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Full Length Research Paper

Trend of antibiotics susceptibility of multidrugs resistance *Pseudomonas aeruginosa* in Jakarta and surrounding areas from 2004 to 2010

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Pseudomonas aeruginosa **is an opportunistic Gram negative microorganism, usually related to serious infections within hospital environment and causes significant increase in patient's morbidity and mortality. This study aimed to report antibiotic susceptibility of** ***P. aeruginosa*** **originated from all kind of specimens received at Clinical Microbiology Laboratory of the Faculty of Medicine, University of Indonesia, Jakarta, from 2004 to 2010, and evaluate their trend of susceptibility to certain antibiotics. Culture and identification of specimens were performed according to standard microbiology procedures. Antibiotic susceptibility tests were carried out according to performance standards for antimicrobial susceptibility testing from the Clinical and Laboratory Standards Institute. The data was processed using WHO-NET Version 5.6 program.** ***P. aeruginosa*** **was constantly found between 12 to 19% among other Gram negative bacteria. A significant decrease of susceptibility against ceftazidim, cefepime, cefoperazone, gentamicin, amikacin, tobramycin, ciprofloxacin, levofloxacin, meropenem and imipenem was observed. Susceptibility to aztreonam and piperacilline-tazobactam was decreased, though it was not statistically significant. In 2010, among the anti-pseudomonas antibiotics, imipenem showed good activity (80%). Overall, declining trend of susceptibility to all antibiotic tested was significantly observed. Imipenem was found to be the only anti-pseudomonas antibiotic with good activity (80%).**

Key words: Gram negative bacteria, *Pseudomonas aeruginosa* and antibiotic susceptibility.

INTRODUCTION

Bacterial infections are becoming more difficult to treat. At present, 70% of nosocomial infections are resistant to at least one antimicrobial drug that previously was effective for the causative pathogens. Microbes that are notorious for their virulence and able to develop resistance include *Staphylococcus aureus, Enterococcus* spp., members of the *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter* species (Carmeli, 2008). Recent data from the U.S. National Healthcare Safety Network indicated that Gram negative bacteria was responsible for more than 30% of health care associated infection (HAI), and these bacteria were found predominately in cases of ventilator-associated pneumonia (47%) and urinary tract infections (45%) (Peleg and Hooper, 2010). A multicenter study showed an increasing number of Gram negative bacteria isolated from blood both from hospital and community acquired infection cases. Of the 12,781 causative organisms, Gram negative aerobic bacteria

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were 47.4%, whereas Gram positives accounted for 43.9% (Luzzaro et al., 2011). Likewise a study carried out by Moehario et al. (2009) in Jakarta found that *P. aeruginosa* was the second most isolated after *Acinetobacter anitratus* from blood specimen. Among all positive isolates from blood specimen, Gram negative bacteria was found approximately 68% with eight most frequent species isolated were *A. anitratus* (25.8%), *P. aeruginosa* (19.5%), *Klebsiella pneumoniae subsp. pneumoniae* (14.5%), *Enterobacter aerogenes* (8%), *Salmonella Typhi* (7.5%), *Escherichia coli* (6.2%), *Alcaligenes faecalis* (5.6%) and *Klebsiella oxytoca* (3.2%). This earlier study also showed *P. aeruginosa* resistant to antibiotic ceftriaxone, cefotaxime, amikacin, gentamycin, and susceptible to ciprofloxacin (77.8%), levofloxacin (92.2%) and cefepime (88.9%)(Moehario et al., 2009).

P. aeruginosa is an opportunistic pathogen and most commonly present serious infection and therapeutic threat within hospital environment. Infections caused by multidrug resistance (MDR) *P. aeruginosa* have been associated with significant increase in patients' morbidity and mortality, length of hospital stay, requirement for additional medical procedure and surgery, chronic care, and overall cost (Lister and Wolter, 2011). As persist else where, more and more cases due to the MDR *P. aeruginosa* occur in hospitals in Jakarta and the adjacent areas and give rise to serious problems. Thus, information on antibiotics for treatment of these patients is seriously needed. Emphirical therapy should, however, be in line with local condition.

This study aimed to report antibiotic susceptibility of *P. aeruginosa* originated from all kind of specimens received in our laboratoryin 2004 to 2010, and evaluate their trend of resistance to certain antibiotics.

MATERIALS AND METHODS

Specimens

All kinds of specimens, that is, blood, sputum, pus, urine and throat swab were received in the laboratory of Clinical Microbiology, Faculty of Medicine, University of Indonesia (CML-FMUI). The specimens' derived from hospitals, among other was National Hospital Cipto Mangunkusumo (a tertiary general public hospital), private practices and individuals.

Cultures and antibiotic susceptibility tests

Cultures and susceptibility tests to antibiotics were performed according to CML-FMUI standard practices (CML-FMUI, 2004, 2009) and performance standards for antimicrobial susceptibility testing from the Clinical and Laboratory Standards Institute (CLSI, 2012). Cultures were performed using Bactec 9050 (Becton Dickinson) and microorganism identification was determined using standard biochemical reactions; in recent years API20E biochemical identification system (BioMerieux) was used instead. The susceptibility of microorganisms to antibiotics was assessed using disc diffusion method. Antimicrobial susceptibility results were categorized in to three groups: sensitive (S), intermediate (I) and resistant (R) according to CLSI. The antibiotics susceptibility data was processed and analyzed using WHO-NET Version 5.6 program.

