



Plasmid Mediated Drug Resistance in *Acinetobacter baumannii* Isolated from Clinical Samples

KEYWORDS

Acinetobacter baumannii, multidrug resistant, plasmid

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ABSTRACT *Acinetobacter baumannii* is a nosocomial pathogen of increasing importance. Prevalence of the multidrug resistant phenotype (MDR) *Acinetobacter baumannii* has increased during the last decade worldwide. Isolation, characterization of plasmids among resistant isolates are very important as they can be disseminated horizontally and contribute to global spread of resistance. Aim of the study to determine the antibiotic susceptibility of the isolates. Detection of plasmids from multidrug resistant *A.baumannii* by alkaline lysis method. A total of 107 clinically significant *Acinetobacter* species from clinical samples and plasmid extraction. *A.baumannii* from clinical samples were 73%, multidrug resistant isolates were 69.2% and plasmid extracted were 59.5%. Awareness of plasmid mediated drug resistant and molecular profiling of these strains are important in current era of drug resistance.

INTRODUCTION:

The genus *Acinetobacter* are ubiquitous, pleomorphic Gram negative coccobacilli. They are opportunistic pathogen being associate with wound infection, urinary tract infection and secondary meningitis. Multi-drug-resistant (MDR) *Acinetobacter baumannii* is a nosocomial pathogen of increasing importance which is rarely found in community isolates of *A. baumannii*. The prevalence of the MDR phenotype among hospital isolates has increased during the last decade and MDR *Acinetobacter baumannii* have turned out to be a leading pathogen in many hospitals worldwide. Hospital outbreaks have been described from various geographical areas, and in some areas this organism has become endemic. Since these patients are often transferred to tertiary care centers, they may become a source of transmission in previously non-endemic hospitals [1].

Acinetobacter species tend to be resistant to a variety of antibiotics and the presence of resistance plasmids (R- plasmids) is a significant features of this organism. Plasmid profiling has been proposed as a method of epidemiological typing of *Acinetobacter*. Most reported cases of indigenous transmissible antibiotic resistance from *Acinetobacter* spp. have been associated with plasmids belonging to broad-host-range incompatibility groups [2]. Plasmid mediated drug resistance to penicillin [3], beta lactamase resistance to penicillin in *H.influenzae*, *N.gonorrhoeae*, metallo beta lactamase (MBL) production in *Acinetobacter* sps and *Pseudomonas* spp [4], tetracycline resistance in *Gonococci* are other examples of plasmid mediated antibiotic resistance [5].

OBJECTIVES:

Isolation and speciation of *Acinetobacter* from clinical samples. To determine the antibiotic susceptibility and resistance pattern of the isolates. Detection of plasmids from the *Acinetobacter* species by alkaline lysis method.

MATERIALS AND METHOD:

A total of 107 clinically significant *Acinetobacter* species were collected from January 2012 to December 2012 in Chettinad Hospital and Research Institute. The source of isolates were blood, urine, pus, wound swabs, respiratory samples, and body fluids. The species identification were carried out by API NE analyser. The antimicrobial susceptibility were carried out, the resistance and susceptibility profile were determined by Kirby Bauer disc diffusion method on Muller Hinton agar (Hi-media) and the resistant strains were tested with higher group of drugs of carbapenem and reserve drugs polymyxin B and colistin (E strip) by standard CLSI guidelines[6].

Pure culture grown in Luria broth after incubation at 37°C for 24 hours were used for plasmid extraction. From the bacterial culture, concentrated pellet the cell were taken for separation of the plasmid DNA. Plasmid were extracted from *Acinetobacter baumannii* isolates by the alkaline lysis method and gel electrophoresis were done.

Add 0.2 ml ice-cold Solution 1 to cell pellet and re-suspend cells as much as possible using disposable transfer pipet.(Solution 1 contains glucose, Tris and EDTA). Add 0.4 ml Solution 2, cap tubes and invert five times gently. Let tubes at room temperature for 5 minutes. (Solution 2 contains NaOH and SDS (a detergent).Add 0.3 ml ice-cold Solution 3, cap tubes and invert five times gently. Incubate tubes on ice for 10 minutes. (Solution 3 contains a mixture of acetic acid and potassium acetate). Centrifuge tubes for 5 minutes. Transfer supernatant to fresh micro centrifuge tube using clean disposable transfer pipet. This separates the plasmid DNA from the cellular debris and chromosomal DNA in the pellet. Centrifuge tube is filled with isopropanol, let tube at room temperature for 2 minutes. A milky pellet at bottom of the tube after centrifuge for 5 minutes,

