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Plasmid Profile Analysis of Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Clinical Samples of Hospitalized Patient in Dr M. Djamil Hospital, Padang, West Sumatera

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ABSTRACT

Pseudomonas aeruginosa is a leading causes of nosocomial infections and responsible for 10% of all hospital-acquired infections. P. aeruginosa nosocomial infections is generally difficult to killed because bacterial resistant mechanism. Increasing bacterial resistance to all antibiotics of different classes are often obtained and transmitted by conjugation, transformation, or plasmid transduction. The aimed was conducted to determine the antibiotic resistant patterns of Pseudomonas aeruginosa isolated from clinical samples in West Sumatera, Indonesia. 95 clinical data were collected from M. Djamil Hospital, Padang (Indonesia). Antibiotic susceptibility was determined on Mueller Hinton agar using the disc diffusion method according to the modified Kirby-Bauer technique. Plasmid isolation was done using a commercial plasmid isolation kit QIAprep® Spin Miniprep Kit. The resultant plasmids were separated using gel electrophoresis. In this study, 34 (35.79%) of P. aeruginosa isolates were resistant to three or more classes of antibiotics. The plasmid analysis revealed that sixteen multidrug resistant P. aeruginosa isolates had a plasmid. A isolate with single plasmid band 300bp, twelve of the isolates had a single plasmid band >1kbp and two isolates (PASw7 and PASp11) had two plasmids band > 1kbp.

KEYWORDS: *Pseudomonas aeruginosa, Bacterial Resistance, Antibiotics, Plasmid*

INTRODUCTION

Pseudomonas aeruginosa is a non-fermentative gram negative bacteria widely distributed in nature and can survive on a wide variety of surfaces and in hospital environment, as the wards encourage bacterial growth [1]. *P. aeruginosa* is an important opportunistic pathogens that cause nosocomial infections, especially in patients with decreasing of immune system [2]. *P. aeruginosa* is a leading cause of nosocomial infections and responsible for

10% of all hospital-acquired infections [3]. Infections caused by *P. aeruginosa* are often severe and life threatening and difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of an emergence antibiotic resistance during therapy, thus resulting in severe adverse outcomes [4].

P. aeruginosa nosocomial infections is generally difficult to killed because of the possibility intrinsic resistance and its ability to obtain faster resistance mechanism against many groups of antimicrobials [5]. *P. aeruginosa* is naturally resistant to B-Lactams. Furthermore, they easily acquire resistance to new antibacterial agents by mutational changes or acquisition of genetic material. In other study, *P. aeruginosa* strains isolated was presented resistance to carbenicillin and gentamicin. *P. aeruginosa* is intrinsically less susceptible to the fluoroquinolones and usually it is moderately susceptible or resistant [6].

Multi Drug Resistant *P. aeruginosa* (MDRPA) is a condition that bacteria resistant to three or more classes of antibiotics such as penicillins, cephalosporins, monobactam, carbapenem, aminoglycosides and fluoroquinolones. Inappropriate antibiotics administration can cause *P. aeruginosa* resistant to several classes of antibiotics [7]. MDRPA cases reported varied from 0.6% - 32% according to various research studies conducted in various regions. MDRPA prevalence increased during the last decade and has become a major concern among patients who were hospitalized [8].

Increasing bacterial resistance to all antibiotics of different classes are often obtained and transmitted by conjugation, transformation, or transduction plasmid [9]. DNA plasmid are extra chromosomal element of finite size, usually stably inherited within a bacterial cell line and potentially capable of transfer between strains, species, or genera [10]. They were discovered in enteric bacteria for the first time and from the late 1950s onwards were increasingly associated with antibiotics resistance. Its main function is to carry antibiotics resistant genes, which are responsible for the increase pathogenicity of most bacteria [11]. Therefore, this study is aimed to determine the percentage of multidrug resistance of *P. aeruginosa* strains that are plasmid mediated.

MATERIALS AND METHODS

Isolated Bacteria

A total of 95 clinical data were collected from M. Djamil Hospital, Padang (Indonesia). All of specimens were cultured on Cetrimide agar in order to isolate *P. Aeruginosa* strains. Then plates were incubated at 37°C for 24 h [12].

