ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Plasmid Profile Analysis of Clinical Isolates of *Acinetobacter* species in Malaysia.

Nazmul MHM[1]*, Jamal H[2], Rashid MA[2], Karim Al-Jashamy[1], Saeid Reza Doustjalali[1], and Negar Shafiei Sabet[1].

[1]Faculty of Medicine, SEGi University, Kota Damansara, Petaling Jaya, 47810 Selangor, Malaysia.
[2]Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, Sungai Buloh,47000 Selangor, Malaysia.

ABSTRACT

Forty clinical isolates of *Acinetobacter* species were collected from Selayang hospital, Selangor, Malaysia. The strains were isolated from respiratory tract, urine and pus. All the isolates were re-identified and confirmed as *Acinetobacter* species in our laboratory. Plasmids were detected in 16 isolates with the plasmid occurrence rate (POR) of 40%. These 16 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids. The sizes of the plasmid DNA range from the lowest 1.8 kb to the highest 8 kb. Supercoil DNA marker was used to provide reference plasmids of known molecular weight. This helped in calculating the molecular weight of the plasmids in this study.

Keywords: Plasmid profile, *Acinetobacter* species, clinical isolates.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In the nature *Acinetobacter* species (spp) are widely distributed. Species of the genus *Acinetobacter* is ubiquitous, free-living and fairly stable in the environment. They are strictly aerobic nonfermentative and Gram-negative bacilli that emerge as significant nosocomial pathogens in the hospital setting and are responsible for intermittent outbreaks. The outbreak of *Acinetobacter* is much more in the regions where temperature is hot and humid. Pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis and meningitis are the common infections caused by *Acinetobacter* spp and is a known nosocomial pathogen causing a wide range of clinical diseases including blood stream infections [1]. *Acinetobacter* spp cause infections that are difficult to control due to multi-drug resistance. *Acinetobacter* spp are noted for their intrinsic resistance to antibiotics and for their ability to acquire genes encoding resistance determinants. Foremost among the mechanisms of resistance in this pathogens are the production of beta-lactamases, aminoglycoside-modifying enzymes and plasmids [2]. Nevertheless, little knowledge has been gained on tracing the development of antibiotic resistance in *Acinetobacter* spp. However, there are few recent surveillance studies reporting in Malaysia showed multiple-drug resistance of *Acinetobacter* spp and the development of antibiotic resistance in *Acinetobacter* spp [3].

Plasmid is a small, covalently closed circular, double stranded extra chromosomal DNA element. It is self replicating independent DNA molecule. Plasmid contains the gene which is normally not essential for bacterial survival or growth. Plasmid commonly carries one or more genes which can confer advantage to the bacterial cell harboring them. Plasmid may confer toxin production, drug resistance etc. Plasmid profiling analysis sometime can be used for epidemiological analysis [4]. The size of the plasmid may vary from 1 kb to 250 kb. Different size of plasmids may be found in a single host bacterium and not all bacteria may contain plasmid. Some types of plasmid are found in large number and some are found in small number in bacterial isolates. In same bacterial cell many copies of a single plasmid can be found which can be extracted in the laboratory using different methods. Many plasmid DNA extraction methods have been established to date. No plasmid profile analysis has been carried out to correlate the association of plasmid with antibiotic resistance in this organism in Malaysia. Therefore, this study aims to extract the plasmids from all the collected *Acinetobacter* spp isolated from patients admitted to Selayang hospital, Malaysia and to analyze their profiles.

MATERIALS AND METHODS

Forty clinical isolates of *Acinetobacter* species were used in this study. These were obtained from Selayang Hospital, Selangor, Malaysia. Supercoil DNA was used to provide reference plasmids of known molecular weight which was purchased from Promega, USA. Plasmid DNA extraction kit was purchased from Eppendorf, Germany. Perfectprep Plasmid Mini preparation kit from Eppendorf, Germany, was used to extract Plasmid DNA and the method was followed according to manufacturer's instructions.

