INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

PHENOTYPIC CHARACTERIZATION OF ACINETOBACTER SPP. ISOLATED FROM ICU, S OF A TERTIARY CARE HOSPITAL

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Accepted Date: 04/05/2016; Published Date: 27/06/2016

Abstract: Background: The organism of the genus *Acinetobacter,* has been implicated in recent years as an important cause of nosocomial infections which are difficult to control and treat, because of wide spread antimicrobial resistance possessed by this organism. **Objective**: To isolate and phenotypically characterize *Acinetobacter* species from respiratory samples. **Material and methods**: All the respiratory samples from the patients admitted in the ICUs of Dayanand Medical College and Hospital over a period of one year (March2014- Feb 2015) were processed and the Acinetobacter isolates were identified and their antimicrobial susceptibility pattern was determined. Further these isolates were categorized into multi drug resistant (MDR), extensively drug resistant (XDR) and pan drug resistant (PDR) isolates. **Results:** Out of the total 302 Acinetobacter isolates, maximum isolates were obtained from endotracheal secretions. All the isolates were sensitive to polymixin B and tigecycline, while a high degree of resistance was seen against fluoroquinolones followed by third generation cephalosporins and carbapenems. Most of the *Acinetobacter baumanii* isolates were XDR, however no PDR isolate was obtained. **Conclusion:** The high prevalence of XDR among the *Acinetobacter baumanii* isolates emphasizes the need for an early detection of such organisms so that it can help in providing an appropriate antimicrobial therapy

Keywords: Isolated, *Acinetobacter baumanii,* Hosptital

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PAPER-QR CODE

Access Online On:

www.ijprbs.com

How to Cite This Article:

Rama Gupta, IJPRBS, 2016; Volume 5(3): 30-38

INTRODUCTION

The genus Acinetobacter can presently be defined as Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, and oxidase- negative coccobacillary bacteria with a DNA G and C content of 39% to 47%. [1,2] The taxonomy of the genus Acinetobacter has undergone extensive development over the past few decades. Currently the genus comprises more than 30 species of which *Acinetobacter baumannii*, and to a lesser extent species GS 3 and GS 13TU, are most associated with the clinical environment and nosocomial infections. These three species and *Acinetobacter calcoaceticus*, a species isolated from soil and water and rarely from human clinical specimens has been collectively termed as, *A. calcoaceticus -A. baumannii (Acb)* complex, because of their high phenotypic similarity.[3,4]. Other important species which are responsible for nosocomial infections include *Acinetobacter lwoffii, Acinetobacter haemolyticus* and *Acinetobacter junii.*

Prolonged hospital and ICU stay along with utilization of mechanical ventilators, drainage tubes, and indwelling catheters are important risk factors that predispose individuals to get infected with *Acinetobacter* spp.[5] Currently, these bacteria represent one of the most important agents causing ventilator-associated pneumonia (VAP) together with *S. aureus, P. aureginosa* and Enterobacteriaceae.[6-7] Treatment of *Acinetobacter* infections has conventionally involved the use of ß-lactams, aminoglycosides, and quinolones . However, the increased use of these antibiotics has resulted in a widespread emergence of antibiotic resistant strains [8].

These organisms may lead to therapeutic dead ends if not detected earlier. Many clinical laboratories have problems in detecting the different strains of this organism, in part because of their confused taxonomic status. In India there have been few studies of *Acinetobacter* species characterization and their antibiotic resistance pattern. Therefore study is planned to phenotypically characterize *Acinetobacter* species.

Materials and Methods

The present study was a prospective study conducted over a period of one year (March 2014 - Feb 2015) on patients admitted in various ICUs in Dayanand Medical College and Hospital, Ludhiana. The respiratory samples (ET secretions, Bronchoalveolar lavage, sputum etc) received in the department of microbiology, were processed as per the standard protocols and Acinetobacter isolates obtained from these samples were further characterized.

Identification of Acinetobacter Spp.

Growth was identified by the colony morphology, Gram's staining, motility and oxidase reaction. The non-lactose fermenting, Gram negative cocco bacilli, non-motile, oxidase

negative, isolates were primarily labeled as Acinetobacter. These isolates were then further characterized.

Phenotypic Characterization of Acinetobacter was done on the basis of glucose oxidation, gelatinase production, citrate utilization, β haemolysis on blood agar, growth at 44°C, 41°C, 37°C and susceptibility to penicillin and chloramphenicol [9].