Antibiotics

Not all antibiotic discs were tested in all consecutive years due to inconsistent availability (stock shortage and laboratory policy). The following antibiotic discs were used continuously from 2004 to 2010: cefepime (FEP) 30 µg, ciprofloxacin (CIP) 5 µg, amikacin (AMK) 30 µg, gentamicin (GEN) 10 µg and meropenem (MEM) 10 µg. In 2008 to 2010, ceftazidime (CAZ) 30 µg, cefoperazone (CFP) 75 µg, tobramycin (TOB) 10 µg, levofloxacin (LVX) 5 µg, piperacillin-tazobactam (TZP) 110 µg, and imipenem (IPM) 10 µg were also tested. Aztreonam (ATM) 30 µg was evaluated for two years, 2009-2010. Susceptibility of *P. aeruginosa* to antibiotics were tabulated and good activity *in vitro* was defined by antimicrobial susceptibility of 80% or greater.

Statistical analysis

Chi-square test was employed to analyze the significant decrease or increase susceptibility to particular antibiotics between years using space plans systems (SPS) version 16.

RESULTS

P. aeruginosa countinously persisted, 12 to19% from all specimens of total Gram negative bacteria (Figure 1). Among all Gram negative microorganisms isolated from 2004 to 2010, eight most frequent ones were presented in Table 1. Overall, *P. aeruginosa* was in the big five most frequent Gram negative bacteria found in all specimens. It was the second most frequent in 2004, the third in 2005, 2007 to 2009, and the fourth in 2006 and 2010. Antipseudomonas antibiotics were tested against all *P. aeruginosa* isolates, and the susceptibility patterns in 2010 was presented in Figure 2. It appreared that only antibiotic imipenem had good activity *in-vitro*, that is 80%, while meropenem, amikacin and piperacillin-tazobactam were less active.

Over period of 7 years until 2010, antibiotic susceptibility patterns of *P. Aeruginosa* showed declining trend to most antibiotic tested except for gentamicin and ciprofloxacin (Figure 3). Some fluctuation of susceptibility were observed within those periods for cefepime, amikacin, gentamicin, ciprofloxacin, meropenem. Statistically, however, either the increase or the decreasesuscepticility of each antibiotics was significant that is AMK $p = 0.000$, GEN $p = 0.002$, CIP $p = 0.003$, FEP $p = 0.000$, MEM $p = 0.000$. Despite the apparent increase trend of susceptibility observed for GEN and CIP from 2007 to 2010, there was a significant decrease of susceptibility of these antibiotics (Figure 3).

Susceptibility of *P.aeruginosa* to cephalosporins as presented in Figure 4 showed decreasing trendof susceptibility for ceftazidime and cefepime of more than 10%, and was statistically significant (CAZ $p = 0.000$ and FEP $p = 0.006$). Interestingly, for cefoperazone there was

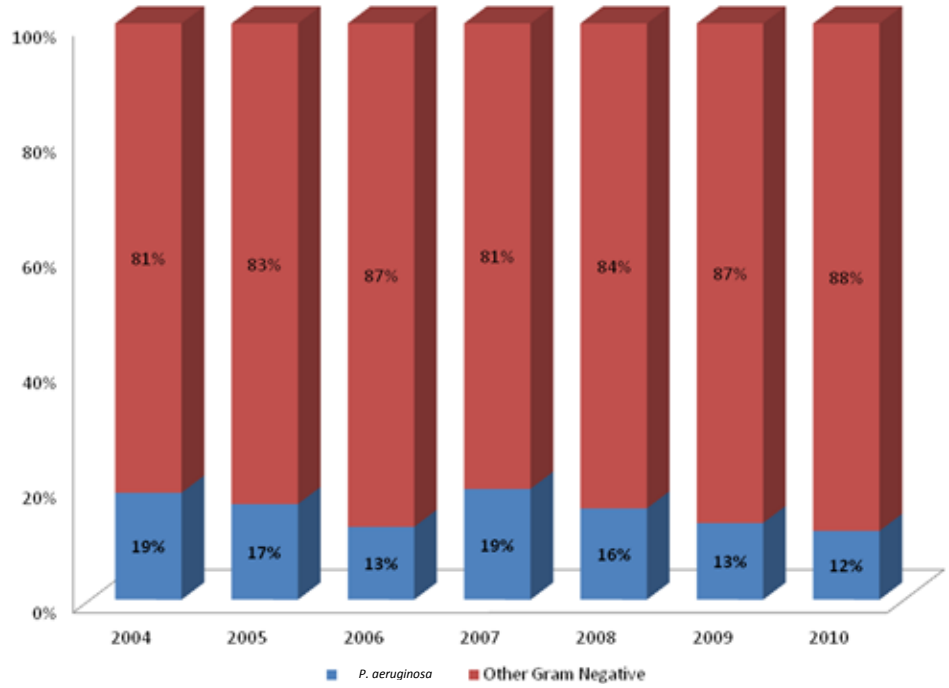

Figure 1. Percentage of *P. aeruginosa*isolatescomparedto other Gram negative bacteria from 2004 to 2010.

Table 1. Eight most frequent Gram negative bacteria isolates in 2004 to 2010.

