Resistance Patterns *Pseudomonas aeruginosa* Isolated from Clinical Patient Samples RSUP. Dr. M. Djamil Padang, West Sumatra, Indonesia

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ABSRACT

Pseudomonas aeruginosa is an opportunistic pathogen that has an innate resistance to some antibiotics and disinfectants. These bacteria are one of the most common cause of nosocomial infections, commonly infects patients who experienced a decrease in the immune system and can cause various types of infections include surgical wound infections, burns, and infections of the urinary tract. The problem that almost occurred throughout the world this is bacterial resistance to many types of antibiotics. Some bacteria found become resistance to multi drug, which makes it difficult to select the antibiotics for the treatment of infections. This study aimed to determine the resistance pattern Pseudomonas aeruginosa isolated from clinical samples of patients treated at RSUP. Dr. M. Djamil Padang, West Sumatra Indonesia from August to October 95, 2015. Total value of bacteria test derived from sputum (35), swabs (22), Pus (23), urine (10), blood (3) and stool (2). The results of resistance tested by the agar diffusion method of Kirby-Bauer is ceftriaxone (43.16%), cefotaxime (42.10%), cefoperazone (36.84%), ofloxacin (31.58%), ciprofloxacin (28.42%), gentamycin (28.42%), ceftazidime (25,26%), Meropenem (24.21%), Ticarcilin (23.16%), Imipenem (23.16%), levofloxacin (20.0%), piperacillin (14.74%) and amikacin (8.42%). Out of 95 isolates, 30 isolates including multidrug resistant. The most effective antibiotics are amikacin (91.51%) and piperacillin (81.05%). The most resistant is ceftriaxone (43.16%) and cefotaxime (42.10%).

Keywords: *Pseudomonas aeruginosa*, Antibiotic resistance, Multidrug resistant

INTRODUNCTION

Pseudomonas aeruginosa is an opportunistic pathogenic bacterium, one cause of nosocomial infections [1]. The incidence of nosocomial infections in the world are caused by *P. aeruginosa* bacteria approximately 10-15% and 10-20% in the Iintensive Care Unit (ICU), usually occurs in patients with septicemia, cystic fibrosis, burns and wound infection [2,3]. Successful treatment of infectious diseases is largely determined by rational antibiotic use, precise and safe. Lately, many reported that the bacteria causing the infection are resistant to the antibiotics used [4]. Bacteria become resistant to antibiotics with different mechanisms, among others by producing β-lactamase enzyme that can destroy the antibiotic, change intracellular targets of antibiotics and efflux pump [5]. Nowadays, almost all the world's part have major problem in *P. aeruginosa* bacteria are growing microorganisms that resistant to many types of antibiotics (MDRPA).

Multi Drug Resistant *P. aeruginosa* (MDRPA) is a condition in which bacteria resistant to three or more classes of antibiotics such as penicillins, cephalosporins, monobactam, carbapenem, aminoglycosides, fluoroquinolones and others. From various studies reported cases MDRPA varied from 0.6%-32%. Prevalence MDRPA increased over the last decade in patients who are hospitalized, resulting in fewer choices for treatment [6]. *Pseudomonas sp*. at RSUD. dr. M. Djamil Padang included into germ MDR with a considerable percentage within a period of 3 years, i.e., 88% in 2010, 61% in 2011 and 66% in 2012 [7].

This study aimed to look at the pattern of *P. aeruginosa* bacterial resistance and to know the percentage of *P. aeruginosa* bacteria that are MDRPA isolated from urine, sputum, swabs, pus, feces and blood of hospitalized patients in RSUD. Dr. M. Djamil Padang. The method used Kirby Bauer agar diffusion using 13 types of antibiotics.

MATERIAL AND METHODS

A total of 95 isolates of *Pseudomonas aeruginosa* bacteria isolated from urine, sputum, swabs, pus, feces and blood inpatients at RSUP. Dr. M. Djamil Padang. Isolation using selective culture medium for *P. aeruginosa* is Cetrimide Agar (CA). Greenish or yellow-green fluorescence after incubation for 24 h indicate a positive isolates of *P. aeruginosa. P. aeruginosa* ATCC 27853 was used as a positive control. The tested activity of antibiotics using Mueller Hinton agar medium.

The antibiotics used are seftazidime (30 g), cefotaxime (30 g), ceftriaxone (30 g), sefoperazone (30 g), ciprofloxacin (5 g), levofloxacin (5 g), ofloxacin (5 g), gentamicin (10 g), amikacin (30 g), piperacillin (100 g), ticarcillin (75 g), meropenem (10 g) and imipenem (10 g).

Blocked diameter produced compared to the standard according to the Clinical Laboratory Standard Institute (CLSI) [8]. *P. aeruginosa* bacteria concluded that MDRPA resistant to three or more classes of antibiotics.

