonas maltophilia	7	2.2
Klebsiella oxytoca	4	1.3
Morganella morganii	4	1.3
Providencia stuartii	4	1.3
Others	23	7.4

ance is on the increase, particularly due to the combination of the following mechanisms: beta-lactamase production, a strong barrier to diffusion at the outer bacterial membrane and bacterial efflux. Selective pressure of antimicrobial drugs has an important impact on the development of bacterial resistance (Gaynes, 1997; Allegranzi *et al*., 2002; Loeffler *et al*., 2003; Urbanek *et al*., 2007). Falagas *et al*., in their systematic review of the literature, considered previous antibiotic use to be the main risk factor for the occurrence of multiresistant *Pseudomonas aeruginosa* strains (Falagas *et al*., 2006). Hsueh *et al*. proved a statistically significant correlation between an increase in the occurrence of meropenem resistant Pseudomonas aeruginosa strains and increased utilization of extended-spectrum cephalosporins, carbapenems, fluoroquinolones, aminoglycosides and beta-lactam antibiotics with beta-lactamases inhibitors combinations (Hsueh *et al*., 2005).

TABLE 5 - *Infections induced by Pseudomonas aeruginosa in ICU patients.*

Infection type	*Patients*	*Proportion [%]*
Pneumonia	11	42.3
Post-surgery infections	6	23.1
Bloodstream infections and sepsis	4	15.4
Urinary tract infection	2	7.7
Others	3	11.5

Our results show that *P. aeruginosa* strains become gradually resistant to all antibiotics used in the treatment of the infections caused by them, with an exception of amikacin and piperacillin/tazobactam. A lower selection pressure of these antibiotics was also described in our previous paper (Kolář *et al*., 2001).

In our survey, no statistical significance was found in the correlations between individual antibiotics or antibiotic groups and the development of bacterial resistance to them, although positive trends were found in the majority of the drugs. The exception of amikacin and piperacillin/tazobactam implies the relative safety and usefulness of these two antibiotics for use in hospital intensive care. The study documents the importance of antibiotic utilization and monitoring of *Pseudomonas aeruginosa* resistance in ICUs.

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