Agarose gel electrophoresis

After extraction, plasmid DNA were resolved by electrophoresis in submerged horizontal agarose slab gel (0.7%) in Tris-Acetate buffer (TAE) (pH 8.3). The agarose (Sigma Chemical Co., USA) was dissolved by boiling in 1X Tris-Acetate (TAE) buffer (pH 8.3) followed by cooling at 50^0C. Ethidium bromide (0.5 µg/ml) was added before casting. A 25 µl aliquot of extracted plasmid DNA was mixed with 5-10µl gel loading buffer. The DNA-dye mixture was then loaded into the well. The tank was filled with 1X Tris-Acetate (TAE) buffer (pH 8.3) to completely submerge the gel. Electrophoresis was supplied by a power pack (Model Vokam 400, Shandon Co. Ltd, England). Electrophoresis was carried out from the cathode (-) to anode (+) at a constant voltage at room temperature. The voltage used was in the range of 70 -90. Electrophoresis was stopped when the tracking dye (loading buffer) was 5-10 mm from the anode end of the gel. The DNA-ethidium bromide complex was visualised using UV transilluminator (Model TFX, Vilber Lourmat, France). Gloves were worn at all times when handling agarose slab gels containing ethidium bromide. Photography was carried out with a Polaroid camera and Polaroid 665 black and white film. Exposure time was between 30-90 seconds. Digital camera was also used for photography and downloaded the image directly to the computer. The brightness and contrast of the image was adjusted before printing.

ISSN: 0975-8585

RESULTS

Plasmids were detected in 16 isolates with the POR of 40%. These 16 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids. Twenty four *Acinetobacter* spp (60%) did not carry any plasmids. The overall sizes of the plasmid DNA range from the lowest 1.8 kb to the highest 8 kb. (Figure 1, 2)

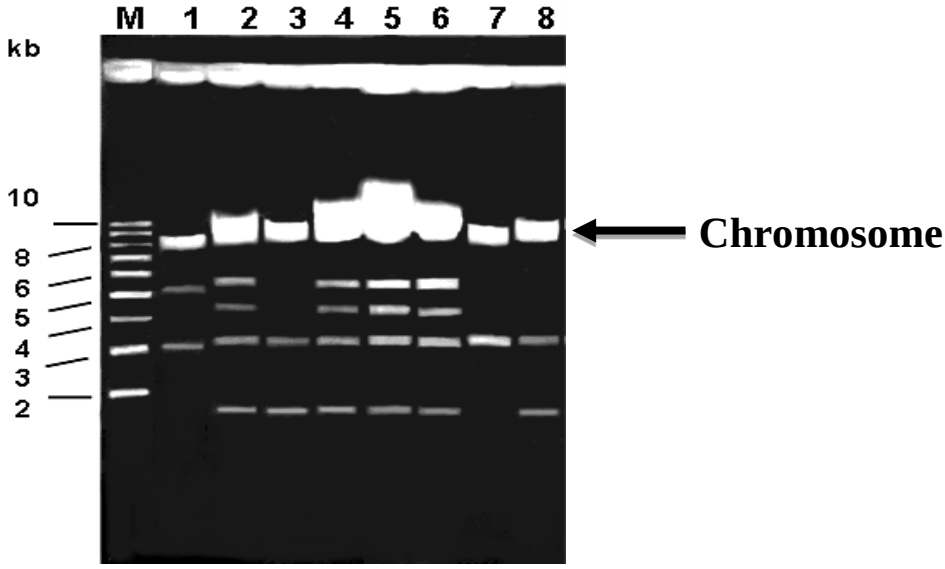

Figure 1: Agarose (0.7%) gel electrophoresis of plasmid DNA extracted from *Acinetobacter* species isolates and supercoil (M) as standard marker.