used for loading wells in the gel. Add 1 ml of ice-cold 70% ethanol, spin tubes for 1 minute. Pour off supernatant, allow tube to dry for 5 minutes. Add 50 μ l TE to tube and plasmid DNA pellet ready for gel electrophoresis [7]. DNA are visible on the gel after electrophoresis by the dye ethidium bromide added to agarose gel along with tracking dye and isolation of bands based on molecular size of DNA determined by examination of the gel under the UV trans illuminator.

RESULTS

Clinical specimen like blood, urine, pus, sputum, CSF were processed and isolates Gram negative, nonmotile coccobacilli based on culture morphology and biochemical reactions were identified as *Acinetobacter* species (107), *Acinetobacter baumannii* (78), *A.haemolyticus* (23), *A.loffii* (6) and confirmed by API NE.

Among the clinical isolates the predominant species were *A.baumannii* (73%). Sputum was most common source of *Acinetobacter* spp. 14 % (n=107). Patients under the age group of 15 to 40 (Figure-1) were highly infected with *Acinetobacter baumannii* (51%).

The gender ratio was not much variable, both male 45.7% (n=49) and female 54.2% (n=58) were equally infected with *Acinetobacter* species. The isolates of multidrug resistant strain (Figure-2) among the female 44.8% (n=48) were greater than in male patient 24.2% (n=26).

The *Acinetobacter* strains that showed different antibiotic susceptibilities pattern, the resistance pattern to the aminoglycosides 18-30% (amikacin-32.7%, gentamycin-35%, tobramycin-19.4%), ciprofloxacin-35%, cephalosporin 40-50% (cefotaxime-45%, cefuroxime-40%, cefipime-48.3%). Out of 107 *Acinetobacter baumannii* 74 (69.2%) shows multi drug resistant (MDR) and 12% (13/36) were multidrug resistant among the isolates from ICU. And resistant to imipenem were 12.4% and meropenem were 21.6%.

The carbapenem resistant strains were tested for the susceptibility pattern to the polymyxin B, colistin and tigecycline susceptibility by both disc diffusion method and E- strip method (100% susceptibility) all strains were susceptible.

Agarose gel electrophoresis of plasmid DNA extracted by alkaline lysis method showed plasmid band pattern. Plasmid extractions (Figure-3) were positive for (44) 59.5% of the isolates of MDR *Acinetobacter baumannii*. The 14 isolates with sharp plasmid bands were observed. Molecular size of the plasmids ranged from 35 to 580 kbp when compared with the standard marker.

DISCUSSION

There are numerous report on *A. baumannii* regarding their emergence, spread of multidrug resistant *Acinetobacter* spp, its genetic potential to carry and transfer diverse antibiotic resistant determinants pose a major threat in hospitals. The overall evolution of antibiotic resistance can be attributed to various factors like spread of transposons or R plasmids to various pathogens mainly because of the selective forces imposed on humans due to the overuse of antibiotics.

In our study the most frequent source of the isolates were from sputum (46.9%) similar to studies of Maria D et al [8] and controversial study of Patwardhan et al [9], *Acinetobacter* with high percentage in urinary tract infection. Analysis of the relationship between incidence of *Acinetobacter* spp. and patients age, indicated the highest incidence in

patients of 15 to 40 years which was controversial to the study of Abdullah A. Al-Arfaj et al [10], with highest incidence in patients of 61 to 70 years.

In our study *Acinetobacter* strains that showed different antibiotic susceptibilities pattern, the resistance pattern to the aminoglycosides 25%, quinolones 35% and cephalosporin 45%. A study by Alessandro Capone et al [11], high level resistant exhibited by *Acinetobacter* to carbapenem with 79% (imipenem and meropenem) when compared with our isolate 44% resistance. Isolates from ICU were more than 10% Patwardhan et al [9] and in our study 12% of ICU isolates were multidrug resistance. The isolates other than *A.baumannii* were found to be susceptible to all drugs.

Susceptibility of *A. baumannii* to polymyxin B were similar to Carl urban et al [12], despite resistance to all other antibacterial agents. Strains of *A. baumannii* were 100% susceptible to tigecycline but controversial to study of Alessandro et al [11].

