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Original Research Article

Relationship between resistance to antibiotics and insusceptibility to biocides of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated in Indonesian hospitals

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ABSTRACT

Background: Several studies have shown that bacteria acquiring resistance to biocides may acquire resistance to antibiotics simultaneously. This study aimed to evaluate the relationship between resistance to antibiotics and insusceptibility to biocides of *S. aureus* and *P. aeruginosa* isolated in Indonesian hospitals.
Methods: 61 isolates of *S. aureus* from nurses' nasal cavities and 46 isolates of *P. aeruginosa* from hospital environments were divided into those with higher minimum inhibitory concentration (MIC) (Higher MIC group) and those with lower MIC (lower MIC group) depending on growth in MIC of chlorhexidine gluconate (CHG) and benzalkonium chloride (BZK) of each standard strain. Afterwards, susceptibility to antibiotics of the 2 groups was compared.
Results: Increases in MICs of CHG were found in both species. Some of *P. aeruginosa* also had higher MICs of BZK. Relationship between antibiotic resistance and insusceptibility to biocides differed among species, biocides and antibiotics. In *S. aureus,* isolates in the Higher MIC group tended to be more resistant to ampicillin (0.167). In *P. aeruginosa*, resistance to aminoglycosides was observed more frequently in the Higher MIC group for CHG and it was significant in amikacin ($p = 0.002$). Further analysis is necessary to determine the mechanisms of the relationship between aminoglycoside resistance and CHG insusceptibility in *P. aeruginosa*.
Conclusions: Increase in insusceptibility to biocides was found in isolated *S. aureus* and *P. aeruginosa* and a relationship between insusceptibility to CHG and resistance to aminoglycosides was observed in *P. aeruginosa*.

Keywords: Antibiotics, Biocides, Insusceptibility, Resistance, *P. aeruginosa*, *S. aureus*

INTRODUCTION

One of the major problems associated with infection control in healthcare settings is a microorganism which is resistant to antibiotics or biocides that are available in healthcare settings.[1,2] The antibiotic resistance is correlated with inappropriate use of antimicrobial medicines.[3] Similarly, improper use of biocides also induces resistance to biocides.

Several laboratory studies have shown that cross-resistance to biocides and antibiotics may occur.[4-9] Cross-resistance has the potential to occur when different antimicrobial agents attack the same target, initiate a common pathway to cell death, or share a common route of access to their respective targets.[10] Relationships between reduced susceptibility to biocides and multidrug resistance among clinical isolates have also been investigated.[11]

There are several mechanisms of bacterial resistance or insusceptibility to antibiotics and biocides, such as mutation or overproduction of target molecules, production of enzymes which inactivate or decompose antibiotics or biocides, or barriers for entry or efflux of antibiotics or biocides.[12] Among these mechanisms efflux proteins in *P. aeruginosa* have been widely studied and shown to be associated with resistance of *P. aeruginosa* to some antibiotics and biocides.[7] Recently induction of mexCD-oprJ operon for a multidrug efflux pump by disinfectants in wild-type *P. aeruginosa*, and efflux mediated cross resistance to ciprofloxacin and benzalkonium chloride in *P. aeruginosa* were reported.[13,14] Non-specific increases in cell impermeability by producing a blanket through outer membrane changes were also considered to be a possible cause of cross-resistance in *P. stutzeri*.[15,16] These studies indicate that bacteria may become resistant to antibiotics when they acquire the mechanism which makes them cross resistant to both biocides and antibiotics by improper use of biocides. It has been revealed experimentally that repeated exposure of bacteria to sub-minimal inhibitory concentrations of biocides resulted in increases in MIC of wild type *P. aeruginosa*.[13] It might occur in clinical isolates when biocides are prepared inappropriately. Therefore, healthcare workers including nurses should be more careful in using biocides not to generate resistant bacteria to antibiotics. In Indonesia, situations which may mimic the exposure to sub-minimal inhibitory concentration of biocides, such as dilution of biocides by the eyes, repeated use of biocide solutions, or immersion of medical instruments which are not dried enough, are not uncommon. Therefore, bacteriological evidences demonstrate that improper use of biocides may induce insusceptibility to biocides and it may be followed by cross resistance to antibiotics making it necessary for promotion of more proper use of biocides in clinical settings in Indonesia.