Identification of *P. aeruginosa* Isolates

Cetrimide Agar is used for the identification of *P. aeruginosa.* Sample to be tested inoculate by spreading on the surface of the plates. Incubate for 24 hours at 37^0C. The presence of growth is indicative of a positive reaction. Examine colonies under short wavelength (254nm) ultraviolet light for the presence of fluorescein.

Kirby- Bauer Disc Diffusion method

Antibiotic susceptibility was determined on Mueller Hinton agar using the disc diffusion method according to the modified Kirby-Bauer technique. All of isolated *P. aeruginosa* strains were tested for their sensitivity to the following Antibiotics: Cephalosporin (Ceftazidime 30µg, Cefotaxime 30µg, Ceftriaxone 30µg, dan Cefoperazone 30µg), Quinolon (Ciprofloxacin 5µg, Levofloxacin5µg, dan Ofloxacin 5µg), Aminoglycoside (Gentamicin 10µg dan Amikacin 30µg), Penicillin (Piperacillin 100µg dan Ticarcillin 75µg), dan Carbapenem (Meropenem 10µg dan Imipenem 10µg). Isolates were considered multidrug resistant if they showed resistance to 3 or more of the tested antibiotics [13].

Plasmid Isolation and Profiling

Plasmid isolation was done using a commercial plasmid isolation kit QIAprep® Spin Minipep Kit according to the manufacturer instructions.

Gel Elecetrophoresis

The resultant plasmids were separated using gel electrophoresis. Agarose gel (1,5%) in 1X TBE Buffer were prepared and placed in gel electrophoresis tank containing 1X TBE buffer. The current supplied about 100V for 30 minutes and the resulting bands were visualized under Gel Documentation and compared with 100 bp and 1kb ladder [14].

RESULTS

Among the 95 *P. aeruginosa* isolates 35 (36,84%) were from sputum, 22 (23,16%) from swab, 23 (24,21%) from pus, 10 (10,53%) from urine, 2 (2,10%) from feces and 3 (3,16%) from blood samples. All the isolates were found to be resistant to Ceftazidime, Cefotaxime, Ceftriaxone, Cefoperazone, Ciprofloxacin, Levofloxacin, Ofloxacin, Gentamicin, Amikacin, Piperacillin, Ticarcillin, Meropenem and Imipenem. The results of antibiotic susceptibility testing are shown in Table 1. Maximum resistance was seen to ceftriaxone (43,16%) and cefotaxime (42,16%) followed by cefoperazone (36,84%), Ofloxacin (31,58%) and Ciprofloxacin (28,42%). Minimum resistance was seen to amikacin (8,42%). In this study, 34 (35.79%) of *P. aeruginosa* isolates were resistant to three or more classes of antibiotics.

The isolates were the antibiotic resistant profile of 34 *P. aeruginosa* isolates shown in Table 2. Isolates number PAU2, PASp26, and PASw21 were resistant with all antibiotic tested. Plasmid mediated analysis of different multidrug resistant P. Aeruginosa from clinical samples were observed by agarose gel which showed plasmid bands in different combinations (Figure 1-3). The plasmid analysis revealed that sixteen multidrug resistant P. Aeruginosa isolates had plasmid. A isolate with single plasmid band 300bp, Twelve of the isolates had single plasmid band >1kbp and two isolates (PASw7 and PASp11) had two plasmids band > 1kbp (Table 3).

DISCUSSION

P. aeruginosa isolates were identified using media Cetrimide Agar (CA), which is a selective medium for the bacteria growth. All Cetrimide Agar media isolates can change to green or greenish yellow indicates that the positive isolates of *P. aeruginosa*. Media Cetrimide Agar contains Cetrimide (cetyltrimethylammonium ammonium bromide) is quartener ammonium can inhibit growth of other microorganisms by breaking (lysis) of the bacterial cell. Cetrimide also can increase of the production of pigment *P. aeruginosa* is pyosianin and pyoverdine that would cause a greenish or greenish-yellow color [15]. The result of antibiotic activity testing showed 34 (35.79%) isolates was MDRPA, marked with the isolates were resistant to three or more classes of antibiotics.