2004		2005		2006		2007		2008		2009		2010	
Isolates	**No.of isolates**	**Isolates**	**No.of isolates**	**Isolates**	**No.of isolates**	**Isolates**	**No.of isolates**	**Isolates**	**No.of isolates**	**Isolates**	**No.of isolates**	**Isolates**	**No.of isolates**
K. pneumoniae ss. Pneumoniae	*416*	*K. pneumoniae ss. Pneumoniae*	*257*	*K. pneumoniae ss. Pneumoniae*	*140*	*K. pneumoniae ss. Pneumoniae*	*111*	*K.pneumoniae ss. Pneumoniae*	*136*	*E. coli*	*103*	*K.pneumonia ss.pneumonia*	*80*
P. aeruginosa	*282*	*E. coli*	*233*	*E. coli*	*106*	*E. coli*	*103*	*E. coli*	*98*	*K. pneumonia ss.pneumonia*	*69*	*E.coli*	*59*
E. coli	*256*	*P. aeruginosa*	*192*	*A. anitratus*	*71*	*P. aeruginosa*	*95*	*P. aeruginosa*	*80*	*P.aeruginosa*	*45*	*A.baumanii*	*47*
A. anitratus	*146*	*A. anitratus*	*153*	*P. aeruginosa*	*67*	*A. anitratus*	*45*	*A. anitratus*	*63*	*A.anitrtus*	*25*	*P.aeruginosa*	*45*
E. aerogenes	*127*	*E. aerogenes*	*95*	*E. aerogenes*	*43*	*P. mirabilis*	*41*	*P. mirabilis*	*37*	*P.mirabilis*	*23*	*E.cloacae*	*16*
P. mirabilis	*100*	*P. mirabilis*	*73*	*P. mirabilis*	*29*	*E. aerogenes*	*24*	*E. aerogenes*	*31*	*K.oxytoca*	*15*	*S.odorifera*	*15*
A. faecalis (odorans)	*54*	*A. faecalis (odorans)*	*67*	*A. faecalis (odorans)*	*24*	*Alcaligenes sp.*	*18*	*K. pneumoniae ss. Ozaenae*	*18*	*K.pneumonia ss.ozaenae*	*12*	*C. luteola*	*12*
K. oxytoca	*34*	*P. agglomerans*	*18*	*K. pneumoniae ss. Ozaenae*	*9*	*P. agglomerans*	*17*	*K. oxytoca*	*12*	*A.baumanii*	*10*	*K.oxytoca*	*12*

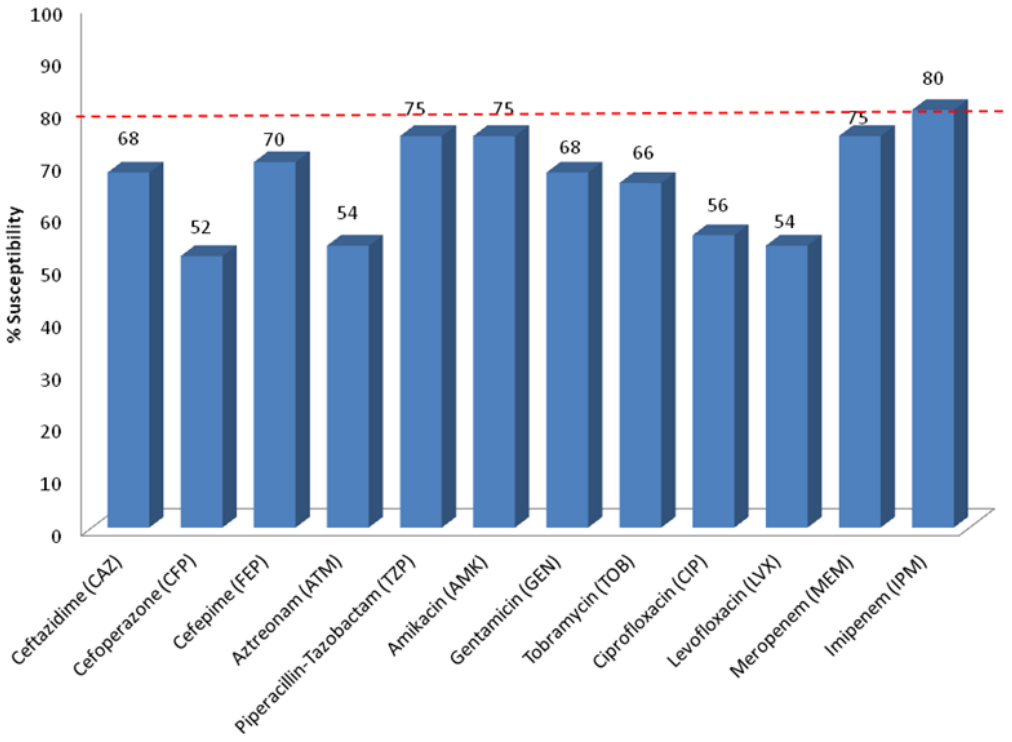

Figure 2. Susceptibility of *P. aeruginosa* to antipseudomonas tested in 2010.

