ORIGINAL ARTICLE

ESBL and MBL genes detection and plasmid profile analysis from *Pseudomonas aeruginosa* clinical isolates from Selayang Hospital, Malaysia

NAZMUL MHM[1], FAZLUL MKK[2], SHAH SAMIUR RASHID[2], *SAEID REZA DOUSTJALALI[1], FARZANA YASMIN, KARIM AL-JASHAMY[1], MUNIRA BHUIYAN[1], NEGAR SHAFIEI SABET[1].*

ABSTRACT

Aim: This study was carried out to detect the ESBL and MBL genes and plasmid profile analysis among the clinical isolates of *Pseudomonas aeruginosa.*

Methods: For this study, 54 *P. aeruginosa* isolates were obtained from blood, skin, pus, respiratory, eyes, urine and sputum of both paediatric and adult patients from Selayang Hospital, Kuala Lumpur. Double Disk Synergy Test (DDST) and E-test were used to detect both ESBL and MBL genes. Plasmids were detected using Close and Rodriguez with modification (1982) method. QIAprep Spin Kit was also used to extract Plasmid DNA and the method was followed according to manufacturer's instructions.

Results: Among all the *P. aeruginosa* isolates, 12.97% were ESBL positive, but none of the isolates were found to produce MBLs. Plasmids were detected in ten isolates with the POR of (18.51%). The overall sizes of the plasmid DNA ranged from the lowest 1.8 kb to the highest 14 kb. These ten isolates appear to harbor one or more plasmids with the maximum of four plasmids. Three isolates possessed single sized plasmids (3400bp- 4600bp) while three isolates had four plasmids (1800bp- 5800bp) and four isolates had two plasmids (220bp-14000bp).

Conclusion: This study shows the prevalence of ESBL and MBL genes and also shows the presence of plasmids among the clinical isolates of *P. aeruginosa.*

Keywords: ESBL, MBL, Plasmid profile, Pseudomonas aeruginosa

INTRODUCTION

*Pseudomnasaeruginosa (P. aeruginosa)*has become increasingly known as an emerging opportunistic pathogen. It is commonly involved in infections of immunosuppressed patients. It can also cause outbreaks of hospital-acquired infections[1]. It always causes nosocomial infections such as bacteremia, urinary tract infections (UTIs), and pneumonia. Sometime Pseudomonas infections are complicated and can be life threatening[2]. In developing countries, *P. aeruginosa* was reported to be the most common pathogen causing wound infection[3]. Unfortunately *P.aeruginosa* develops multi-drug resistance to most of antibiotics[4]. The emerging antibiotic resistance is a serious global problem which resulted treatment failures and increased health care costs. Production of beta-lactamase is the most common cause of bacterial resistance to beta-lactam antibiotics. The problem of metallo-beta-lactamase (MBL) production is increasing with increasing production of extended-spectrum beta-lactamase (ESBL) in hospitals.ESBLs and MBLs production is a significant problem in clinical isolates of *P. aeruginosa.*Both the genes have been reported to spread from *P. aeruginosa* to some members of *Enterobacteriaceae*[5]. The organisms that produce ESBL or MBL are also associated with a higher mortality and morbidity[6]. Plasmids are made of circular double stranded DNA molecules.Plasmid profile analysis examines the total bacterial plasmid content. Plasmid mediated antibiotic resistance is common in *P. aeruginosa*and plasmid plays an important role in bacterial multidrug resistance.

[1]Faculty of Medicine, SEGi University, Kota Damansara, Petaling Jaya, 47810 Selangor, Malaysia.
[2]Faculty of Industrial Sciences and Technology, University Malaysia Pahang, Gambang, 26300 Pahang, Malaysia
Correspondence to Dr Mohammad Nazmul Hasan Maziz, Associate Professor in Microbiology, Faculty of Medicine, SEGi University, Kota Damansara PJU 5, 47810 Petaling Jaya, Selangor, Malaysia Email: poorpiku@yahoo.com

MATERIALS AND METHODS

For this study, 54 *P. aeruginosa* isolates were obtained from blood, skin, pus, respiratory, eyes, urine and sputum of both paediatric and adult patients from Selayang Hospital, Kuala Lumpur.

Double Disk Synergy Test (DDST) and E-test were used to detect both ESBL and MBL genes. In DDST**,**discs of third generation cephalosporins and augmentin were kept 30mm apart from center to center on inoculated Muller-Hinton Agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards augmentin disc was interpreted as positive. The increase in the zone diameter was due to the inhibition of the β lactamase by clavulanic acid. The procedure for E-test was followed according to the manufacturer's instructions.

Plasmids were detected using Close and Rodriguez with modification (1982) method[7]. QIAprep Spin Kit was also used to extract Plasmid DNA and the method was followed according to manufacturer's instructions. Plasmid DNA were resolved by electrophoresis in submerged horizontal agarose slab gel (0.7%) in Tris- Acetate buffer (TAE) at pH 8.3. Power for electrophoresis was supplied by a power pack (Bio-rad). Electrophoresis was carried out at room temperature from the cathode (-) to anode (+) at a constant voltage. The voltage gradient was varied depending on whether the gel was run overnight or short period of time. Supercoil DNA marker was used to estimate the plasmid size.It was used in electrophoresis gel each time along with the plasmids as molecular weight marker (Table 1).

