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Isolation, Characterization and Antibacterial Susceptibility test of *Acinetobacter* species obtained from Tertiary Care Hospital

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A B S T R A C T

The nosocomial infections are most important medical complications in all areas of the world and they remain one of the main sources of morbidity and mortality in hospitalized patients. *Acinetobacter* bacteria has appeared over the last era as significant opportunistic pathogen liable for nosocomial infections, although it is commonly related with benign colonization of hospitalized patients. The present investigation is carried out to isolate, identify *Acinetobacter* species from the different samples collected from tertiary care hospitals. Further susceptibility and resistance of different antibiotics against *Acinetobacter* is performed in the present study. A total of 30 samples from various sources (wound pus, urine, respiratory secretion, blood) were collected from hospital unit. Isolation is done for different samples by using different media as blood agar and MacConkey agar for pus samples, Chrom and CLED for urine samples, Blood agar, MacConkey agar and Chocolate agar for respiratory secretions and isolation from blood samples is done by using automated blood culture system. *Acinetobacter* isolates were initially identified according to the morphological characteristics on MacConkey agar and further identification is performed on the basis of biochemical characterization. The isolates of *Acinetobacter* are identified as Non-fermenter, Gram-negative coccobacilli on the basis of the Catalase positive, Oxidase negative, Urease negative, Nitratenegative, Indole negative and motility test. Antibacterial susceptibility testing was performed by Kirby Bauer disc diffusion method allowing to CLSI guidelines. Most of the isolates were obtained from blood samples. It has showed very high resistance (>75%) to Imipenem, Ceftazidime, Ciprofloxacin, Tobramycin, Doripenem. Only some of isolates have showed to be effective agents against gentamycin, amikacin and ampicillin sulbactam.

Introduction

Acinetobacter species has arisen as significant and challenging human pathogen as it is the causal agent of numerous kinds of infections in humans like nosocomial pneumonia, which is most often related to endotracheal tubes or tracheostomies, endocarditis, meningitis, septicaemia, skin, wound infections, UTI and bacteraemia peritonitis in patients receiving peritoneal dialysis (Winn *et al.*, 2006; Oberoi *et al.*, 2009). *Acinetobacter* sometimes "colonize" or live in a patient without producing infection or symptoms, especially in tracheostomy sites or exposed wounds. Presently, *A. baumannii* is becoming an imperative developing nosocomial pathogen universal and is liable for 2-10 % of all the

Gram-negative infections (Richet *et al.*, 2006). It is characterized as second most offending one after *Pseudomonas aeruginosa* amongst the nosocomial, aerobic, non-fermentative, gram negative bacilli pathogens. *Acinetobacter* species are saprophytic, ubiquitous and an important nosocomial pathogen due to its ability for persistence in the hospital environment on a wide range of dry and moist areas (Mindolli *et al.*, 2010). The genus *Acinetobacter* is now defined as Gram-negative non-fermenting bacilli, strictly aerobic, non-motile, catalase positive, and oxidase negative. *Acinetobacter* species generally form smooth and sometimes mucoid colonies on solid media, with a colour ranging from white to pale yellow or greyish-white. It has been isolated from the samples like human sputum, blood, urine and pus in patients admitted in hospital (Baltimore *et al.*, 1989 and Rosenthal *et al.*, 1975)**.**

Despite of the increasing significance and frequency of antibiotic resistant activity of *Acinetobacter*, numerous clinicians still lacking an appreciation of the potential importance of these organisms in hospitals. Since its first detection, *A. baumannii* has become resistant to numerous common antibiotics due to mutually intrinsic mechanisms and its ability to acquire drug resistance determinants. The accumulative occurrence of multi-drug and pan-drug resistant *A. baumannii* strains found in clinics has extracted it one of the few important nosocomial pathogens, only next to *Pseudomonas aeruginosa* amongst non-fermentative gram-negative bacteria (Dijkshoorn *et al.*, 2007; Navon-Venezia *et al.*, 2005). *A. baumannii* is resistant to dehydration, UV radiation, communal chemical sanitizers, and detergents, making it extremely challenging task to destroy *A. baumannii* contaminations from hospital surroundings, especially catheter-related devices used in intensive care units (ICU).

Antibiotic susceptibility pattern of *Acinetobacter* could differ extensively in nature and between different units of the same hospital at different time points. It is very much necessary to know about the alteration in *Acinetobacter resistogram*, for a periodic observation of these pathogens to get suitable group of treatment (Prashanth *et al.*, 2004 and Lone *et al.*, 2009) Due to random antibiotic resistance patterns of clinical strains of *Acinetobacter*, it is necessary to know the institutional widespread susceptibility profiles of this pathogen. In India a small number of studies on *Acinetobacter* species have been reported especially in Uttar Pradesh and in analysis of their increasing significance in nosocomial infections, more study is needed in this part. Accordingly, this study is undertaken to isolate the *Acinetobacter* species from different clinical samples via simplified phenotypic identification procedure and to determine the antibiotic susceptibility and resistance pattern of these isolates.