Organism	44°C	41°C	Citrate	OF glucose	Geltainase production	Haemolysis
A.baumannii	+	-	+	+	+/-	-
A.lwoffii	-	+	-	-	+/-	-
A.haemolyticus	-	+	+	+	-	+
A.junii	-	+	+	-	-	+/-

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method on Mueller Hinton Agar (MHA) as per CLSI guidelines [10] The (Mueller Hinton Agar (MHA) plate was inoculated using a swab, that has been submerged in a bacterial suspension, by lawn culture method. The surface of the plate was swabbed in three directions to ensure an even and complete distribution of the inoculum over the plate. The antimicrobial discs were applied and then the plates were incubated at 35-37ºC for 24 hour. The antimicrobial disc applied were gentamicin (10µg), amikacin (30µg), ceftazidime (30µg), ciprofloxacin (5µg), cotrimoxazole (1.25/23.75µg), piperacillin(100µg), cefoperazone-sulbactam (75µg/30µg), imipenem (10µg), cefepime (30µg), polymixin B (300 µg), tigecycline (30µg). The zones of inhibition were measured after 24 hours and compared with the performance standards of antimicrobial disc susceptibility test provided by CLSI.[10]

Categorization of Multi drug Resistant Acinetobacter Isolates.:

The isolates were categorized as multidrug resistant (MDR), extensively drug resistant(XDR) and pan drug resistant(PDR), as per definitions[11] given below.

MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories.

XDR: non-susceptible to ≥1 agent in all but ≤2 categories.

PDR: non-susceptible to all antimicrobial agents.

Results

A total of 302 isolates of Acinetobacter were obtained from 2336 respiratory samples received during the study period. Maximum number of the isolates were obtained from endotracheal secretions (76.8%), followed by sputum (55%) and bronchoalveolar lavage (12%). Out of the total Acinetobacter isolates 292 (96.6%) of the isolates were phenotypically identified as *Acinetobacter baumannii and only 10 (3.4%) were identified as Acinetobacter lwoffii* (Table 1).

Fig 1 depicts the antibiotic resistance pattern of all the 292 *Acinetobacter baumannii* isolates obtained. The *Acinetobacter baumannii* showed high degree of resistance towards all the classes of antimicrobials tested except polymixin B and tigecycline. All isolates were sensitive to polymixin B and tigecycline. Maximum resistance was observed towards flouroquinolones (ciprofloxacin) (98%), followed by cephelosporins (96.2-98%) and aminoglycosides (88.7-89.7%). Resistance towards the carbapenam varies between (81-93%) though more resistance was seen towards meropenem (93.1%) as compared to imipenem and ertapenem (81.8%).

A.lwoffii isolates were less resistant towards most of the antibiotics tested as compared to *Acinetobacter baumannii* isolates. Only 5 (50%) of the isolates were resistant to cefepime, ciprofloxacin and meropenem. All the isolates were susceptible to all other drugs tested (Table - 2)

Multidrug resistant (MDR) *Acinetobacter baumannii* is defined as resistance to more than three classes of antibiotics. Of the 292 isolates, 287(98%) were mutidrug resistant, and out of the 287 MDR 241 were XDR, being resistant to all the major group of antibiotics including aminoglycosides, cephalosporins, carbepenems, extended spectrum penicillins, and quinolones except polymixin and tigecycline. None of the isolate was pan-resistant (PDR). 50% of the *A.lwofii* isolates were MDR (Table-3).

Discussion

The epidemiological, clinical, prognostic, and therapeutic characteristics of *A. baumannii* isolated from infected patients have been studied widely in the last decade.[12] Failure to detect these organisms has contributed to their uncontrolled spread and the consequent clinical failures. The most alarming problems encountered during this period are the organism's ability to accumulate diverse mechanisms of resistance and the emergence of strains that are resistant to all commercially available antibiotics coupled with lack of new antimicrobial agents in the pipeline[13] . These resistant organisms may lead to therapeutic dead ends if not detected earlier.

A total of 302 isolates of Acinetobacter were obtained from 2336 respiratory samples received during the one year study period. These isolates were identified on the basis of motility, glucose oxidation, citrate utilization, gelatinase production,growth at 44°C/41°C/37°C and hemolysis on sheep blood agar. The important species identified were *A. baumannii (96%) and A.lwoffii (4%)* This was consistent with the study done by Turton *et al*, which also showed that the majority of the isolates were *A. baumannii* (78%) followed by *A.lwoffii* (8.8%)[14]. In a study done by Jaggi *et al*[15] in a tertiary care hospital, out of the 1632 Gram negative isolates from the entire hospital, 155 were *A. baumannii*. Another study done by Villers *et al* [5] has also reported a predominance of *A. baumannii* in tracheao bronchial secretions as 24.8 - 48.8%.

Acinetobacter baumannii has become an important pathogen in recent years and has been shown to increase morbidity and mortality.[16-18] The defnition of MDR varies in the literature, but several authorities consider an isolate to be multidrug resistant if it is resistant to three or more classes of antibiotics.[19] Multi- drug resistant *A. baumanii* is a significant problem as seen in this study where 98% of isolates were considered multidrug resistant and 78% were XDR. Dent *et al* has also reported 72% of the *A. baumanii* isolates as multidrug resistant.[20]

All these MDR/ XDR isolates in the present study, were resistant to all commonly used antibiotics including aminoglycosides, cephalosporins, carbepenems, extended spectrum penicillins, and quinolones and these findings are similar with previous studies.[21-23]

A distinction was made between carbepenems and the non-carbepenem β-lactam antibiotics because carbapenem resistance is a sentinel event for emerging antimicrobial resistance and in itself confers high resistance and therapeutic challenges.[24] Very high resistance towards cabpenams (88-91%) is a matter of concern in the present study. However, earlier studies from India have reported lower resistance rates (9.8-18.5%) in *A.baumannii*. This clearly explains that our study brings up an important aspect of increasing resistance in *A.baumannii* towards carbapenems, also.