This study aimed to investigate susceptibility to biocides of *S. aureus* isolated from nurses and *P. aeruginosa* isolated from hospital environments and to investigate whether more insensitive isolates to biocides were also more resistant to antibiotics. The reasons why this study focused on clinical isolates of *S. aureus* and *P. aeruginosa* were: 1) The susceptibility and mechanism of resistance to antibiotics are different between Gram-positives and Gram-negatives and both species are representative species of each group; 2) They are important and major pathogens of healthcare-associated infections (HAIs); and 3) Cross-resistance has been reported in both species.

METHODS

S. aureus and P. aeruginosa

S. aureus (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were used as a standard strain for each bacteria. 61 isolates of *S. aureus* and 46 isolates of *P. aeruginosa* were examined. Isolates of *S. aureus* were obtained from nurses' nasal cavities of the intensive care unit (ICU), internal wards and surgical wards of two hospitals in Yogyakarta, Indonesia. Isolates of *P. aeruginosa* were obtained from taps, sinks and tubs of patient and staff rooms in the same wards where *S. aureus* samples were isolated. They were identified biochemically using the VITEK 2 compact system (Sysmex Biomerieux Japan).

Biocides

20% chlorhexidine gluconate (CHG) solution (Wako Pure Chemical Industries, Ltd) and benzalkonium chloride (BZK) (MP Biomedicals, LLC) were used for the experiments. They were diluted to required concentrations with sterilized distilled water or Heart Infusion broth (HI broth) before use. The reasons why we chose CHG and BZK were because their abilities of disinfection were classified in low level and increases in insusceptibility of *P. aeruginosa* were reported by exposure to sub MIC of these biocides.[13] In addition information of biocides used in the wards was obtained.

Determination of MIC of standard strain

MICs of CHG and BZK of standard strain were determined by the following procedures: (1) Bacteria were cultivated in 3 mL of HI broth at 37°C overnight; (2) After centrifugation at 3000 rpm for 10 min, supernatant was removed and precipitated bacterial cells were washed with 6mL of sterilized saline twice to remove traces of growth media; (3) Bacteria were suspended in sterilized saline at the concentration of approximately 1-2x10^9cfu/mL; (4) 5mL of HI broth containing CHG or BZK with different concentrations were dispended to L-shaped tube for shaking culture; (5) 5μL of bacterial suspension prepared in (3) was added to each L-shaped tube, and then incubated at 37°C for 48hr shaking at 50rpm by a shaker (Compact rocking incubator TVS062CA, Advantec Toyo); and (6) Optical densities (OD) of L-shape tubes were continuously monitored and L-shaped tubes whose OD was lower than 0.1 or whose bacterial cell count which was less than inoculated cell count was considered as inhibited bacterial growth.[17] The concentration gradient of biocides was continuously narrowed until MIC for each biocide was determined.

Evaluation of susceptibility to biocides of the isolates

Susceptibility of the isolates to CHG and BZK was evaluated in the same way except that only MIC of CHG or BZK of the standard strains of *S. aureus* or *P. aeruginosa* was used as the concentration of biocides in the HI broth. L-shaped tubes which were obviously cloudy or bacterial cell count which was greater than inoculated cell count, or whose OD was higher than 0.1 for CHG or 0.2 for BZK was evaluated that growth of inoculated isolate was not inhibited at the MIC of

standard strain and inoculated isolate had higher MIC than that of standard strain.

Antibiotics susceptibility test

Microdilution method was used to determine MICs of the isolates to antibiotics. These antibiotics were provided in the condition being adherent to the bottom of 96-well microplate of antibiotic sensitivity test kit (Dry plate, Eiken Chemical Co., Ltd.). DP 32 and DP 35 were used for *S. aureus* and *P. aeruginosa*, respectively. Then susceptibility to antibiotics of isolates was categorized as S (Susceptible), IR (Intermediate Resistant), and R (Resistant) based on the MICs obtained.[18] We also collected the information of antibiotics used in the wards.

Statistical analysis

Chi square tests, Fisher's exact tests, or Kolmogorov-Smirnov tests were used to determine the relationship between susceptibility to antibiotics and biocides. Data were analyzed using SPSS version 22 (IBM-SPSS).