Materials and Methods

Processing of sample

The study was carried out in the Department of Microbiology of Ram Manohar Lohia Institute of Medical Sciences, Lucknow over a period of one year. A total of 1040 clinical samples received in the Department of Microbiology for bacterial culture and sensitivity from indoor and outdoor patients were included in the study.

Isolation of bacteria

Isolation is done for different samples by using different type of media.Blood agar and MacConkey agar was used for pus and sputum samples. CHROM and CLED media were used for urine sample. Blood culture was done by using automated blood culture system.

Identification of bacteria

Primary identification of isolates was based on colonial morphology, and different biochemical characteristics through pattern description in Bergey's manual of determinative bacteriology (Holt *et al.,* 1994 and MacFaddin, 2000). Gram staining was done on isolates. Culture positive isolates were further identified by biochemical tests. *Acinetobacter* species were differentiated by glucose test.

Antimicrobial susceptibility test

Antimicrobial susceptibility test for *Acinetobacter* species was done using most common and therapeutically and commercially important antibiotics by Kirby Bauer disk diffusion method on Mueller Hinton agar allowing to norms of Clinical Laboratory Standards institute (CLSI) CLSI document M100-S17 (Wayne, 2007). The antibiotics used in present study were as Ampicillin sulbactam (As), Cefoperazone-sulbactam (Cfs), Ceftriaxone (Ctx), Ceftazidime (Caz), Gentamycin (G), Chloramphenicol (Cmp), Ciprofloxacin (Cip), Imipenem (Imp), Amikacin (Ak), Tetracycline (Tet), Tobramycin (Tob), Doripenem (Dori), Doxycycline (Do), Levofloxacin (Levo).

Results and Discussion

Out of 1040 samples, 359 (34.5%) were found to be Gram-negative bacteria. Of total Gram-negative organisms, 176 (49%) were non-fermenter. Of total non-fermenter organisms, *Acinetobacter* species was 30 (34%).Out of total 1040 samples, 30 (2.8%) infections were found to be due to *Acinetobacter*. In this study the isolates of *Acinetobacter* species were isolated from different samples blood samples 10 (33.3%) followed by urine sample 9 (30%), pus 7 (23.3%), and sputum 4 (13.3%) (Table 1) and different environment, i.e. 15 (50%) isolates were from ICU, 9 (30%) from surgical ward and 6 (20%) from gastro ward (Table 2). This study reveals that *Acinetobacter* infection were common in patients in age group >50 years 13 (43.3%) followed by >30 years 17 (56.6%) and in male patients 18 (60%) followed by female patients 12 (40%). In the present study different antibiotics showed resistance and susceptibility against isolates of *Acinetobacter* (Table 3 and Fig. 1). All the antibiotics have variation in their resistance and susceptibility patterns against isolates obtained from different sources and from different environment (Table 4). Maximum resistance was recorded for Imipenem (76%), followed by Ceftazidime (70%), Tobramycin (66.6%), Chloramphenicol (63 %), Doripenem (60%) Ciprofloxacin (60 %) Tetracycline (56.6 %), Ceftriaxone (56.6%), Doxycycline 16 (53.6%), Levofloxacin 15 (50%), Cefoperazone 13 (43 %), Ampicillin 8 (26.6%), Amikacin (10 %) and Gentamycin (6.6%). Less resistance was observed in Gentamycin (Table 3 and Fig. 1).

Of the "newer" pathogens currently identified, *Acinetobacter* plays an important role in colonization and infection of patients admitted to hospitals. They have been concerned in a variety of nosocomial infections, including bacteraemia, urinary tract infections and secondary meningitis and nosocomial pneumonia, especially ventilator associated pneumonia in patients restricted to intensive care units (ICU's). Such infections are frequently very complicated to treat for the clinician because of extensive resistance of the virulent organism to a hugefigure of antibiotics (Bergone Berezin E and Towner KJ1996). Normally *Acinetobacter* isolated from normal skin and mucous membranes are reported to cause serious and sometimes fatal infections (Pal RB and Kale VV1981). In present study, *Acinetobacter* accounted for359 (34.5%) of total positive culture, 176 (49%) of non-fermenter, 88 (50%) of non-

motile and 2.8% of total samples cultures. The present study is supported by previously, published studies which have accounted 12.9% (Lahiri *et al.*, 2004) and 4.8% (Lone *et al.*, 2009) of *Acinetobacter* isolates from total infected samples, respectively.