This result is higher than Akin bade research at 2012 which 20% of isolates was MDRPA [13]. Plasmid known as one factor that can lead bacterial resistance to antibiotics in the presence of R plasmids that carry genes for resistance and become a medium to facilitate the rapid spread of bacterial resistance of antibiotics, thereby its contribute resulting MDRPA [10]. Plasmid identification of 34MDRPA isolates showed that 16 isolates had plasmid and characterized by band plasmid fluoresce under UV light and Gel Documentation instrument. The amount 13 isolates had a band with a size above 1 kb, PASp29 isolates had a band with a size of 300 bp, and 2 isolates (PASw7 and PASp11) had 2 plasmids with two band of different sizes above 1 kb (Figure 2). It shown that almost all MDRPA isolates had plasmid with band size exceeding 1 kb which shows that plasmids are large.

PAU2 isolate had a band with 1 kb size was resistant to all antibiotics tested, while PAU6, PAP9 and PAP17 isolates was resistant to 8 antibiotics tested and PASw2 and PAP16 isolates resistant to 7 antibiotics tested. PASp29 isolate had plasmid with band 300 bp was resistant to 12 antibiotics tested, whereas PASw7 and PASp11 isolates that has two band of plasmids with different sizes above 1 kb was resistant to 8 and 11 antibiotics. The results obtained that the plasmid size does not ensure high or low the antibiotic resistance. The number of antibiotic resistant genes contained in plasmid which can affect more or at least the bacteria resist to antibiotics. Overall, all plasmid MDRPA isolates was resistant to the class of cephalosporins, such as ceftazidime, cefotaxime, ceftriaxone, and sefoperazon.

The results of this study indicate that 47.06% MDRPA isolates had a plasmid, the plasmids can cause resistance of bacteria to antibiotics (extra chromosomal resistance) and accelerate the spread of antibiotics resistance that will cause MDRPA. Conversely, 18 MDRPA isolates with no plasmid showed that the isolates were resistant not caused by extra chromosomal resistance, but due to other resistance mechanisms, such as non-genetic, chromosomal resistance or cross-resistance. Plasmids are DNA circular element lies free in a bacterial cell (extra chromosomal) which replicates itself independently and not depend on the bacterial chromosome [10]. Plasmid known as a mediator that facilitates the rapid spread of bacterial resistance to antibiotics because it can be transferred not only in one species of bacteria, but also in one genus [9].

Bacteria that had plasmid (R plasmid) had two groups of genes, which includes the factor of resistance transfer genes for plasmid replication &conjugation and determinant R that have the resistance gene and encode the production of enzymes for inactivation of antibiotics. The study on the burn unit patients at University Hospital Menoufiya showed a total of 18 isolates (39.1%) of the total 46 isolates have the plasmid, of which 8 strains (44.4%)

had 3 plasmids, 7 strains (38.9%) had one plasmid, 2 strains (11.1%) has 2 plasmids and one strain (5.5%) had 5 plasmids [16]. The result of that study as lower than this study (47.06% isolates had plasmid)