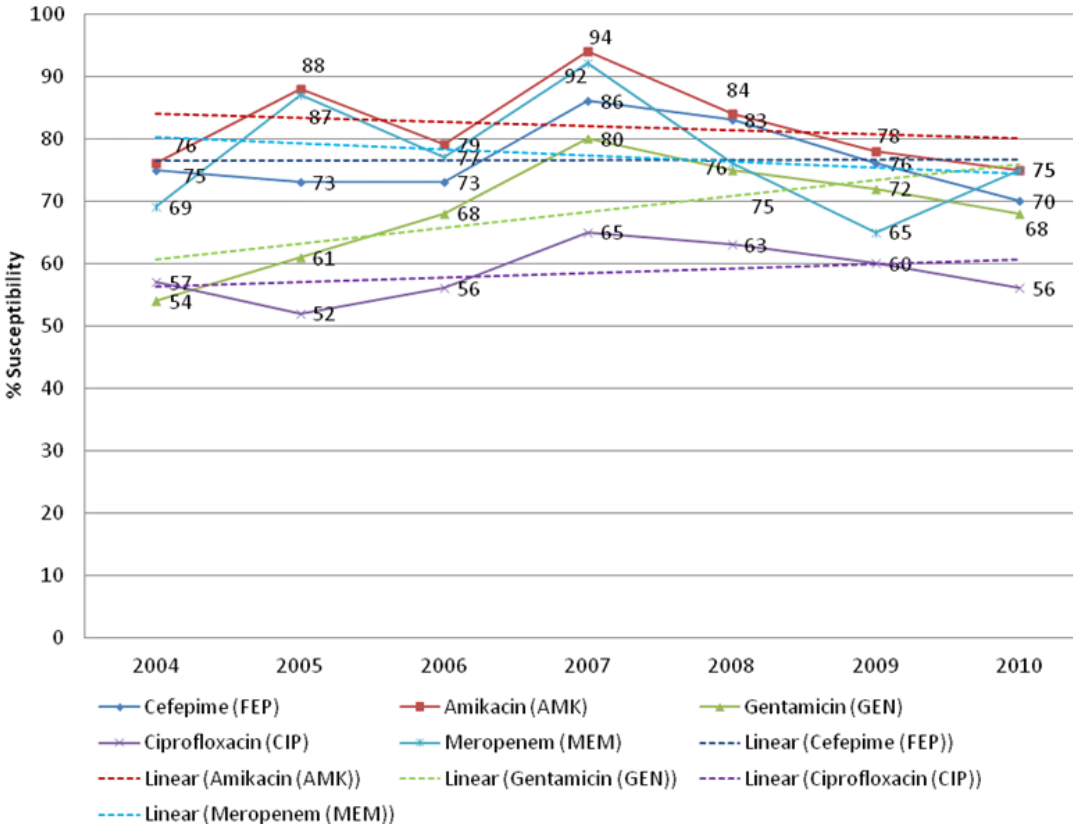

Figure 3. Changing pattern of *P. aeruginosa* susceptibility to antipseudomonas antibiotics used from 2004 to 2010.

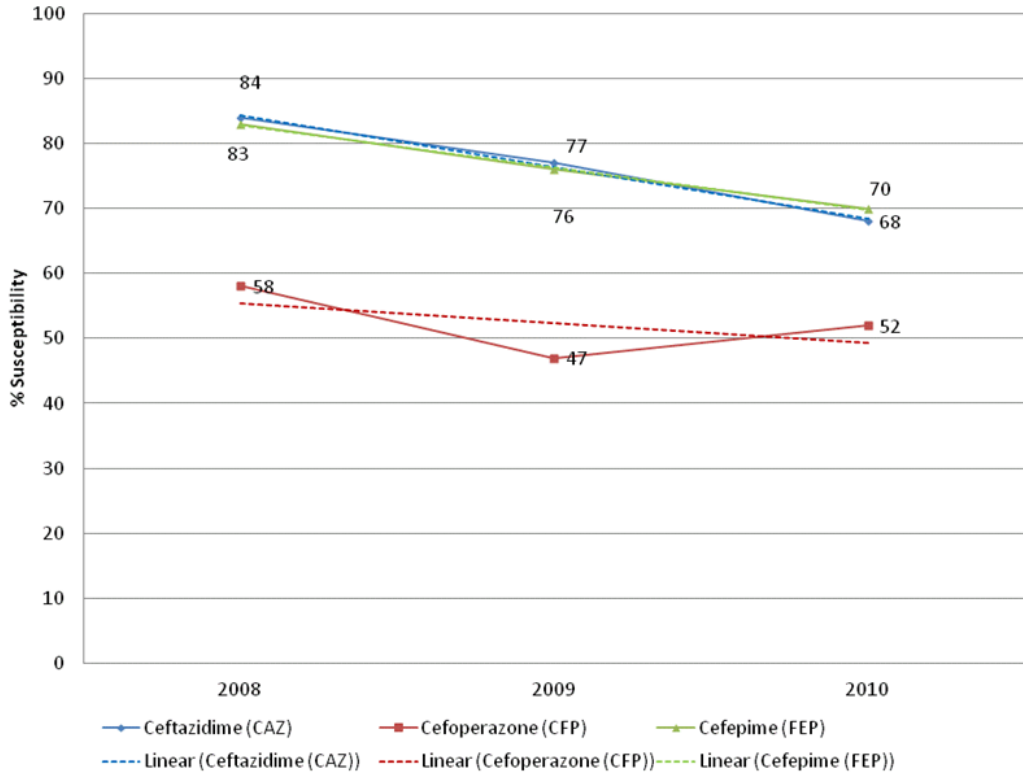

Figure 4. Trend of *P. aeruginosa* susceptibility to Cephalosporins from 2008 to 2010.