RESULTS

Among all the *P. aeruginosa* isolates, 12.97% were ESBL positive, but none of the isolates were found to produce MBLs.Plasmids were detected in 10 isolates with the POR of (18.51%). The overall sizes of the plasmid DNA ranged from the lowest 1.8 kb to the highest 14 kb. These 10 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids.Three isolates possessed single sized plasmids (3400bp- 4600bp) while 3 isolates had four plasmids (1800bp- 5800bp) and 4 isolates had 2 plasmids (220bp-14000bp) (Table 2).

Table 1:Sizes of Supercoil DNA standard marker used for molecular weight estimation of plasmid DNA.(Purchased from Promega, USA).

Plasmid No.	Molecular weight (kb)
1	10.0
2	9.0
3	8.0
4	7.0
5	6.0
6	5.0
7	4.0
8	3.0
9	2.0

Table 2: Frequency of specific sites from which P. aeruginosa was isolated

pecimen	Number of specimens	% of Total
Pus	22	40.74
Urine	11	18.51
Resp.	9	16.67
Blood	6	11.12
Tissue	4	7.41
Genitalia	2	3.70

Fig. 1: Double Disk Synergy Test showing ESBL positive.

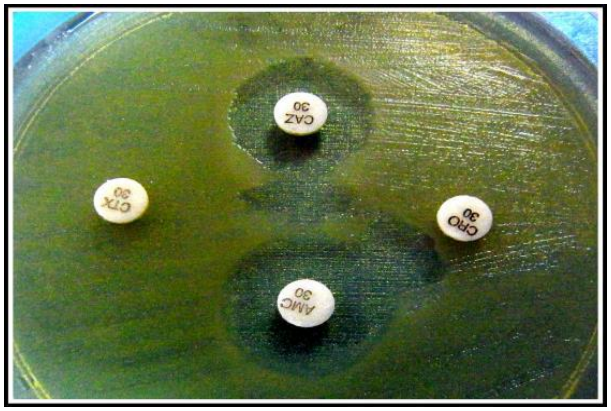

Fig. 2: Agarose (0.7%) gel electrophoresis of plasmid DNA extracted from *P. aeruginosa* 9 using plasmid isolation methods and kits.

Lane 1: Close and Rodrigue modified method, 1982 2: QIAprep Spin Kit

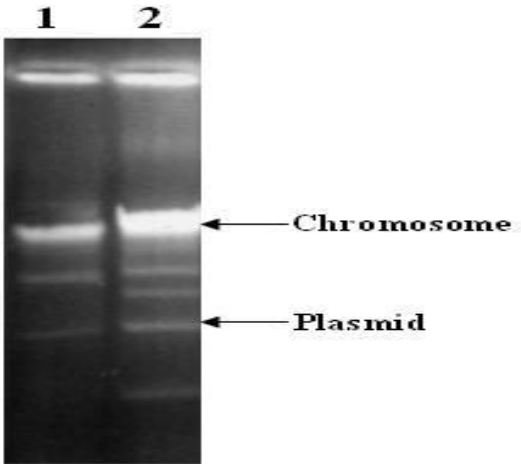

Fig. 3: Agarose (0.7%) gel electrophoresis of plasmid DNA
Lane M: Supercoli DNA marker
1: *P. aeruginosa* 7 4: *P. aeruginosa* 27
2: *P. aeruginosa* 9 *5: P. aeruginosa* 19
3: *P. aeruginosa* 14 *6: P. aeruginosa* 21

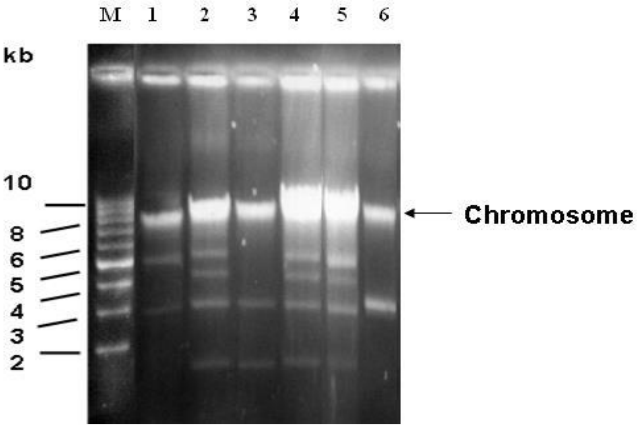

Table 3: The different size plasmids in all P. aeruginosa isolates

Sizes of plasmids DNA(kb)
14
5.8
5
4.6
3.4
2.2
1.8

Table 4: Plasmid occurrence rate (POR) in P. aeruginosa

No. of Plasmid DNA	Isolates No.	POR%
0	44	81.48
1	3	5.55
2	4	7.41
3	0	0
4	3	5.55
5	0	0

DISCUSSION

The DDST was based on the synergistic effect (Figure 1) between clavulanate and ceftazidime as a result of the inhibition of ESBLs in the presence of clavulanic acid. It is one of the most common and confirmation test to detect the presence of ESBL.