RESULTS

MIC of standard strain

MICs of CHG and BZK of *S. aureus* standard strain were determined to be 2.5μg/mL and 20μg/mL, respectively. MICs of CHG and BZK of *P. aeruginosa* standard strain were determined to be 25μg/mL and 200μg/mL, respectively. *P. aeruginosa* standard strain was much more insusceptible to both biocides (Table 1).

Table 1: MICS of standard strain.

Biocides	*S. aureus* (ATCC 25923)	*P. aeruginosa* (ATCC 27853)
CHG	2.5 μg/mL	25 μg/mL
BZK	20 μg/mL	200 μg/mL

Evaluation of susceptibility to biocides

Table 2: Number of isolates and insusceptibility to biocides.

Isolate	N	MIC group	CHG (%)	BZK (%)
S. aureus	61	Lower MIC	48 (78.7)	61 (100)
		Higher MIC	13 (21.3)	0
P. aeruginosa	46	Lower MIC	14 (30.4)	34 (73.9)
		Higher MIC	32 (69.6)	12 (26.1)

Only CHG was used in all wards. In *S. aureus*, MICs of CHG of 13 isolates (21.3%) were higher than the standard strains and no isolates exhibited higher MICs against BZK than the standard strains. In *P. aeruginosa*, the number of isolates which had higher MICs of CHG and BZK were 32 (69.6%) and 12 (26%), respectively. 10 (21.7%) isolates had higher MICs of both CHG and BZK (Table 2).

Comparison of susceptibility to antibiotics between the isolates with higher MICs and lower MICs of biocides

For *S. aureus*, most isolates were sensitive to the antibiotics tested except ampicillin. Resistance to ampicillin was observed in 48 (78.7%) of the isolated *S. aureus*. Regarding the antibiotics used in the ward, 7 (11.5%) isolates were classified to be resistant to vancomycin and 4 (6.6%) isolates were resistant to gentamycin and levofloxacin (Table 3).

Table 3: Susceptibility to antibiotics of isolated *S. aureus* (n=61).

Antibiotics	Interpretive criteria[#]		
	S, N (%)	IR, N (%)	R, N (%)
MPIPC	60 (98.4)	0	1 (1.6)
ABPC*	13 (21.3)	0	48 (78.7)
CEZ	60 (98.4)	1 (1.6)	0
CMZ	58 (95.1)	3 (4.9)	0
IPM	60 (98.4)	0	1 (1.6)
GM*	57 (93.4)	2 (3.3)	2 (3.3)
MINO	60 (98.4)	1 (1.6)	0
EM*	46 (75.4)	11(18.0)	4 (6.6)
CLDM	54 (88.5)	2 (3.3)	5 (8.2)
VCM*	54 (88.5)	5 (8.2)	2 (3.3)
TEIC	56 (91.8)	2 (3.3)	3 (4.9)
LZD	50 (82.0)	0	11 (18.0)
LVFX*	57 (93.4)	0	4 (6.6)
ST	61 (100)	0	0

#Interpretive criteria as defined by the Clinical and Laboratory Standards Institute. *Antibiotics used in the ward

In contrast to *S. aureus*, antibiotic resistance of isolated *P. aeruginosa* was obviously common in 8 of 14 antibiotics tested (Table 4). High prevalence of resistant isolates to the antibiotics which were not used in the ward such as tazobactam/piperacillin, doripenem, tobramycin was observed.

Next, susceptibility to antibiotics of the isolates with higher MICs of biocides (higher MIC group) were compared with that of the isolates with lower MICs (lower MIC group) (Tables 5, 6 and 7). There were three patterns of relationship between resistance of the isolates in the higher MIC group and the isolates in the Lower MIC group. The first pattern was that the isolates in the higher MIC group were more resistant than isolates in the Lower MIC group. The second pattern was that isolates in the higher MIC group were less resistant than the isolates in the Lower MIC group. The third pattern was that susceptibility of isolates in the higher MIC group were similar to the lower MIC group.

For *S. aureus*, resistance to ampicillin, cefazolin, cefmetazol, imipenem and teicoplanin were categorized

into the first pattern. Most antibiotics in the first pattern were β-lactams. Although statistical significance was not found (p = 0.167), more isolates in the higher MIC group (12; 92.3%) were resistant to ampicillin than isolates in the lower MIC group (36; 75%). The resistance to oxacillin, erythromycin, clindamycin, vancomycin and levofloxacin were categorized into the second pattern. The resistance to gentamycin, minocyclin, linezolid and sulfematoxazole trimethropim were categorized into the third pattern (Table 5). One isolate in the higher MIC group exhibited resistance to multiple antibiotics including β-lactams (ampicillin, imipenem), aminoglycoside (gentamicin), glycopeptide (teicoplanin), and oxazoidinones (linezolid).