Table.1 Sources of *Acinetobacter* species

Samples	No. of Samples	% of sample
Blood	10	33.3%
Urine	9	30%
Pus	7	23.3%
Sputum	4	13.3%

Table.2 Percentage of isolates' environment

Environment	No. of isolates	% of isolates
ICU ward	15	50%
Surgical ward	9	30%
Gastro ward	6	20%

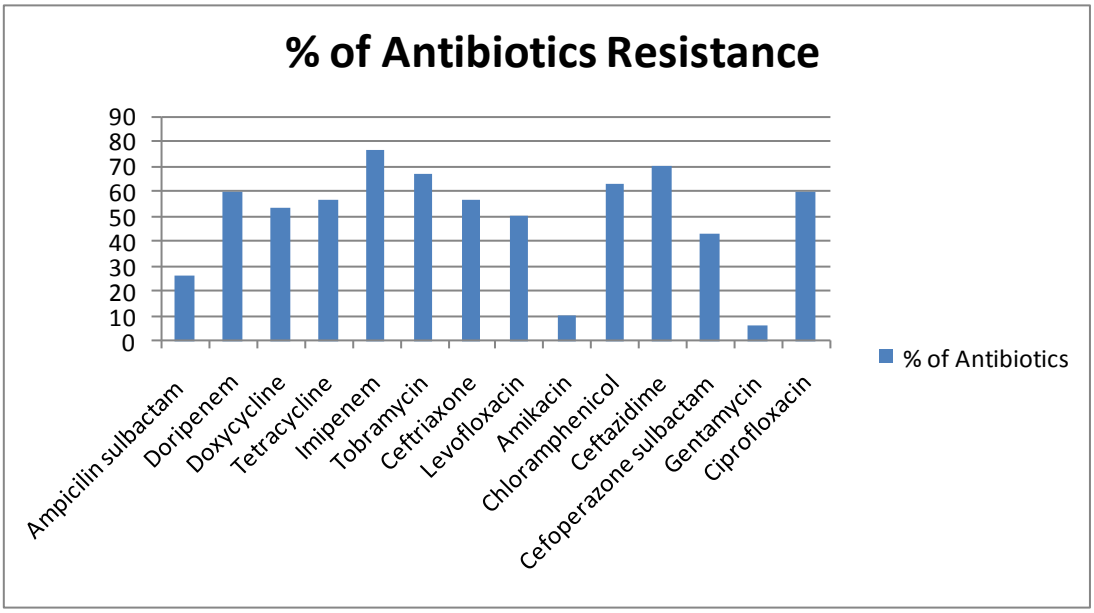
Table.3 Antibiotic resistance pattern of *Acinetobacter* species

Antibiotics	No. of Resistance (n=30)	% of Resistance
Imipenem	23	76.6%
Ceftazidime	21	70%
Tobramycin	20	66.6%
Chloramphenicol	19	63.3%
Doripenem	18	60%
Ciprofloxacin	18	60%
Tetracycline	17	56.6%
Ceftriaxone	17	56.6%
Doxycycline	3	53%
Levofloxacin	16	50%
Cefoperazone sulbactam	13	43.3%
Ampicillin sulbactam	8	26.6 %
Amikacin	3	10 %
Gentamycin	2	6.6%

Table.4 Antibiotics resistances pattern

Source	No. of *Acinetobacter* isolates (n=30)	Resistances pattern
Blood	10	Imipenem >Tobramycin >Doripenem
Urine	9	Imipenem> Chloramphenicol> Ceftriaxone
Pus	7	Ceftazidime> Doripenem> Levofloxacin
Sputum	4	Imipenem>Doripenem>Tobramycin

Fig.1 Antibiotics resistance (%) for different isolates of *Acinetobacter* species



Pseudomonas species was the most common nonfermenter isolated in previous studies (Neetu Gupta *et al.,* 2015). Fontana *et al.*, 2008 found that the *A. baumannii* was the most common pathogen (7%) among Gram negative bacteria isolated from patients of intensive care units in France hospitals. While, it has been always considered as less virulent amongst other pathogenic microbes however (Joly-Guillou, 2005), recommend that occasionally it can be extremely pathogenic and cause invasive diseases. The members of this genus are usually inhabitants of human skin, throat, respiratory and intestinal tract of hospitalized patients; additional reservoirs may involve the medical equipment used in the hospital environment besides the patients and hospital staff may also be act as reservoir for this pathogen (Towner, 2006).