The production of ESBLs confers resistance at various levels to expanded spectrumcephalosporins, such as cefotaxime and ceftazidime, and to aztreonam, but not normally to the cephamycins and carbapenems[8].

Among the 54 isolates of *P. aeruginosa* 12.97% of isolates shows the ESBL positive. Studies in Iran showed 8.1% ESBL production among *P. aeruginosa*isolates[9]. The studies conducted by others depicted low rates, 3.7%[10], 4.2%[11], 7.7%[12] respectively, of ESBL production in *P. aeruginos*a. However, some studies showed high rate, 20.3%, 39.41%[8] and 35.85%[13] of ESBLs productions from *P. aeruginosa* isolates.

After screening all the *P.aeruginosa* isolates, none of the isolates was found to produce MBL. We did not find any isolates produce MBL. Recent studies detected MBL from twelve isolates using DDST suggesting the efficacy of this method for the phenotypic detection of MBL[14].

A plasmid is a small, circular, independent, self-replicating double-stranded DNA molecule that is extrachromosomal genetic elements carries only a few genes naturally exist in bacterial cells as well as in some eukaryotes. It is known that several important properties of enteropathogenic bacteria are plasmid mediated which can be distinguished from one another on the basis of the sizes expressed as the number of kilo base pairs (kb) of DNA or mega Daltons (Md) and determined by electrophoretic migration in agarose gel.

Scientists have taken advantage of plasmids to use them as tools to clone, transfer, and manipulate genes but the main problem is its separation from chromosomal DNA, since the plasmid usually compromise not more than 5% of the total DNA. To simplify the isolation of plasmid DNA there are several protocols have been developed in recent years. When selecting one procedure over another, factors such as the size of the plasmid to be isolated, the simplicity and reproducibility of the procedure, the bacterial genus to be lysed, the DNA yield and the subsequent use of plasmid DNA, one should aim for as few manipulations as possible. It should be noted that for most of these rapid procedures will invariably be contaminated to lesser or greater extent with chromosomal DNA. The detection of plasmids depends on the proper methods[15].

Other than using Close and Rodriguez with modification (1982) method[7], plasmid isolation kit (QIAprep Spin) was also used to extract and purify plasmid DNA from the bacterial isolates (Fig. 2). The plasmid DNA extraction kit purchased gave more purified plasmid without contamination with genomic DNA and was able to purify even small size plasmids. The kit was found to be consistent, *i.e.,* reproducible recovery of all plasmid DNA from *P. aeruginosa* was observed.

Supercoil DNA marker was used to estimate the *P. aeruginosa* plasmid size and was used as molecular weight markers in each gel. Plasmids were detected in 10 isolates (Fig. 3) with the POR of (18.51%) by using Close and Rodriguez method with modification (1982)[7] and QIAprep Spin Kit (Table 4). This finding is in agreement with Nazmul *et al.,* (2007)[15] where they detected plasmids in 3(5.56%) of the isolates.

These 10 isolates appear to harbour 1 or more plasmids with the maximum of 4 plasmids. Various plasmid profiles were observed in the isolates. Three isolates were found to carry only one plasmid DNA, 4 isolates were found to harbor 2 different plasmid DNA and 3 isolates were found to harbor 4 different plasmid DNA. The overall sizes of the plasmid DNA ranged from the lowest 1.8 kb to the highest 14 kb (Table 3). Forty four isolates (81%) did not carry any plasmids.

Studies in Nigeria showed the plasmid DNA ranged from the lowest 0.662 kb to the highest 0.83 kb[16]. In another study, Edward Raja *et al.,* 2009[17], detected *P. aeruginosa* plasmid of approximately 23 kb, which is larger plasmid compared to our current studies. Similar finding was observed in Zimbabwe where the plasmid DNA ranged from the lowest 1.80kb to the highest 8.88 kb[18]. Another study by

Chikwen du *et al.,* (2011)[19] showed plasmids ranging in size from 794-11845bp in more than 50% of all the tested isolates.

Studies have shown the involvement of plasmid in multidrug resistance. Therefore, plasmids found in our studies may play an important role in ESBL productions and multidrug resistance. The different sized plasmids found in our studies might be involved in multidrug resistance. Further studies on each plasmids are necessary to confirm the association of these plasmids in multidrug resistance.

CONCLUSION

This study shows the prevalence of ESBL and MBL genes and also shows the presence of plasmids among the clinical isolates of*P. aeruginosa.* These plasmids may possess multidrug resistant genes and also may be related to the production of ESBL genes but needs further study to confirm this.

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