RESULTS

A total of 95 isolates of *Pseudomonas aeruginosa* in the study came from sputum (35), swab (22), pus (23), urine (10), blood (3) and stool (2). Antibiotic activity test carried out following CLSI standards. Before the activity test isolated *P. aeruginosa*, the test was conducted by prior activity of bacteria *P. aeruginosa* ATCC 27853, the test results in Table 1. Further test isolates of *P. aeruginosa* activity test, the tests performed may know the percentage and number of isolates Resistant (R), Sensitive (S) and Intermediates (I), the results presented in Table 2.

Table 1: Test activities of 13 antibiotics against the bacterium *Pseudomonas aeruginosa* ATCC27853

Antibiotic	Blocked diameter (mm)	Blocked diameter according to CLSI (mm)	Antibiotic	Blocked diameter (mm))	Blocked diameter according to CLSI (mm)
Ceftazidime	22.00	22-29	Gentamicin	20.00	16-21
Cefotaxime	20.25	18-22	Amikacin	24.00	18-26
Ceftriaxone	26.00	17-23	Piperacilin	29.50	25-33
Cefoperazone	26.00	23-29	Tikarcilin	26.50	21-27
Ciprofloxacin	38.50	25-33	Meropenem	40.25	27-33
Levofloxacin	36.00	19-26	Imipenem	33.68	20-28
Ofloxacin	32.25	17-21			

Table 2: Percentage of *Pseudomonas aeruginosa* bacteria activity test against 13 type of antibiotics

S. No.	Antibiotic	Amount isolate	R (%)	I (%)	S (%)
1	Ceftazidime (CAZ)	95	24 (25.26)	5 (5.26)	66 (69.47)
2	Cefotaxime (CTX)	95	40 (42.10)	36 (37.90)	19 (20)
3	Ceftriaxone (CRO)	95	41(43.16)	13(13.68)	41 (43.16)
4	Cefoperazone (CFP)	95	35 (36.84)	9 (9.47)	50 (52.63)
5	Ciprofloxacin (CIP)	95	27 (28.42)	4 (4.21)	66 (69.47)
6	Levofloxacin (LEV)	95	19 (20.00)	10 (10.52)	66 (69.47)
7	Ofloxacin (OFX)	95	30 (31.58)	2 (2.10)	63 (66.32)
8	Gentamicin (CN)	95	27 (28.42)	6 (6.31)	62 (65.26)
9	Amikacin (AK)	95	8 (8.42)	-	87 (91.58)
10	Piperacilin (PRL)	95	14 (14.74)	-	77 (81.05)
11	Tikarcilin (TIC)	95	22 (23.16)	4 (4.21)	69 (72.63)
12	Meropenem (MEM)	95	23 (24.21)	1 (1.05)	71 (74.74)
13	Imipenem (IPM)	95	22 (23.16)	1 (1.05)	72 75.79)

R: Resistance; I: Intermediate; S: Sensitive

The result of the activity of 95 isolates of *P. aeruginosa* to antibiotics 13 showed 34 (35.79%) isolates had MDRPA properties marked with the isolates were resistant to three or more classes of antibiotics derived from the urine of three isolates (30.00%), sputum 11 isolates (31.54%), swab 6 isolates (27.27%), pus 13 isolates (52.52%), stool isolates 0 isolate (0%), and blood 1 isolate (33.33%). Please also note 17 (17.89%) isolates of bacteria are resistant to one or two classes of antibiotics and 44 (46.32%) isolates were sensitive (Table 3).

Table 3: Number and percentage of resistance bacteria *Pseudomonas aeruginosa* obtained from patient of RSUP. Dr. M. Djamil Padang

S. No.	Source of Isolate *Pseudomonas aeruginosa* (n)	Sensitive n (%)	Resistant one/two group AB n (%)	Multi drug resistant n (%)
1	Urin (10)	5 (50)	2 (20)	3 (30,00)
2	Sputum (35)	19 (54,29)	5 (14,29)	11 (31,43)
3	Swabs (22)	9 (40,90)	7 (31,82)	6 (27,27)
4	Pus (23)	7 (30,43)	3 (13,04)	13 (56,52)
5	Feces (2)	2 (100)	0 (0)	0 (0)
6	Blood (3)	2 (66,67)	0 (0)	1 (33,33)
	Total 95	44 (46,32)	17 (17,89)	34 (35,79)

DISCUSSION

In this study, the determination of antibiotic activity carried out by the agar diffusion method. In this method the diameter measured is inhibition of growth, which is a clear area around the disc. Size diameter resistor is proportional to the antibacterial activity [9]. One of 95 isolates tested showed the highest resistance against ceftriaxone 41 (43.16%) isolates. The percentage of such resistance is greater than the results of research conducted at three hospitals in South West Nigeria, 34.5% for ceftriaxone [10]. Test isolates most sensitive to amikacin 87 (91.58%) isolates. This result is lower than the results of research conducted on patients in the burn unit at the University Hospital Menoufiya 91.3% to amikacin [11], but most of them higher than the results of a study of patients with nosocomial infections in Menoufiya University Hospital, Egypt namely for amikacin 80.05% [12].