Resistance to antibiotics of *P. aeruginosa* isolates was shown in the Table 6 and 7. For CHG, resistance to tazobactam/piperacillin, cefepime, ceftazidime, imipenem, gentamycin, tobramycin, amikacin, and colistine were categorized into the first pattern.

Table 4: Susceptibility to antibiotics of isolated in *P. aeruginosa* (n= 46).

Antibiotics	Interpretive criteria[#]		
	S, N (%)	IR, N (%)	R, N (%)
PIPC	36 (78.3)	5 (10.9)	5 (10.9)
CFPM*	31 (67.4)	9 (19.6)	6 (13.0)
IPM	28 (60.9)	3 (6.5)	15 (32.6)
MEPM*	26 (56.5)	4 (8.7)	16 (34.8)
DRPM	30 (65.2)	3 (6.5)	13 (28.3)
TAZ/PIPC	29 (63.0)	7 (15.2)	10 (21.8)
AZT	33 (71.7)	10 (21.8)	3 (6.5)
GM*	35 (76.1)	4 (8.7)	7 (15.2)
TOB	30 (65.2)	2 (4.4)	14 (30.4)
AMK*	32 (69.5)	1 (2.2)	13 (28.3)
CL	34 (73.9)	1 (2.2)	11 (23.9)
LVFX*	28 (60.9)	7 (15.2)	11 (23.9)
CPFX	31 (67.4)	3 (6.5)	12 (26.1)
CAZ	28 (60.9)	12 (26.1)	6 (13.0)

#Interpretive criteria as defined by the Clinical and Laboratory Standards Institute. *Antibiotics used in the wards.

Table 5: Relationship of susceptibility to CHG and antibiotics in *S. aureus* (n=61).

Classification of Antibiotics		MIC Group		Interpretive criteria[#]			p
				S	I[##]	R	
				N (%)	N (%)	N (%)	
β-laktams: Penicillins	MPIPC	Lower MIC	48	47 (97.9)	0	1 (2.1)	0.787
		Higher MIC	13	13 (100)	0	0	
	ABPC	Lower MIC	48	12 (25)	0	36 (75)	0.167
		Higher MIC	13	1 (7.7)	0	12 (92.3)	
Cephalosporins	CEZ	Lower MIC	48	48 (100)	0	0	0.213
		Higher MIC	13	12 (92.3)	1 (7.7)	0	
	CMZ	Lower MIC	48	47 (97.9)	1 (2.1)	0	0.112
		Higher MIC	13	11 (84.6)	2 (15.4)	0	
Carbapenems	IPM	Lower MIC	48	48 (100)	0	0	0.213
		Higher MIC	13	12 (92.3)	0	1 (7.7)	
Aminoglycosides	GM	Lower MIC	48	45 (93.7)	2 (4.2)	1 (2.1)	0.627
		Higher MIC	13	12 (92.3)	0	1 (7.7)	
Tetracycline	MINO	Lower MIC	48	47 (97.9)	1 (2.1)	0	0.787
		Higher MIC	13	13 (100)	0	0	
Macrolide	EM	Lower MIC	48	35 (72.9)	9 (18.8)	4 (8.3)	0.318
		Higher MIC	13	11 (84.6)	2 (15.4)	0	
Lincosamide	CLDM	Lower MIC	48	41 (85.4)	2 (4.2)	5 (10.4)	0.169
		Higher MIC	13	13 (100)	0	0	
Glycopeptides	VCM	Lower MIC	48	41 (85.4)	5 (10.4)	2 (4.2)	0.169
		Higher MIC	13	13 (100)	0	0	
	TEIC	Lower MIC	48	45 (93.7)	1 (2.1)	2 (4.2)	0.287
		Higher MIC	13	11 (84.6)	1 (7.7)	1 (7.7)	
Oxazolidinones	LZD	Lower MIC	48	39 (81.2)	0	9 (18.8)	0.570
		Higher MIC	13	11 (84.6)	0	2 (15.4)	
New quinolones	LVFX	Lower MIC	48	44 (91.7)	0	4 (8.3)	0.373
		Higher MIC	13	13 (100)	0	0	
Sulfonamide-trimethoprim	ST	Lower MIC	48	48 (100)	0	0	-
		Higher MIC	13	13 (100)	0	0	

#Interpretive criteria as defined by the Clinical and Laboratory Standards Institute was used. ##Intermediate resistance was included to resistance and 2x2 table analysis was used

Table 6: Relationship between susceptibility to CHG and antibiotics in P. aeruginosa (n=46).