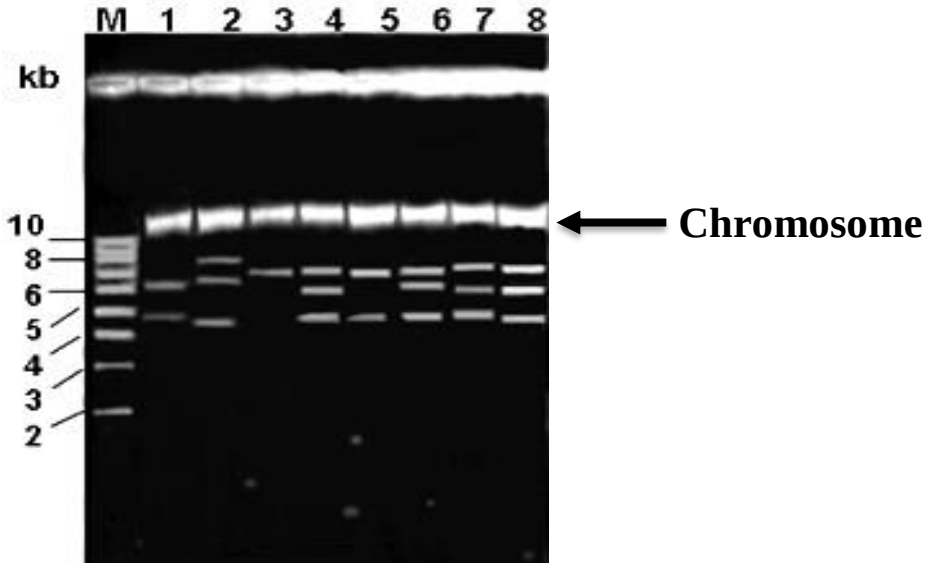

Figure 2: Agarose (0.7%) gel electrophoresis of plasmid DNA extracted from *Acinetobacter* species isolates and supercoil (M) as standard marker.

DISCUSSION

Perfectprep Plasmid Mini preparation kit from Eppendorf, Germany, was used to extract Plasmid DNA and the method was followed according to manufacturer's instructions. The exact procedure was followed and no further modification was done. This isolation technique gave plasmid DNA bands in patterns, and this technique was found to be stable, i.e., reproducible recovery of all plasmid DNA was observed from all the *Acinetobacter* spp isolates. Therefore, this kit was used for plasmid profiling in this study. Firstly, the plasmids

of all the 40 *Acinetobacter* spp isolates were extracted. The experiment was repeated few times for the confirmation of the findings. After purification, the plasmids were analyzed to estimate the approximate size by using the graphical method of Aiij and Borst, 1972 [5].

The largest plasmid observed was 8 kb (60 Md) plasmid. This plasmid was carried by 1 *Acinetobacter* spp isolates. Six *Acinetobacter* spp isolates revealed the presence of a 7.2-kb plasmid DNA. This result is in agreement with Wan Himratul (1999) findings where different bacteria were used. This 7.2-kb plasmid from non-O157 *E. coli* was previously shown to be associated in VT1 production. DNA hybridization studies showed that, this 7.2 kb plasmid to be hybridized against VT1 probe [6].

Three of the *Acinetobacter* spp isolates harbored a 6.6 kb plasmid. This size plasmid was also reported by Wan Himratul, (1999) and a plasmid of similar size was also reported by Scaletsky *et al*., (1995) [6, 7]. Scaletsky *et al.,* (1995) reported the 6.6 kb plasmid to be very significant in its pathogenesis. Bacteria containing this plasmid synthesize a protein of 32 kDa (pl 4.93) which seemed to be required for cell invasion. They isolated this 6.6 kb plasmid from an O111:H- EPEC that is capable of conferring to an avirulent, non-adherent *E. coli* k12 strain (DK1) the capacity to invade epithelial cells [7]. Similar studies are required to observe the pathogenesis of the 6.6 kb plasmid harbored by the *Acinetobacter* spp isolates in this study.

The most common plasmid found was a 4.6 kb plasmid, which was harbored by 11 *Acinetobacter* spp isolates. Wan Himratul, (1999) also showed a similar finding in Malaysia when tested different isolates, where 16 diarrheagenic *E. coli* were found to harbor a 4.6-kb plasmid [6]. Another plasmid found was 5.8 kb plasmid. The significance of these plasmids is yet to be known. Gyles *et al.* (1974) found plasmids coding for heat stable enterotoxins was in the range of 3.2-12 kb [8]. It suggests that any of this plasmid might be involved in producing heat stable enterotoxin but requires further study to confirm its association with the toxin production.

