

Isolation and identification of *Acinetobacter* species with special reference to antibiotic resistance

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Abstract

Background: *Acinetobacter* is clinically important pathogen with widespread resistance to various antibiotics. We assessed the incidence of *Acinetobacter* infection at a tertiary care hospital, analyze their resistance pattern and identify the production of extended spectrum β -lactamases (ESBLs) and metallo β -lactamases (MBLs). **Materials and Methods:** The study was conducted in tertiary care hospital, India over a period of 2 years. *Acinetobacter* species were isolated from various clinical samples received in Department of Microbiology. After identification, *Acinetobacter* isolates were speciated and antibiotic susceptibility was determined by the standard disc diffusion method. ESBL and MBL production was detected by the double disc synergy test and combined disc diffusion test respectively. **Results:** Of 3298 infected samples, 111 (3.36%) were found to be *Acinetobacter*. The most predominant species was *Acinetobacter calcoaceticus*-*A. baumannii* (Acb) complex (72%). High incidence of resistance was recorded for piperacillin (55%), followed by ceftriaxone (46%) and ceftazidime (46%). Isolation rate and antibiotic resistance was higher in the Intensive Care Units (ICUs) of the hospital. ESBL and MBL production was detected in 31.5% and 14.4% of the isolates respectively. **Discussion and Conclusion:** A high level of antibiotic resistance was observed in our study and maximum isolation rate of *Acinetobacter* was in the ICUs. Acb complex was the most predominant and most resistant species. The analysis of susceptibility pattern will be useful in understanding the epidemiology of this organism in our hospital setup, which will help in treating individual cases and controlling the spread of resistant isolates to other individuals.

Key words: Acb complex, *Acinetobacter spp.*, Antibiotic resistance, intensive care units

INTRODUCTION

Acinetobacter species are saprophytic, ubiquitous and have emerged as an important nosocomial pathogen due to its ability for survival in the hospital environment on a wide range of dry and moist surfaces.^[1] Human infections caused by *Acinetobacter* species include pneumonia, which is most often related to endotracheal tubes or tracheostomies, endocarditis, meningitis, skin and wound infections, peritonitis in patients receiving peritoneal dialysis, UTI and bacteremia.^[2,3]

Antibiotic susceptibility pattern of *Acinetobacter* may vary widely geographically and between various units of the same hospital at various time points. The variations in *Acinetobacter* resistogram, necessitates a periodic surveillance of these pathogens to achieve appropriate selection of therapy.^[4,5] Due to unpredictable multidrug resistance patterns of clinical strains of *Acinetobacter*, it is imperative to know the institutional prevalent susceptibility profiles. Hence, this study was conducted to isolate the *Acinetobacter* species from various clinical samples by a simplified phenotypic identification protocol and to determine the antibiotic susceptibility pattern of these isolates.

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MATERIALS AND METHODS

The study was conducted in the Department of Microbiology over a period of 2 years. The study was approved by the Ethical Committee of our institute

(Padmashree Dr. D.Y Patil Medical College and Research Center). A total of 6007 clinical specimens received in the Department of Microbiology for culture and sensitivity from indoor and outdoor patients were included in the study. Samples were processed for culture by standard conventional methods and susceptibility testing were determined by Kirby Bauers disc diffusion method.^[2,5,6] Antibiotics and their strength used was according to the Clinical and Laboratory Standards Institute guidelines: Piperacillin (100 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg) amikacin (30 µg).^[6] Genus *Acinetobacter* was identified by Gram staining, cell and colony morphology, positive catalase test, negative oxidase test and absence of motility.^[5] Speciation of *Acinetobacter* was performed on the basis of glucose oxidation, gelatin liquefaction, beta hemolysis, growth at 37°C and 42°C, arginine hydrolysis and susceptibility to chloramphenicol [Table 1].^[1,7-11]

Detection of Extended spectrum β-lactamases production

The double disc synergy test (DDST) was used to determine the prevalence of extended spectrum β-lactamases (ESBL) production in *Acinetobacter* as per previously reported protocols.^[7,12,13]

Detection of metallo β-lactamases production

Two 10 µg imipenem discs were placed on a lawn culture of the isolates to be tested and 10 µl of 0.5 M ethylenediaminetetraacetic acid (EDTA) (750 µg of disodium salt, dehydrate) solution was added to one IMP disc. The zone of inhibition around IMP discs alone and those with EDTA were compared after 16-18 h. An increase in zone size of at least 7 mm around the IMP-EDTA disc when compared to IMP disc alone was recorded as a positive result.^[14]