Classification of antibiotics		MIC Group		Interpretive criteria[#] S N (%)	IR[##] N (%)	R N (%)	*p*
β-laktam	PIPC	Lower MIC	14	9 (64.3)	3 (21.4)	2 (14.3)	0.130
		Higher MIC	32	27 (84.4)	2 (6.2)	3 (9.4)	
Penicillins	TAZ/ PIPC	Lower MIC	14	11 (78.6)	1 (7.1)	2 (14.3)	0.133
		Higher MIC	32	18 (56.2)	6 (18.8)	8 (25)	
Cephems	CFPM	Lower MIC	14	10 (71.4)	2 (14.3)	2 (14.3)	0.489
		Higher MIC	32	21 (65.6)	7 (21.9)	4 (12.5)	
	CAZ	Lower MIC	14	11 (78.6)	1 (7.1)	2 (14.3)	0.095
		Higher MIC	32	17 (53.1)	11 (34.4)	4 (12.5)	
Carbapenems	IPM	Lower MIC	14	11 (78.6)	1 (7.1)	2 (14.3)	0.095
		Higher MIC	32	17 (53.1)	2 (6.3)	13 (40.6)	
	MEPM	Lower MIC	14	5 (35.7)	2 (14.3)	7 (50)	0.060
		Higher MIC	32	21 (65.6)	2 (6.3)	9 (28.1)	
	DRPM	Lower MIC	14	7 (50)	1 (7.1)	6 (42.9)	0.137
		Higher MIC	32	23 (71.9)	2 (6.3)	7 (21.9)	
Monobactams	AZT	Lower MIC	14	7 (50)	5 (35.7)	2 (14.3)	0.037
		Higher MIC	32	26 (81.3)	5 (15.6)	1 (3.1)	
Aminoglycosides	GM	Lower MIC	14	12 (85.7)	0	2 (14.3)	0.269
		Higher MIC	32	23 (71.9)	4 (12.5)	5 (15.6)	
	TOB	Lower MIC	14	12 (85.7)	0	2 (14.3)	0.05
		Higher MIC	32	18 (56.2)	2 (6.3)	12 (37.5)	
	AMK	Lower MIC	14	14 (100)	0	0	0.002
		Higher MIC	32	18 (56.2)	1 (2.2)	13 (40.6)	
Lincomycins	CL	Lower MIC	14	12 (85.7)	1 (7.1)	1 (7.1)	0.077
		Higher MIC	32	22 (68.7)	0	10 (31.3)	
New quinolones	LVFX	Lower MIC	14	8 (57.1)	2 (14.3)	4 (28.6)	0.404
		Higher MIC	32	20 (62.5)	5 (15.6)	7 (21.9)	
	CPFX	Lower MIC	14	10 (71.4)	1 (7.1)	3 (21.4)	0.489
		Higher MIC	32	21(65.6)	2 (6.3)	9 (28.1)	

[#]Interpretive criteria as defined by the Clinical and Laboratory Standards Institute was used. [##]Intermediate resistant was included to resistant and 2x2 table analysis was used.

Resistance to aminoglycosides were categorized into the first pattern, and statistical significance was found in amikacin (p = 0.002). 14 (42.8%) isolates in the Higher MIC group were resistant to amikacin whereas no isolates in the lower MIC group exhibited resistance. Resistance to β-lactams in higher MIC group were different depending on the antibiotics. The resistance to piperacillin, meropenem, doripenem and aztreonam were categorized into the second pattern and the ratio of resistant isolates to aztreonam in the lower MIC group was significantly greater than the higher MIC group (*p* = 0.037). Resistance to new quinolones (levofloxacin and ciprofloxacin) were categorized into the third pattern (Table 6).