up and down patterns in which a significant decrease of susceptibility occurred from 2008 to 2009 (p = 0.000). In 2010, an increase of susceptibility was apparent but statistically was not significant (p = 0.753). Ceftazidime and cefepime showed good activitiesin 2008 that is 84 and 83%. These antibiotics became less active in 2009 to 2010, that is 68 and 70%. Susceptibility of *P. aeruginosa* to ceftazidime and cefepime was similar, but higher than cefoperazone.

Antibiotic susceptibility patterns of *P. aeruginosa* to aminoglycosides was shown in Figure 5. It showed decrease susceptibility for all three aminoglycosides tested which statisticaly significant (AMK p = 0.000; GEN p = 0.002; TOB p = 0.000).The microorganism appeared to be more susceptibel to amikacin than gentamicin. Earlier in 2008, amikacin was less active compared to tobramycin, however, it had better activity in 2009 and 2010. Amikacin showed better activity than gentamicin and tobramycin, that is 75% versus 68 and 65%, respectively in 2010. Susceptibility patterns of *P. aeruginosa* tociprofloxacin and levofloxacinwas rather similar in three consecutive years from 2008 to 2010, that is 63, 60 and 56% versus 66, 62 and 54%, respectively; significant declining susceptibilities were observed that is, CIP p = 0.003, LVX p = 0.000 (Figure 6). In carbapenem group, depletion of susceptibility for both meropenem and imipenem in 2008 to 2009 was significant (p = 0.000), however, an insignificant increase of susceptibility of these antibiotics was observed in later years(p = 0.692) (Figure 7). Imipenem showed better activity than meropenem in 2010 which was 80% against 75%. Susceptibility of *P. aeruginosa* to piperacillin-tazobactam and aztreonam was shown in Figure 8. A declining trend was observed for both antibiotics from 2008 to 2010 which was not significant statistically (TZP p = 0.055, and ATM p = 0.346). In 2010, piperacillin-tazobactam showed nearly good activity, that is 75%.

DISCUSSION

Our study showed *P. aeruginosa* was constantly present as one of the most Gram negative bacteria isolated from clinical specimens in 2004 to 2010. Despite much lesser specimen received in our laboratory in recent years due to more microbiology laboratories available in the area, *P. aeruginosa* isolates found were rather consistent that is 12 to 19%. The same condition was reported by one private hospital in Jakarta in 2005 and 2010 (Soebandrio, 7th National Symposium of Indonesia Antimicrobial Resistance Watch Symposium, Jakarta, 2011), and also by one hospital in Solo, Central Java (Priyambodo and Saptawati, Dr. Moewardi General Hospital-Solo, Indonesia, unpublished, 2010). Though many more hospitals detected high occurence of MDR *P. aeruginosa* in our country, published data is hardly available. The

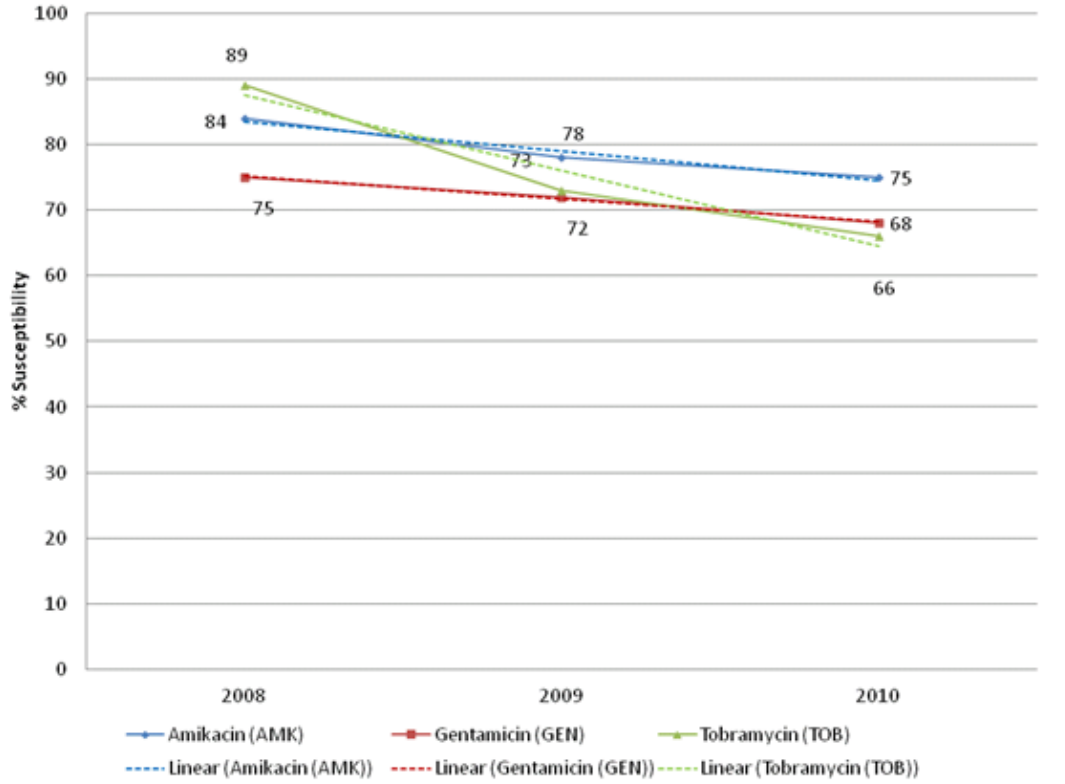

Figure 5. Trend of *P. aeruginosa* susceptibility to Aminoglycosides from 2008 to 2010.