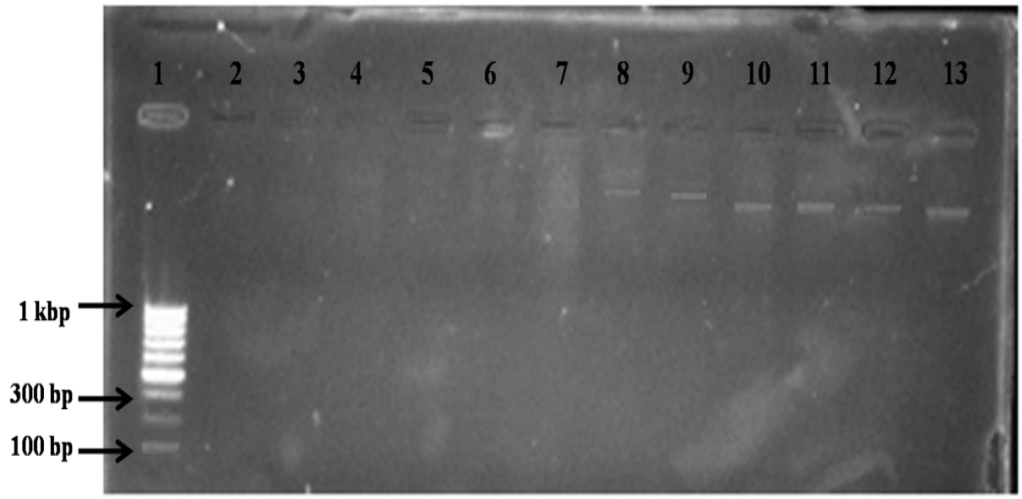
Other studies on the surgical unit patients of the teaching hospital Ahmadu Bello University, Zaria, Nigeria, 14 strains known to had the plasmid in which eight strains had the same pattern of plasmid with 1-3 band [18]. The results of another study in South West Nigeria, the determination of plasmid using Miniprep kit and a 0.8% agarose gel result 8 strains (36.4%) of the total 22 strains had plasmid band, 6 strain has a single plasmid band and two strains has two band with sizes ranging from 662 to 830bp [13]. From this study result, the case of MDRPA resistance which had plasmid was high in patients of Dr. M. Djamil hospital. It was happened because R plasmid can be transferred horizontally by conjugation method so that the recipient cell will be resistance, similar to the donor cell.

The plasmid displacement occurs not only in one species of bacteria but also can occur in a single genus, with the result that increase bacterial resistance and reduce the effectiveness of treatment. Early detection will greatly assist in the control of hospital infections caused by these bacteria [19].

Tabel-1: Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates

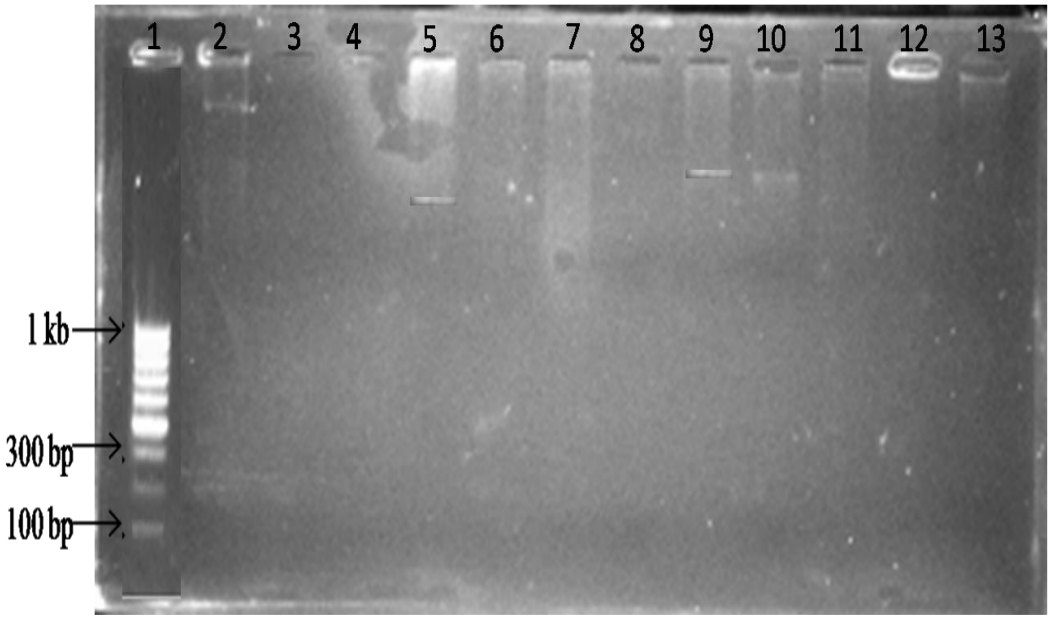
No.	Antibiotic	Resistant No.	Percentage (%)
1	Ceftazidime(CAZ)	24	25.26
2	Cefotaxime(CTX)	40	42.10
3	Ceftriaxone(CRO)	41	43.16
4	Cefoperazone(CFP)	35	36.84
5	Ciprofloxacin (CIP)	27	28.42
6	Levofloxacin (LEV)	19	20.00
7	Ofloxacin (OFX)	30	31.58
8	Gentamicin (CN)	27	28.42
9	Amikacin (AK)	8	8.42
10	Piperacillin (PRL)	14	14.74
11	Ticarcillin (TIC)	22	23.16
12	Meropenem (MEM)	23	24.21
13	Imipenem (IPM)	22	23.16