RESULTS

Of the total 6007 samples, 3298 (55%) were found to be culture positive. Of the 3298 positive culture, Gram-negative bacteria were 1376 (42%). Of total Gram-negative organisms, 291 (21%) were nonfermenters and the isolation rate of *Acinetobacter* from total nonfermenters was 111 (38%). Out of total culture positive samples, 111 (3.36%) infections were found to be due to *Acinetobacter*. *Acinetobacter* species were predominantly isolated from blood samples 41 (36.9%) followed by pus 25 (22.5%), respiratory samples 16 (14.4%), urine 13 (11.7%), other body fluids 10 (9%) and various catheter tips 6 (5.4%). Maximum *Acinetobacter* species isolated were from Intensive Care Units (ICUs) 42 (38%) followed by surgery ward 22 (20%), medicine ward 16 (14%), orthopedics ward 12 (11%), pediatric ward 11 (10%), gynecology ward 4 (4%) and out patients department 4 (4%). Statistically significant difference was noted in infections in ICU caused by other nonfermenters 29 (16.1%) and *Acinetobacter* species 42 (37.8%). In the present study, maximum isolated species were *Acb* (*Acinetobacter calcoaceticus-A. baumannii*) complex (80 [72%] of total *Acinetobacter* isolates), non-*Acb* complex (*Acinetobacter lwoffii* 16 (14%), *Acinetobacter haemolyticus* 13 (12%), *Acinetobacter junii* 1 (1%), *Acinetobacter radioresistans* 1 (1%). *Acinetobacter haemolyticus* species were predominantly found in pus samples. There was a higher incidence of infection among males. *Acinetobacter* infection was more common in patients in age group >50 years (31 [28%]) followed by <10 years (26 [23%]). Infection in neonates was higher within 14 days postpartum. Isolation rate of *Acinetobacter* species was maximum (52 [46.84%]) during July to September period (95% confidence interval [37.39-56.51]). The disc diffusion susceptibility testing shows the percentages of resistance and sensitivity among all isolates. Maximum resistance was recorded for piperacillin 61 (55%), ceftriaxone 51 (46%), ceftazidime 51 (46%),

Table 1: Phenotypic characteristics of *Acinetobacter* species

Name of test	<i>Acinetobacter</i> species				
	<i>Acb. complex</i>	<i>A. lwoffii</i>	<i>A. haemolyticus</i>	<i>A. junii</i>	<i>A. radioresistans</i>
Gram staining	Gram negative cocci or coccobacilli				
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Motility	-	-	-	-	-
Urease	V	V	-	-	-
Citrate	+	-	+	+	-
OF glucose	+	-	V	-	-
Nitrate reduction test	-	-	-	-	-
Hemolysis	-	-	+	-	-
Gelatin hydrolysis	-	-	+	-	-
Growth at 42°C	+	-	-	-	-
Chloramphenicol sensitivity	R	S	R	R	R
Arginine hydrolysis	+	-	+	+	+

V: Variable, S: Sensitive, R: Resistant, *A. lwoffii*: *Acinetobacter lwoffii*, *Acb*: *Acinetobacter calcoaceticus-baumannii*, *A. haemolyticus*: *Acinetobacter hemolyticus*, *A. junii*: *Acinetobacter junii*, *A. radioresistans*: *Acinetobacter radioresistans*, OF: Oxidation-fermentation

cefepime 49 (44%), cefotaxime 48 (43%), amikacin 47 (42%), ciprofloxacin 26 (23%) and imipenem 24 (22%).