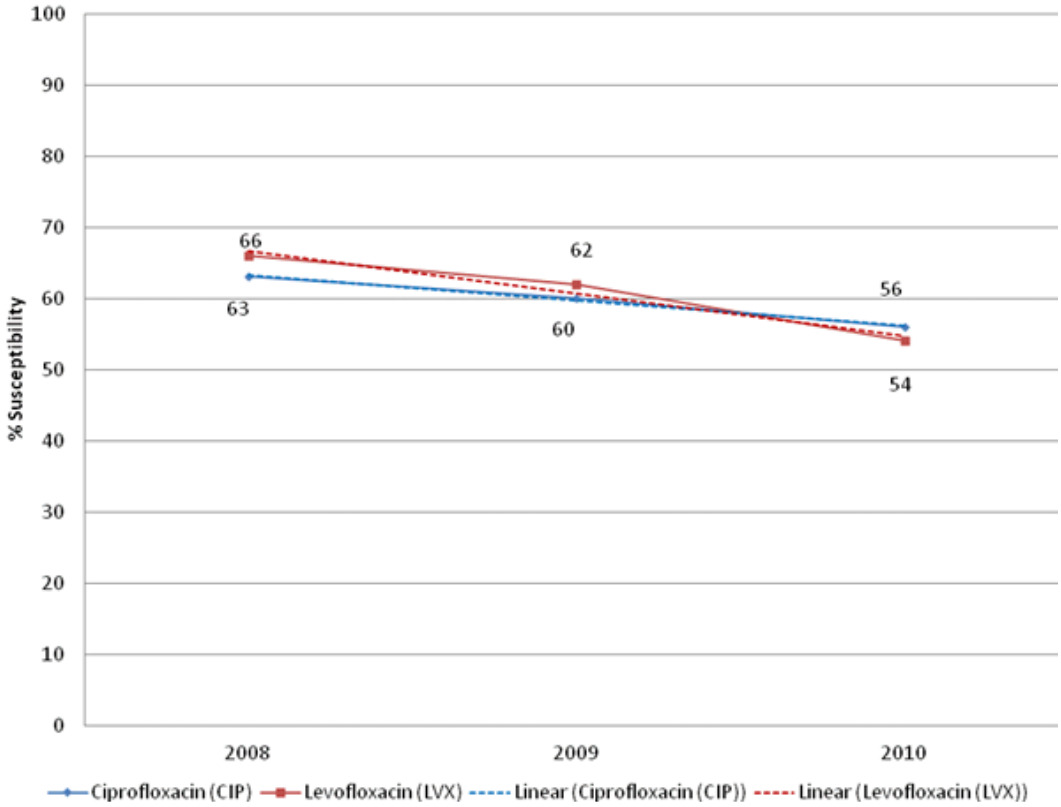

Figure 6. Trend of *P. aeruginosa* susceptibility to Quinolones from 2008 to 2010.