Figure-1: Plasmid profiles of multidrug resistant *Pseudomonas aeruginosa* isolates



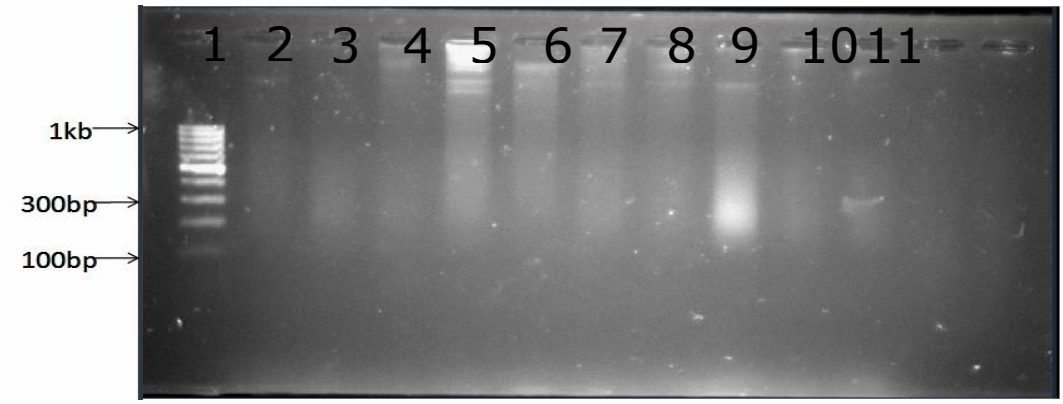
1: DNA Ladder; 2: PASp22 isolate; 3: PAP7isolate; 4: PAU3 isolate; 5: PAP12isolates; 6: PASp16 isolate; 7: PAP13 isolate;8: PAP22 isolate;9: PAD3 isolate;10: PASp26 isolate; 11: PAU6 isolate;12: PAP17 isolate;13: PAP18 isolate)

Figure-2: Plasmid profiles of multidrug resistant *Pseudomonas aeruginosa* isolates



1: DNA Ladder; 2: PASw2 isolate; 3: PASp2isolate; 4: PAP8 isolate; 5: PAP9isolates; 6: PASp9 isolate; 7: PAP21 isolate; 8: PASp34 isolate; 9: PAP14 isolate;10: PAP16 isolate; 11: PASp28 isolate; 12:PASp30 isolate; 13:PAP19 isolate.

Figure-3: Plasmid profiles of multidrug resistant *Pseudomonas aeruginosa* isolates



1: DNA Ladder; 2: PASw1 isolate; 3: PASw4isolate; 4: PAP3 isolate; 5: PASw7isolates; 6: PASw21 isolate; 7: PAU2 isolate; 8: PASp11 isolate;9: PASp35 isolate; 10: PASp26 isolate; 11: PASp29

Table-3: Plasmid profiles of multidrug resistant *Pseudomonas aeruginosa* isolates

No	Isolates	Plasmid band number	Band size
1	PASw2	1	> 1kbp
2	PASw7	2	> 1kbp
3	PASw21	1	> 1kbp
4	PAU2	1	> 1kbp
5	PAP9	1	> 1kbp
6	PASp11	2	> 1kbp
7	PASp35	1	> 1kbp
8	PAP14	1	> 1kbp
9	PAP22	1	> 1kbp
10	PAD3	1	> 1kbp
11	PASp26	1	> 1kbp
12	PAP16	1	> 1kbp
13	PAU6	1	> 1kbp
14	PASp29	1	300 bp
15	PAP17	1	> 1kbp
16	PAP18	1	> 1kbp

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