Of 111 isolates of *Acinetobacter* species, 35 (31.5%) were found to be producing ESBL by the DDST with one or more of the cephalosporins used. A total of 16 (14.4%) isolates were metallo β -lactamases (MBL) producers by combined disc diffusion test. The statistically increased resistance of antibiotics for ICUs isolates in comparison to wards isolates was analyzed by Chi-square test [Table 2]. Of 111 isolates, 44 (39.63%) isolates were multidrug resistant strains (isolates resistant to at least one agent in three or more antimicrobial categories-penicillins, cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems).^[15,16]

DISCUSSION

Acinetobacter is a nosocomial pathogen. Its ability to infect healthy hosts and its propensity to develop antimicrobial drug resistance is a cause for concern among infectious disease specialty. *Acinetobacter* isolated from normal skin and mucous membranes are reported to cause serious and sometimes fatal infections.^[17] In our study, *Acinetobacter* accounted for 38% of total nonfermenters and 3.36% of total positive cultures. *Pseudomonas* spp. was the most common nonfermenter isolated in our study. Previously, published studies have accounted 12.9%^[10] and 4.8%^[5] of *Acinetobacter* isolates from total infected samples, respectively. In various countries, studies on *Acinetobacter* isolation have shown predominance in urine (21-27%) and tracheobronchial secretions (24.8-48.8%)^[10] nevertheless there is an increase in occurrence of *Acinetobacter* in hemocultures in some hospital departments.^[11] Bacteremia 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.^[18] In our study, maximum *Acinetobacter* isolates were from blood samples and from

ICUs, which is consistent with previous reports.^[19] We 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.*^[11] isolates *Acinetobacter* were in age group >45 years possibly due to weakened immune system and associated chronic diseases in these age groups. In this study, maximum resistance was observed to piperacillin (55%), followed by ceftriaxone (46%) and ceftazidime (46%). Rahbar *et al.*^[20] found high rate of resistance to *A. baumannii* for ceftriaxone (90.9%), piperacillin (90.9%), ceftazidime (84.1%), ciprofloxacin (90.9%) and imipenem was the most effective antibiotic, which is consistent with our observation. 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 their study Shakibaie *et al.*^[21] they found that many isolates of *Acinetobacter* species were resistant to almost all antibiotics routinely used in the ICUs of their hospital. There is limited data on β -lactamase producing *Acinetobacter* species from India. In our study, 31.5% of *Acinetobacter* species were ESBL producer by the DDST and 14.4% were MBL producers by the combined disc diffusion test. Kansal *et al.*^[22] and Kumar *et al.*^[23] found the 75% of ESBL producing and 21% of MBL producing isolates in their study respectively. Due to different antimicrobial susceptibility pattern in different hospitals, these surveillance studies are valuable in deciding the most adequate therapy for *Acinetobacter* infections. In our study, 44 (39.6%) isolates were MDR strains. *Acinetobacter* appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of its long term evolutionary exposure to antibiotic producing organisms in soil environment.^[24] The emergence of antibiotic resistant strains in ICUs is because of higher use of antimicrobial agents per patient and per surface area.^[18] *Acinetobacter* species obtained were maximum during July to September period, which is consistent with previous reports.^[25-27] The reason for this seasonality correlated with atmospheric temperature changes (high isolation rates especially in regions where temperature is hot and humid). Nevertheless, it is essential to periodically perform such prevalence and sensitivity assays as it will help clinicians in better management of *Acinetobacter* infections.

Table 2: Comparison of antibiotic susceptibility in ICUs and wards

Antibiotics	Susceptibility in ICUs (%)	Susceptibility in wards (%)	χ^2 (df = 1)	P value
Ciprofloxacin	29 (69)	56 (81)	1.513	0.219
Imipenem	27 (64)	60 (86)	6.637	0.010
Ceftazidime	18 (43)	42 (61)	2.724	0.099
Ceftriaxone	16 (38)	44 (64)	5.934	0.015
Cefepime	15 (36)	47 (68)	9.841	0.002
Amikacin	15 (36)	49 (71)	13.325	0.0002
Cefotaxime	14 (33)	49 (79)	13.608	0.0001
Piperacillin	12 (29)	38 (55)	6.375	0.012
Total samples tested	42	69		

ICUs: Intensive care units

CONCLUSION

The occurrence of *Acinetobacter* species among nonfermenters is high in hospital settings. Rationale use of antibiotics is important and necessary to prevent microbial resistance catastrophe. Definitive identification and characterization of ESBLs can only be confirmed by molecular techniques. However, these techniques are not

available in all laboratories. Therefore simple phenotypic methods can be used to recognize these enzymes. Resistant antibiotic after sensitivity report should be discontinued and in place a sensitive drug should be given. A continued awareness of the need to maintain good housekeeping and control of the environment, including equipment decontamination, strict attention to hand washing should undertake to control the spread of *Acinetobacter* in hospitals.

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