Resistance to cephalosporin class of antibiotics (ceftriaxone) occurs because of a mutation that results in the production of Penicillin Binding Proteins (PBP) which is different that cephalosporins not inhibit PBP again. In addition resistance can also occur because of mutations that altered porin involved in transport passing through the outer membrane; these resulted cephalosporins can't reach the cytoplasmic membrane (location PBP). Ability lactamase produced bacteria and their genes may encode lactamase also lead to bacteria resistant to antibiotics is because the hydrolysis of the bond lactam ring resulted in the inactivation of antibiotics [13].

From the results of the activity test is seen that 34 (35.79%) isolates were MDRPA, which is resistant to more than three classes of antibiotics. This result is smaller than a study of patients with burn injuries in hospital Motahari, Tehran. Of the 220 clinical isolates of *P. aeruginosa* obtained 112 (50.9%) isolates are MDRPA [1]. Other research results show of 180 clinical isolates of *P. aeruginosa* note 41 (22.7%) isolates are MDRPA [14]. Furthermore, the results of a study of 316 clinical isolates of *P. aeruginosa* showed 141 (44.62%) isolates were MDRPA [15]. MDRPA highest bacteria derived from pus that 13 isolates.

The difference in the percentage *P. aeruginosa* isolates resistant to antibiotics in different places due to the irrational use of antibiotics, such as: antibiotics are not appropriate dose, incorrect diagnosis, and incorrect causing bacteria. Improper administration of antibiotics is a risk factor that will make the bacteria mutate and become resistant. Besides antibiotic-resistant can also be caused by lack of patient compliance in using antibiotic drugs, as well as the lack of information and knowledge about the drug patients [16]. Early detection will greatly assist in the control of hospital infections caused by these bacteria [17].

CONCLUSION

The highest percentage of resistance of 95 isolates of *P. aeruginosa* to cceftriaxone 43.16% (41 isolates) and least sensitive to amikacin 91.58% (87 isolates). MDRPA percentage is 35.79% (34 isolates), with the greatest percentage of pus which obtained 56.52% (13 of 23 isolates).

REFERENCES

[1] M. Vahdani, L. Azimi, B. Asghari, F. Bazmi, L.A. Rastegar, *Ann. Burns. Fire. Disasters.,*, **2012**, 25, 2, 78-81.

[2] T. Strateva, D. Yordanov, *J. Med. Microbiol.*, **2009**, 58, 1133-1148.

[3] I. Biswal, S.A. Balvinder, K. Dimple, Neetushree, *J. Clin. Diag. Res.*, **2014**, 8, 5, 26-29.

[4] M. Soleha, H.L. Elvistra, N. Fitri, Triyani, *Proceeding Puslitbang Biomedik dan Farmasi*, **2009**.

[5] S. Gunawan, R. Setiabudy, Nafrialdi, *Pharmacol. Ther.,* **2009.**

[6] R. Kalaivani, P. Shashikala, D. Sheela, K. Prashanth, R. Saranathan, *Int. J. Cur. Res. Rev.*, **2013**, 5, 17, 28-35.

[7] N.R. Sjahjadi, R. Rasyid, E. Rustam, L. Restusari, *Andalas. Med. J.,* **2014**, 3, 3.

[8] Clinical Laboratory Standards Institute (CLSI), Document M02-A11, **2012**.

[9] E. Jawetz, J. Melnick, E. Adelberg, *Med. Microbiol.*, **2008**.

[10] O.A. CAkingbade, S.A. Balogun, D.A. Ojo, R.O. Afolabi, B.O. Motayo, P.O. Okerentugba, I. Okonko, *World Appl. Sci. J.*, **2012**, 20, 6, 766-775.

[11] B.A. Koura, H.E.Z. Mohammed, M. Noha, El. *Molecular Diagnosis and Vaccine*, **2003**, 1, 1, 59-70.

[12] A.B. Mahmoud, A.Z. Wafaa, R.H. Ghada, Z.I. Aza, G. Rasha, *J. Virol. Microbiol.*, **2013**, 1-13.

[13] S.T. Pratiwi, *Pharm. Microbiol.*, **2008**.

[14] M.M. Gill, J. Usman, F. Kaleem, A. Hassan, A. Khalid, R. Anjum, Q. Fahim, *J. Coll. Phys. Surg. Pak.*, 2009, 21, 9, 531-534.

[15] S. Jayanthi, M. Jeya, *J. Clin. Diag. Res.*, **2014**, 8, 6, 16-19.

[16] R.J. Lamont, R.A. Burne, M.S. Lantz, D.J. Leblanc, *Oral. Microbilol. Immunol.*, **2006**.

[17] S. Thenmozhi, K. Moorthy, B.T. Sureshkumar, M. Suresh, *Int. J. Pure. Appl. Biosci.,* **2014**, 2, 3, 207-226.