Six *Acinetobacter* spp isolates harbored two plasmids of 3.4 kb and 1.8 kb. One possible explanation for this 3.4 kb plasmid, is that may be a dimer of 1.8 kb plasmid, in which there is only one plasmid DNA that is present in two possible forms namely super helical of covalently closed circular (CCC) and relaxed form or open circular (OC). Two *Acinetobacter* spp isolates were found to harbor only 3.4 kb plasmid without harboring 1.8. Wan Himratul (1999) also showed that these two plasmid 3.4 kb and 1.8 kb were harbored by two different *E. coli* isolates [6]. These findings indicate that these two 3.4 kb and 1.8 kb plasmids may be of different origin and unrelated, not a dimer. But the significance of these two plasmids is yet to be reported.

Four *Acinetobacter* spp isolates were observed to have identical plasmid profiles carrying four different size plasmids of 5.8, 4.6, 3.4 and 1.8 kb. Another 4 *Acinetobacter* spp isolates also showed to harbor identical plasmid profiles carrying 3 different size plasmids of 7.2, 6.6 and 4.6 kb. Many other isolates also showed identical plasmid profile among them. Identical plasmid profiles have been reported when the isolates are in same serogroups [9]. Fernandes *et al.,* (1992) reported the usefulness of plasmid profile analysis to differentiate strains of *Acinetobacter* spp. They showed no common plasmid profiles among strains of distinct serotypes. However, they grouped most of the strains within a few major profiles. They also reported that plasmid profile analysis is very useful to differentiate strains within specific serotypes [9]. All the *Acinetobacter* spp isolates in this study need to be further serotyped to provide a finer plasmid profiling analysis as to differentiate the strains within the specific serotype. This will also lead to a better understanding of the plasmid profiles among the respective serotypes. There are several common small plasmids of similar sizes among many of the *Acinetobacter* spp isolates, but the significance of these plasmids is yet to be determined.

No plasmids were detected in 24 (60%) of the *Acinetobacter* spp isolates. This finding also agrees with the findings of Wan Himratul (1999) where 22% different strains of non-O157 *E. coli* isolates were shown to be plasmidless. Plasmid occurrence rate is normally significant for the epidemiological studies where large number of isolates is tested. Plasmids are known to be associated with different virulence property of the bacterial isolates. In this study POR is significant for the basis of further studies and each plasmid needs to be characterized to detect their role in pathogenesis (if any).

CONCLUSION

POR in this study was found to be 40%. POR is usually more significant for epidemiological studies where a large number of isolates are tested. Large number of isolates is needed to be tested to use this POR as an epidemiological tool for *Acinetobacter* species 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.

ACKNOWLEDGEMENT

Would like to thank the laboratory staff for their support. This study was funded by UiTM internal DANA grant.

REFERENCES

[1] Dijkshoorn L, Nemec A. The diversity of the genus *Acinetobacter*. In: Gerischer U editors. *Acinetobacter* molecular biology. Norfolk: Caister Academic Press 2008; 1–34.
[2] Robert AB, Dora S. Oxford Journal 2006; 43: S49-S56.
[3] Nourkhoda S, Reza R, Javad Z, Mohammad YA, Sobhan G, Mohammad R, Ahmed SA, Ali D, Reza M, Fatimah AB. Asian Biomed 2010; 4: 901-911.
[4] Bratoeva MP, JF John, and NL Barg. J Clin Microbiol 1992; 30: 1428-1431.
[5] Aiij C. and Borst P. Biochem Biophys Acta 1972; 269: 197-200.
[6] Wan Himratul A. Detection and molecular analysis of non-O157 diarrheagenic *Escherichia coli* isolates by using VT1 probe. 1999; Master's thesis, University of Malaya, Malaysia.
[7] Scaletsky, IC, Gatti MS, JF. da Silveira, DeLuca IM, Freymuller E and L.R. Travassos. Microb Pathog 1995; 18: 387-99.
[8] Gyles C, M So. and Falkow S. J Infect Dis 1974; 130: 40-49.
[9] Fernandes RM, SR Ramos, V Rassi, PA Blake, and TA Gomes. Braz J Med Biol Res 1992; 25: 667-72.