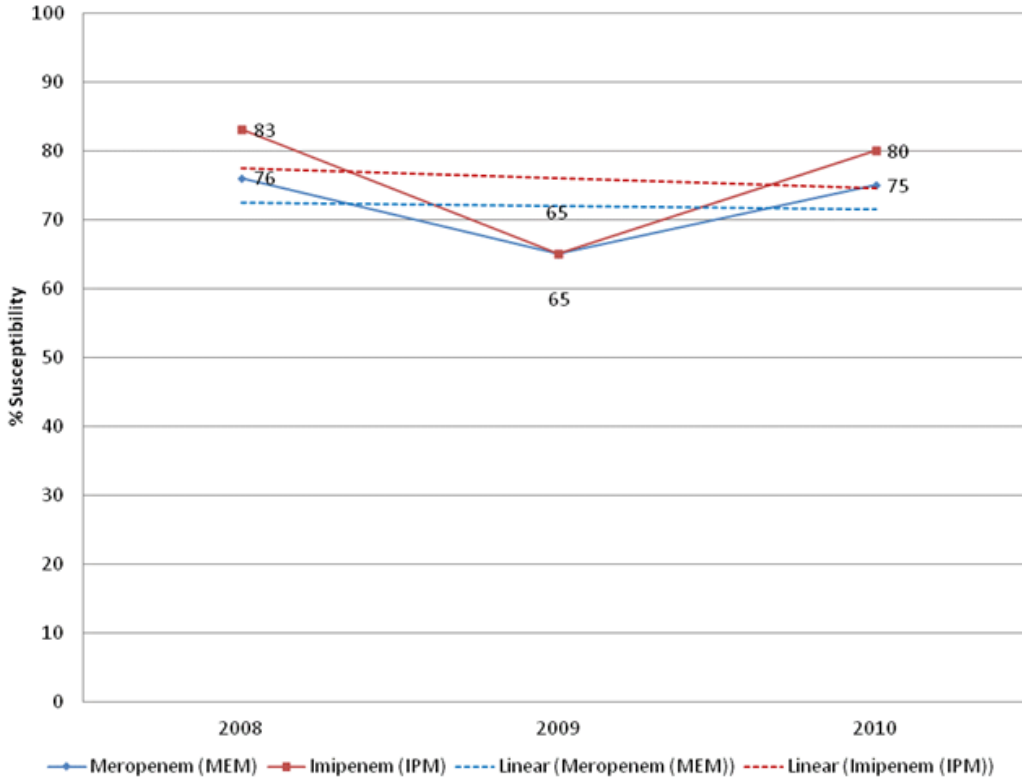

Figure 7. Trend of *P. aeruginosa* susceptibility to Carbapenem from 2008 to 2010.

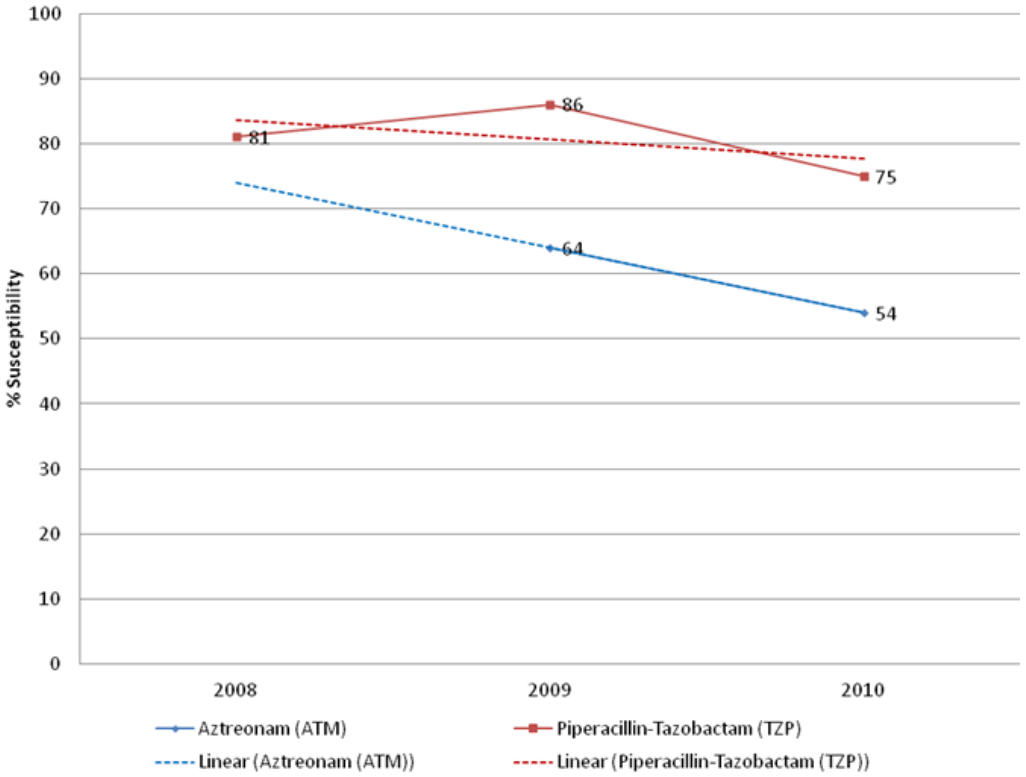

Figure 8. Trend of *P. aeruginosa* susceptibility to Aztreonam and Piperacilin-Tazobactam from 2008 to 2010.

problem with MDR *P. aeruginosa* was also reported from many countries, such Thailand (Dejsirilert et al., 2009), France, Germany, Italy, Canada, the United States (Jones et al., 2004), and Saudi Arabia (Al Johani et al., 2010).

The decrease of susceptibility of *P. aeruginosa* to amikacin, gentamicin, cefepime, ciprofloxacin and meropenem, were likely correlate to inadequacy of the use of these antibiotics.In cephalosporin group, susceptibility patternsof *P. aeruginosa* to ceftazidime and cefepime in the present study was similar to that reported by Pathmanathan et al. (2009) from Malaysia. Better susceptibility to ceftazidime than cefoperazone observed in our study was also found in Sudan (Saeed and Awad, 2009). Superiority of amikacin compared to gentamicin found in 2008 to 2010 (Figure 5) was confirmed by Mohanasoundaram (2011) which reported that susceptibility to amikacin was higher as compared to gentamicin that is 63, 41 and 46% versus 36, 32 and 32%, respectively. In 2009 and 2010, amikacin remained superior in comparison to tobramycin, and this was similar to that of Gad et al. (2010).