Isolation, characterization of plasmids are very important as they can be disseminated horizontally through the resistant determinants located in plasmids, transposons contributing to the global spread of resistance mechanisms [4]. Plasmid DNA extracted by alkaline lysis method and plasmid bands documented by gel electrophoresis in 59.45% (n=44) and controversial to study of Saranathan et al [13]

CONCLUSION

These isolates are important because of nosocomial infection and their resistance transmitted by plasmid or mutation. Plasmid transmission are more dangerous and burden to clinicians so awareness of plasmid mediated drug resistant and molecular profiling of these strains are important in current era of drug resistance. future plan to design a genomic study in multidrug resistant *A.baumannii*.

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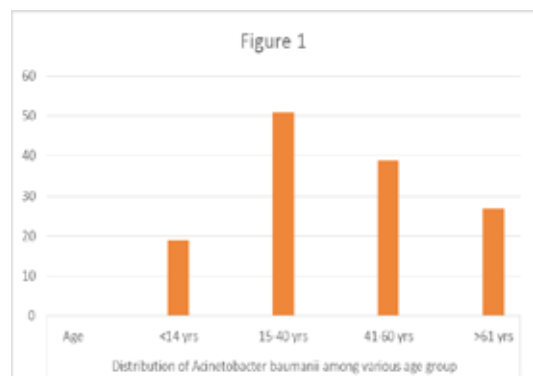


Figure 1 : Distribution of *Acinetobacter baumannii* among various age groups.

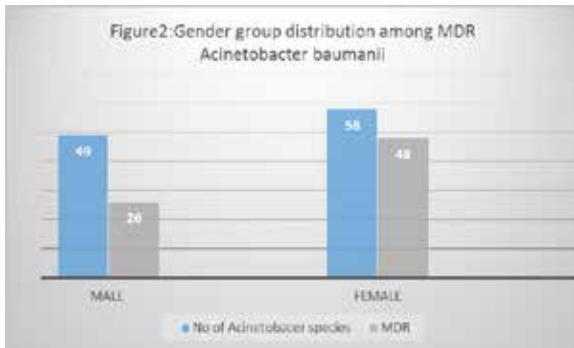


Figure 2: Gender distribution of multidrug resistant (MDR) *Acinetobacter baumannii*.

Figure 3: Gel documentation of plasmid DNA

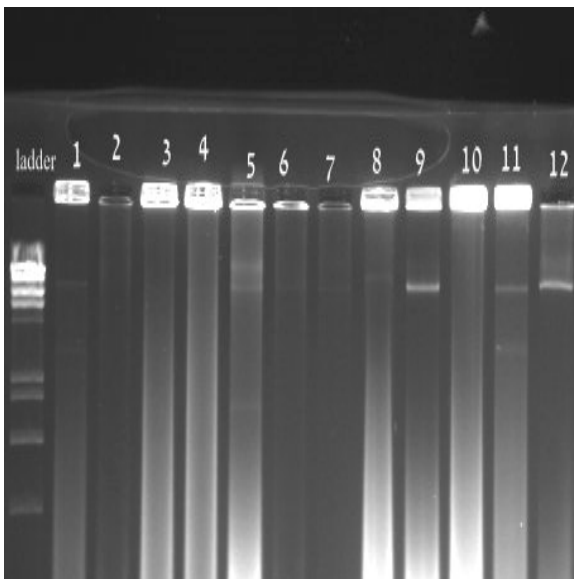


Figure 3: Gel documentation of plasmid DNA of *Acinetobacter baumannii*.

Lane 5, 9, 11, 12 – positive for plasmid showing sharp band pattern. Other lanes were negative for plasmid band pattern.

Table: 1
Distribution of *Acinetobacter* species among the clinical samples

Samples	No of isolates	No of Nonfermentors(%)	No of <i>Acinetobacter</i> species (%)
Blood	873	42 (4.8)	11(1.2)
Pus	2246	107(4.7)	49(2.1)
Sputum	198	93(46.9)	28(14.1)
Urine	1321	86 (6.5)	19(1.4)
Total	4638	328 (7.07)	107(2.3)

TABLE:2
Prevalence of plasmid among MDR *Acinetobacter baumannii*:

MDR <i>Acinetobacter baumannii</i> (n=74)	No of isolates (%)
Plasmid isolation Positive	44 (59.45)
Plasmid isolation Negative	30 (40.54)

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