For BZK, resistance to piperacillin, tazobactam/piperacillin, ceftazidime, gentamycin and levofloxacin were categorized into the first pattern. However, no significant differences were found. The resistance to meropenem, amikacin and colistin were categorized into the second pattern and statistical significance was found in resistance to meropenem (p = 0.030). Only 2 (16.7%) isolates in the higher MIC group were resistant to meropenem whereas 18 (53%) isolates in the lower MIC group exhibited resistance. The resistance to cefepime, imipenem, doripenem, aztreonam, tobramycin, and ciprofloxacin were categorized into the third pattern. Increased resistance to aminoglycoside of the isolates in the higher MIC group was not observed for BZK except resistance to gentamycin (Table 7). Resistance to new quinolones in the higher MIC group differed among each of the antibiotics.

DISCUSSION

Increases in insusceptibility to CHG were found in both *S. aureus* and *P. aeruginosa* isolates. It was remarkable in *P. aeruginosa* and approximately 70% of the isolates had higher MIC than the standard strain. In the present study, *P. aeruginosa* isolates were obtained from hospital environments whereas *S. aureus* were obtained from nurses' nasal cavities. The hospital environments seemed to be exposed to biocides more frequently than the nasal

cavity, and it resulted in increased insusceptibility of *P. aeruginosa* in the hospital environments.

Unlike CHG, insusceptibility to BZK was observed only in *P. aeruginosa* isolates. Moreover, 10 isolates were insusceptible to both CHG and BZK. There are two possibilities to explain why insusceptibility to BZK was found only in *P. aeruginosa* although BZK was not used in the wards. First, *P. aeruginosa* isolates with higher MIC to BZK were innately insensitive to BZK. Secondly, increases in MIC to BZK were induced by repeated exposure to CHG due to sharing the same mechanism of resistance to CHG and BZK in *P. aeruginosa*. The latter means that MICs of BZK of the isolates with Higher MIC increased through cross-resistance between CHG and BZK. In fact, the induction of mexCD-oprJ operon for multidrug efflux pump in wild-type *P. aeruginosa* by CHG and BZK was reported. Exposure to CHG in the hospital environment might induce mexCD-oprJ operon in *P. aeruginosa* and the efflux pump also might work for the exclusion of BZK.[13]

Table 7: Relationship between susceptibility to BZK and antibiotics in P. aeruginosa (n=46).

Classification of Antibiotics		MIC of BZK		Interpretive criteria[#] S N (%)	IR[##] N (%)	R N (%)	*p*
β-laktam	PIPC	Lower MIC	34	28 (82.4)	3 (8.8)	3 (8.8)	0.229
		Higher MIC	12	8 (66.7)	2 (16.7)	2 (16.7)	
Penicillins	TAZ/PIPC	Lower MIC	34	23 (67.6)	5 (14.7)	6 (17.6)	0.228
		Higher MIC	12	6 (50)	2 (16.7))	4 (33.3)	
Cephems	CFPM	Lower MIC	34	23 (67.6)	7 (20.6)	4 (11.8)	0.608
		Higher MIC	12	8 (66.7)	2 (16.7)	2 (16.7)	
	CAZ	Lower MIC	34	22 (64.7)	8 (23.5)	4 (11.8)	0.288
		Higher MIC	12	6 (50)	4 (33.3)	2 (16.7)	
Carbapenems	IPM	Lower MIC	34	20 (58.8)	3 (8.8)	11 (32.4)	0.452
		Higher MIC	12	8 (66.7)	0	4 (33.3)	
	MEPM	Lower MIC	34	16 (47)	4 (11.8)	14 (41.2)	0.030
		Higher MIC	12	10 (83.3)	0	2 (16.7)	
	DRPM	Lower MIC	34	21 (61.8)	3 (8.8)	10 (29.4)	0.323
		Higher MIC	12	9 (75.0)	0	3 (25)	
Monobactams	AZT	Lower MIC	34	24 (70.6)	7 (20.6)	3 (8.8)	0.543
		Higher MIC	12	9 (75)	3 (25)	0	
Aminoglycosides	GM	Lower MIC	34	27 (79.4)	2 (5.9)	5(14.7)	0.302
		Higher MIC	12	8 (66.7)	2 (16.7)	2(16.7)	
	TOB	Lower MIC	34	22 (64.7)	1 (2.9)	1(32.4)	0.597
		Higher MIC	12	8 (66.7)	1 (8.3)	3 (25)	
	AMK	Lower MIC	34	22 (64.7)	1 (2.9)	11 (32.4)	0.203
		Higher MIC	12	10 (83.3)	0	2 (16.7)	
Lincomycins	CL	Lower MIC	34	23 (67.6)	1 (2.9)	10 (29.4)	0.139
		Higher MIC	12	11 (91.7)	0	1 (8.3)	
New quinolones	LVFX	Lower MIC	34	22 (64.7)	5 (14.7)	7 (20.6)	0.302
		Higher MIC	12	6 (50)	2 (16.7)	4 (33.3)	
	CPFX	Lower MIC	34	23 (67.6)	2 (5.9)	9 (26.5)	0.608
		Higher MIC	12	8 (66.7)	1 (8.3)	3 (25)	

