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 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.⁵ Relationships between reduced susceptibility to biocides and multidrug resistance among clinical isolates have also been investigated.⁶

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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. 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. 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. 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*. 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*. 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 (HAI); 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. 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 1-2x10⁹cfu/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. 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 2.5 μg/mL and 20 μg/mL, respectively. MICs of CHG and BZK of *P. aeruginosa* standard strain were determined to be 25 μg/mL and 200 μg/mL, respectively. *P. aeruginosa* standard strain was much more insusceptible to both biocides (Table 1).

**Evaluation of susceptibility to biocides**

**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                     |

**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 criteriaa | 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, minocycline, linezolid and sulfamethoxazole trimethoprim 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 oxazolidinones (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 colistin were categorized into the first pattern.

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

| Antibiotics | Interpretive criteria\(^a\) |
|-------------|-----------------------------|
|             | S, N (%) | IR, N (%) | R, N (%) |
| PIPC        | 36 (78.3) | 5 (10.9) | 5 (10.9) |
| CFP\(^*\)   | 31 (67.4) | 9 (19.6) | 6 (13.0) |
| IPM         | 28 (60.9) | 3 (6.5) | 15 (32.6) |
| MEP\(^*\)   | 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) |
| CFX         | 31 (67.4) | 3 (6.5) | 12 (26.1) |
| CAZ         | 28 (60.9) | 12 (26.1) | 6 (13.0) |

\(^a\)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\(^b\) | \(P\) |
|------------------------------|-----------|-----------------------------|-------|
| \(\beta\)-lactams: Penicillins | MPPIC     | Lower MIC 48, 47 (97.9), 0, 1 (2.1) | 0.787 |
|                              |           | Higher MIC 13, 13 (100) | 0, 0 |
| Cephalosporins               | CEZ       | Lower MIC 48, 48 (100) | 0, 0 |
|                              |           | Higher MIC 13, 12 (92.3) | 1 (7.7) |
|                              | CMZ       | Lower MIC 48, 47 (97.9) | 1 (2.1) |
|                              |           | Higher MIC 13, 11 (84.6) | 2 (15.4) |
| Carbapenems                  | IPM       | Lower MIC 48, 48 (100) | 0, 0 |
|                              |           | Higher MIC 13, 12 (92.3) | 0, 1 (7.7) |
| Aminoglycosides              | GM        | Lower MIC 48, 45 (93.7) | 2 (4.2) |
|                              |           | Higher MIC 13, 12 (92.3) | 0, 1 (7.7) |
| Tetracycline                 | MINO      | Lower MIC 48, 47 (97.9) | 1 (2.1) |
|                              |           | Higher MIC 13, 13 (100) | 0, 0 |
| Macrolide                    | EM        | Lower MIC 48, 35 (72.9) | 9 (18.8) |
|                              |           | Higher MIC 13, 11 (84.6) | 2 (15.4) |
| Lincosamide                  | CLDM      | Lower MIC 48, 41 (85.4) | 2 (4.2) |
|                              |           | Higher MIC 13, 13 (100) | 0, 0 |
| Glycopeptides                | VCM       | Lower MIC 48, 41 (85.4) | 5 (10.4) |
|                              |           | Higher MIC 13, 13 (100) | 0, 0 |
|                              | TEIC      | Lower MIC 48, 45 (93.7) | 1 (2.1) |
|                              |           | Higher MIC 13, 11 (84.6) | 1 (7.7) |
| Oxazolidinones               | LZD       | Lower MIC 48, 39 (81.2) | 0, 9 (18.8) |
|                              |           | Higher MIC 13, 11 (84.6) | 0, 2 (15.4) |
| New quinolones               | LVFX      | Lower MIC 48, 44 (91.7) | 0, 4 (8.3) |
|                              |           | Higher MIC 13, 13 (100) | 0, 0 |
| Sulfonamide-trimethoprim     | ST        | Lower MIC 48, 48 (100) | 0, 0 |
|                              |           | Higher MIC 13, 13 (100) | 0, 0 |

\(^b\)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\(^a\) | \(p\) |
|------------------------------|-----------|-------------------------------|-------|
| \(\beta\)-laktam            | PIPC      | S (\%) | IR\(^b\) (\%) | R (\%) |       |
|                              | 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 (24.9) | 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) |
| Lincomycins                  | AMK       | Lower MIC | 14 | 14 (100) | 0 | 0 | 0.002 |
|                              | Higher MIC | 32 | 18 (56.2) | 1 (2.2) | 13 (40.6) |
| New quinolones               | 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) |
|                              | 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) |

\(^a\)Interpretive criteria as defined by the Clinical and Laboratory Standards Institute was used. \(^b\)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 \(\beta\)-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 cavities.
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 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 criteriaa | S | IRb | R | p |
|------------------------------|------------|------------------------|---|-----|---|---|
| **β-laktam**                 |            |                        |   |     |   |   |
| Penicillins                  | 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) |
| 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 (33.3) | 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 (3.8) | 3 (25) |
| AMK                         | Lower MIC  | 34 | 22 (64.7) | 1 (2.9) | 11 (32.4) | 0.203 |
|                             | Higher MIC | 12 | 10 (33.3) | 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) |
| CFX                         | 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) |

*aInterpretive criteria as defined by the Clinical and Laboratory Standards Institute was used. bIntermediate resistant was included to resistant and 2x2 table analysis was used. M=gentamicin, MINO=minocycline, EM=erythromycin, CLDM=clindamycin, VCM=vancomycin, TEIC=teicoplanin, LZD=linezolid, LVFX=levofloxacin, ST=sulfamethoxazole trimethoprim, PIPC=piperacillin, CFPM=cefpime, MEP=meropenem, DRPM=doripenem, TAZ/PIPC=tazobactam/piperacillin, AZT=aztreonam, TOB=tobramycin, AMK=amikacin, CL=colistine, CPFX=ciprofloxacin, CAZ=ceftazidine

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 \textit{P. aeruginosa} were isolated from clinical samples in these studies.\textsuperscript{21-23} Relationships between antibiotic resistance and insusceptibility to biocides differed among antibiotics.

However, significant decrease in susceptibility to amikacin of isolated \textit{P. aeruginosa} with higher MICs of CHG was observed. Susceptibilities to other aminoglycosides were also reduced in \textit{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 \textit{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. \textit{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.\textsuperscript{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.\textsuperscript{25,28} On the other hand, increased susceptibility to CHG is considered to be mediated by the mexCD-oprJ system.\textsuperscript{13} Therefore, increases in resistance to amikacin in isolated \textit{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 \textit{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 \textit{S. aureus}.

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

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