In quinolone group, the susceptibility of *P. aeruginosa* to ciprofloxacin and levofloxacin in the present study was higher compared to that of Javiya et al. (2006) which was only 26.76 and 35.71% for ciprofloxacin and levofloxacin, respectively. Adhikari et al. (2010) reported 47.76 and 44.78% susceptibility to those antibiotics. Activity of imipenem and meropenemto *P. Aeruginosa* shown in our study correlated with others which found imipenem had a better activity than meropenem (Gupta et al., 2006). Nonetheless, it was not in agreement to that reported by Tan et al. (2008), who found that meropenem was better than imipenem. In 2010, piperacillin-tazobactam showed nearly good activity that is 75% despite other study performed by Tan et al. (2008) which demonstrated 88.3%. The susceptibility of *P. aeruginosa* to aztreonam was 54%, which in fact was higher than the one reported by Pieboji et al. (2006) which is 33%.

CONCLUSION

In the past seven years until 2010, *P. aeruginosa* was one of the most Gram negative bacteria found from all kind of specimens received in CML-FMUI, Jakarta. Overall, there was a tendency of decrease susceptibility to all antibiotic tested. In 2010, the only anti-pseudomonas antibiotic showed good activity (80%) was imipenem, and so was suggested as drug of choice in pseudomonas infection.

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REFERENCES

Adhikari L, Roy K, Tsering DC, Pal R, Kar S(2010). Susceptibility Rates of Pseudomonas Aeruginosa Strains to Quinolones. J. Lab. Physicians, 2(2): 121

Al Johani SM, Akhter J, Balkhy H, El-Saed A, Younan M, Memish Z (2010). Prevalence of antimicrobial resistance among gam-negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. Ann. Saudi Med., 30(5): 364-369

Carmeli Y (2008).Strategies for managing today's infections.Clin Microbiol Infect., 3(Suppl14): 22-31.

CLSI (2004-2010). Performance Standards for Clinical Laboratory Standard Institute.

CML-FMUI (2009). Standard Operating Procedure for Clinical Microbiology Examination. Laboratory of Microbiology of Department Microbiology, Fac. of Medicine Univ. Indonesia, Jakarta.

CML-FMUI (2004). Standard Operating Procedure for Clinical Microbiology Examination. Laboratory of Microbiology of Department Microbiology, Fac. of Medicine, Univ. Indonesia, Jakarta.

Dejsirilert S, Suankratay C, Trakulsomboon S, Thongmali O, Sawanpanyalert P, Aswapokee N, Tantisiriwat W (2009). National Antimicrobial Resistance Surveillance, Thailand (NARST) Data among Clinical Isolates of Pseudomonas aeruginosa in Thailand from 2000 to 2005. J. Med. Assoc. Thai., 92(Suppl 4): S68-75.

Gad GF, Mohamed HA, Ashour HM (2010). Aminoglycoside Resistance Rates, Phenotypes, and Mechanisms of Gram-Negative Bacteria from Infected Patients in Upper Egypt. PLoS ONE 6(2): e17224. doi:10.1371/journal.pone.0017224.

Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A (2006). Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J. Med. Res., 124: 95-98

Javiya VA, Ghatak SB, Patel KR, Patel JA (2008). Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian J. Pharmacol., 40: 230-4.

Jones ME, Draghi DC, Thornsberry C, Karlowsky JA, Sahm DF, WenzelRP (2004). Emerging resistance among bacterial pathogens in the intensive care unit –A European and North American Surveillance study (2000–2002). Ann. Clin. Microbiol. Antimicrob., 3: 14.

Lister PD, Wolter DJ (2011). Resistance Challenges Threatening the Treatment of *Pseudomonas aeruginosa* Infections with Levofloxacin: The Role of a Levofloxacin-Imipenem Combination for Prevention of Resistance. Accessed on Mei 7th from www.infectweb.com/only/artsrv2008_3.pdf

Luzzaro F, Ortisi G, Larosa M, Drago M, Brigante G, Gesu G (2011). Prevalence and epidemiology of microbial pathogens causing bloodstream infections: results of the OASIS multicenter study. DMID., 69(4): 363-369.

Moehario LH, Tjoa E, Kiranasari A, Ningsih I, Rosana Y, Karuniawati A (2009). Trends in antimicrobial susceptibility of Gram-negative bacteria isolated from blood in Jakarta from 2002 to 2008. J. Infect. Dev. Ctries., 3(11): 843-848.

Mohanasoundaram KM (2011). The Antimicrobial Resistance Pattern In The Clinical Isolates Of Pseudomonas Aeruginosa In A Tertiary Care Hospital; 2008-2010 (A 3 Year Study). J. Clin. Diag. Res., 491-494

Pathmanathan SG, Samat NA, Mohamed R (2009). Antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa from a Malaysian Hospital. Malaysian, J. Med. Sci., 16(2): 27-32.

Peleg AY, Hooper DC (2010). Hospital-Acquired Infections Due to Gram-Negative Bacteria. N Engl. J. Med., 362: 18 04-13.

Pieboji JG, Shiro SK, Ngassam P, Adiogo D, Ndumbe P. (2006). Antimicrobial activity against Gram negative bacilli from Yaounde Central Hospital, Cameroon. Afr. Health Sci., 6(4): 232-235.

Saeed HA, Awad AA (2009). Susceptibility of *pseudomonas aeruginosa* to third generation cephalosporins. J.Sc. Tech., 10(2): 195-200

Tan TY, Hsu LY, Koh HT, Lily SY Ng, Tee NWS (2008). Antibiotic Resistance in Gram-negative Bacilli: A Singapore Perspective. Ann. Acad. Med. Singapore., 37: 819-25.