CONCLUSION

Amongst all the *Acinetobacter baumanii* isolates, most of the isolates were resistant to various antimicrobials tested. The high prevalence of such resistant starains emphasizes the need for an early detection of such organisms so that it can help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistance strains.

Table-1 Distribution of the *Acinetobacter spp* isolated from respiratory samples (N=2336)

Organism	No.
A.baumannii	292 (96.6)
A.lwoffii	10 (3.4)
Total Acinetobacter spp	302

Table-2: Comparison of antimicrobial resistance profile of *Acinetobacter baumannii* (n=292) and Acinetobacter. lwoffii (n=10), from respiratory samples

Antibiotics	*A.baumannii* No. (%)	*A.lwoffii* No.(%)
Gentamicin	262(89.7)	0
Amikacin	259(88.7)	0
Piperacillin	282(96.5)	0
Ceftazidime	286(98)	0
Cefotaxime	281(96.2)	0
Ceftriaxone	282(96.5)	0
Cefepime	285(97.7)	5(50%)
Ciprofloxacin	286(98)	5(50%)
Cotrimoxazole	281(96.2)	0
Piperacillin- tazobactam	255(87.3)	0
Cefoperazone-sulbactam	224(76.7)	0
Ertapenem	239(81.8)	0
Imipenem	239(81.8)	0
Meropenem	272(93.1)	5(50%)

Polymixin B	0	0
Tigecycline	0	0

Figure-1: Antimicrobial resistance profile of *Acinetobacter baumannii* isolates from respiratory samples (n=292)

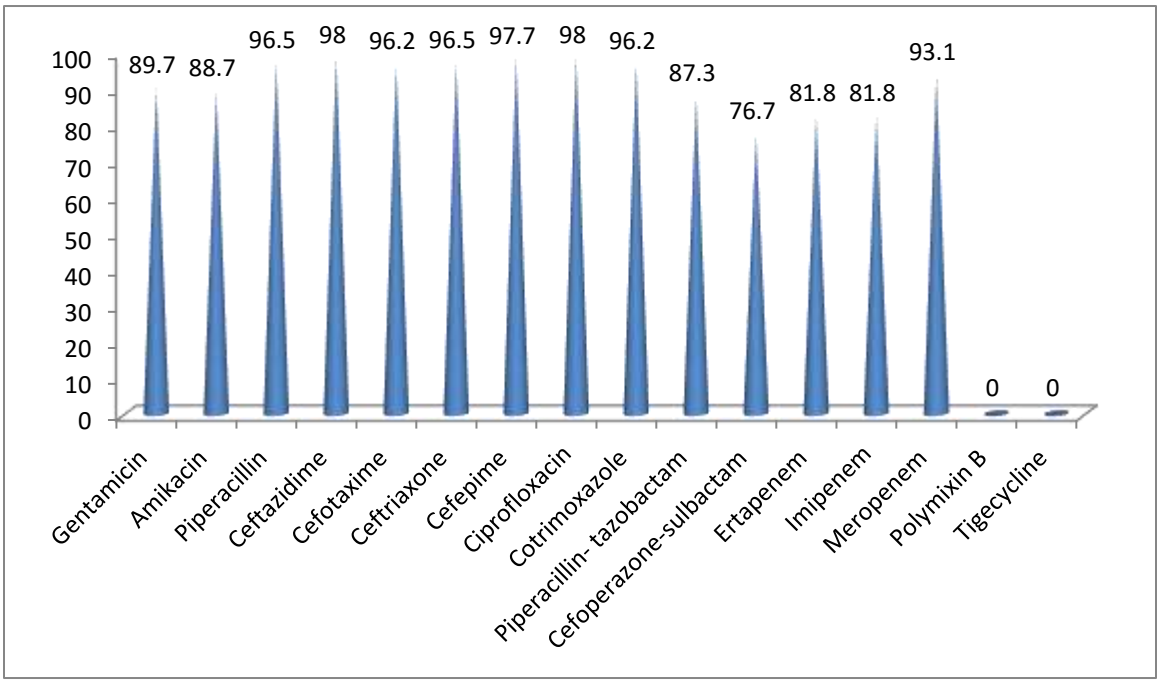

Table 3: Categorization of the isolates as MDR, XDR and PDR (n=302)

Organism	Isolates	MDR	XDR	PDR
A.baumannii	292	287(98.3%)	241(79.8%)	Nil
A.lwoffii	10	5(50%)	Nil	Nil

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