This study showed that large number of isolates were isolated from blood samples (33.3%), followed by urine (30%), pus (23.3%) and sputum (13.3%)while in various countries, studies on *Acinetobacter* isolation have shown predominance in urine (21-27%) and tracheobronchial secretions (24.8-48.8%) (Lahiri *et al.,* 2004). According to various studies *Acinetobacter* were commonly obtained from patients admitted in ICU and who have undergone invasive process or surgery or on advanced generation antimicrobial drugs. In this present study also most of the isolates were obtained from ICU which is related to the research conducted by Mindolli *et al.,* (2010), in present study 15 (50%) *Acinetobacter* isolates were obtained from ICU out of them 10 (33.3 %) from blood which is supported with previous reports of Seifert *et al.,* (1995) who observed maximum *Acinetobacter* isolates were obtained from blood samples and from ICU. Bacteraemia due to *Acinetobacter* occur most frequently in critically ill patients particularly admitted in ICUs as these patients usually require prolonged hospital stay, need repeated invasive procedures and frequently receive treatment with broad spectrum antimicrobials (Cisneros and Rodríguez-Baño, 2002).

The distribution of *Acinetobacter* in clinical samples perhaps is due to its capability to cause various nosocomial infections and resistance to a broad variety of antibiotics. Moreover, some authors stated that the source

of infection maybe from endogenous routes slightly than the exogenous routes by sink, taps and hands of hospital staff (Thomson, 2004). In India *Acinetobacter* is recorded for about 13.2% of nosocomial infection in ICU patients (Patwardhan *et al.,* 2008). In our study *Acinetobacter* infection was more common in patients in age group >50 years [13 (43.3%)] followed by >30 years [17 (56.6%)]. Neeta Gupta *et al.,* (2015) also observed that the infection was common in patients of age group >50 years followed by 0-10 years age group. In the study by Mindolli *et al.,* (2010) isolates *Acinetobacter* were in age group >45 years possibly due to weakened immune system and associated chronic diseases in these age groups.

One of the most prominent properties of genus *Acinetobacter* is the ability to develop antibiotic resistance very hastily in response to challenge through new antibiotics. In this present study, maximum resistance is observed to Imipenem (76%), Ceftazidime (70%), Tobramycin (66.6%), Chloramphenicol 63.3%), Doripenem (60%), Ciprofloxacin (60 %) Ceftriaxone (56.6%) Levofloxacin (56.6%) and less resistance to Ampicillin sulbactam (26.6%), Amikacin (10%), Gentamycin (6.6%), respectively.

Neetu Gupta *et al.,* (2015) observed that *Acinetobacter* showed maximum resistance to piperacillin (55%), followed by Ceftriaxone (46%) and Ceftazidime (46%). However Rahbar *et al.,* (2010) found high rate of resistance to *A. baumannii* for Ceftriaxone (90.9%), Piperacillin (90.9%), Ceftazidime (84.1%), Ciprofloxacin (90.9%) which is similar to our study. Rahbar *et al.,* (2010) observed that Imipenem was the most effective antibiotic, which is inconsistent with our observation. In our study Imipenem (76%) showed highest resistance to *Acinetobacter* species.

Maximum resistance was observed in ICU isolates in comparison to wards where Acb complex was most prevalent. In ICUs most sensitive drug was ciprofloxacin (69%) followed by Imipenem (64%). In the study conducted by Cisneros and Rodríguez-Baño (2002) they found that many isolates of *Acinetobacter* species were resistant to almost all antibiotics routinely used in the ICUs of their hospital. In the antibiotic sensitivity studies conducted in Pakistan resistance rates of *A. baumannii* are reported as 32-100% against ciprofloxacin, 91-100% against Cefepime, 90-92% against Piperacillin-tazobactam, 24-94% against Amikacin and 18-85% against Gentamycin (Gozutok *et al.,* 2013). It is in support of our study which showed minimum resistance against Gentamycin and Amikacin. In some previous studies it was found that the resistance rates were 84.9 against Ciprofloxacin, 95.5% against Cefepime, 89.2% against Piperacillin-tazobactam, 86.3% against amikacin and 76.5% against Gentamicin and these results will remove these drugs from being an option for treatment. These results were not in support of our results which showed Gentamycin is least resistance (Necati Hakyemez *et al.,* 2013).

In conclusion, *A. baumannii* is a pathogen with rapid spread and extended resistance to even newer antimicrobial agents. Due to its ability to survive in the hospital environment, *A. baumannii* has the immense potential to cause nosocomial infectionsand also *Acinetobacter* is now a day a common threat in hospital acquired infection especially in critically ill patients admitted to intensive care unit. Therefore the main aim of this study was to assess the distribution of *Acinetobacter* species and to determine the detection rate of this microorganism in comparison to other pathogens in different patients suffering from significant infections *Acinetobacter* species were found to be resistant to most commonly

used antibiotics. Therefore, identification and expressive antibiotic sensitivity pattern of *Acinetobacter* helps in developing antibiotic policy against hospital acquired infections. In the present study it was observed that all isolates were resistant to almost all the antibiotics used in the present study except Amikacin and Gentamycin which are susceptible to the *Acinetobacter* species showing susceptibility in 28 (93.3%) and 27 (90%) patients. Finally it is evident from present study in ICU most sensitive drugs are Gentamycin and Amikacin.

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