[#]Interpretive criteria as defined by the Clinical and Laboratory Standards Institute was used. [##]Intermediate resistant was included to resistant and 2x2 table analysis was used. MPIPC=oxacillin, ABPC=ampicillin, CEZ=cefazolin, CMZ=cefmetazol, IPM=imipenem, GM=gentamycin, MINO=minocycline, EM=erythromycin, CLDM=clindamycin, VCM=vancomycin, TEIC=teicoplanin, LZD=linezolid, LVFX=levofloxacin, ST=sulfematoxazole trimethoprim, PIPC=piperacillin, CFPM=cefepime, MEPM=meropenem, DRPM=doripenem, TAZ/PIPC=tazobactam/piperacillin, AZT=aztreonam, TOB=tobramycin, AMK=amikacin, CL=colistine, CPFX=ciprofloxacin, CAZ=ceftazidime

Susceptibility of the isolates to the antibiotics were also different between the isolated *S. aureus* and *P. aeruginosa*. Compared to *P. aeruginosa*, isolates of *S. aureus* were sensitive to tested antibiotics except ampicillin, to which 78.7 % of isolates were resistant. High incidence of ampicillin resistance in *S. aureus* is also common in other countries.[19,20] Isolated *P. aeruginosa* exhibited resistance to more antibiotics than *S. aureus*. In the present study, rate of antibiotic resistance of isolated *P. aeruginosa* in 8 antibiotics tested

were almost more than 30%. These rates of resistance seemed to be similar to those reported in India and USA although most *P. aeruginosa* were isolated from clinical samples in these studies.[21-23] Relationships between antibiotic resistance and insusceptibility to biocides differed among antibiotics.

However, significant decrease in susceptibility to amikacin of isolated *P. aeruginosa* with higher MICs of CHG was observed. Susceptibilities to other aminoglycosides were also reduced in *P. aeruginosa* isolates with higher MICs of CHG although statistical significance was not found. As mentioned above, expression of multidrug efflux system is related to resistance to certain antibiotics and biocides in *P. aeruginosa*. If the multidrug efflux systems which can exclude both CHG and aminoglycosides are expressed in the isolates with higher MIC of CHG, these isolates become resistant to aminoglycosides. *P. aeruginosa* has several multidrug efflux systems, of which MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY are significant determinants of multidrug resistance in laboratory and clinical isolates.[24-27]

Among these systems the MexXY system has been comprehensively studied and has been recognized as one of the primary determinants of aminoglycoside resistance.[25,28] On the other hand, increased susceptibility to CHG is considered to be mediated by the mexCD-oprJ system.[13] Therefore, increases in resistance to amikacin in isolated *P. aeruginosa* with higher MIC of CHG observed in the present study may be due to the expression of different multidrug efflux systems induced independently by exposure to CHG and amikacin, since both CHG and amikacin were frequently used in the wards where the isolates were collected. Further analysis about the mechanisms by which the isolates exhibited higher MICs of CHG or resistance to amikacin is necessary. In *S. aureus* more isolates (92.3%) with higher MICs of CHG exhibited resistance to ampicillin. However, it was also high (75%) in the isolates with lower MICs although statistical significance was not found. Therefore, it was suggested that there was no significant relationship between resistance to ampicillin and insusceptibility to CHG in isolated *S. aureus.*

In the present study, only 61 *S. aureus* isolates and 48 *P. aeruginosa* isolates were examined. Further studies with more isolates are necessary.

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