Original Research Article

Bacteriological profile and antimicrobial resistance pattern of Acinetobacter species isolated from patients of tertiary care hospital of Gujarat

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Abstract

Introduction: *Acinetobacter* spp. is an emerging important nosocomial pathogen. This opportunistic bacterium is quickly becoming resistant to commonly prescribed antimicrobials. Emergence of MBLs and ESBLs is becoming a therapeutic challenge as these enzymes leads to degradation of higher generation antibiotics.

Aim and Objectives: The study aimed at identification and antimicrobial resistance pattern of the common *Acinetobacter* species prevalent in our setup and to correlate with different clinical conditions.

Materials and methods: All the specimens received in a Clinical Microbiology Laboratory for bacterial culture processed to obtain *Acinetobacter* during period of June 2014 to May 2015. Identification and species differentiation of *Acinetobacter* was done by different biochemical tests. They were performed according to standard procedures. Antibiotic susceptibility test was done by Modified Kirby Bauer disk diffusion technique. The ESBL production was examined by phenotypic confirmatory disk diffusion method (PCDDT) and phenotypic expression of MBL was examined by combined disc diffusion test (CDDT).

Results: All the clinical samples received in Clinical Microbiology Laboratory for bacterial culture were included in our study. These samples were processed to obtain *Acinetobacter* during period of June 2014 to May 2015. A total of 64 *Acinetobacter* were identified from 360 non-lactose fermenting bacteria isolated from various specimens. Out of 64 isolates, 61 were *A. baumannii*, 2 were *A. lwoffii* and 1 was *A. calcoaceticus*. Most of the isolates were resistant to Cefuroxime (96.87%) followed by Amoxicillin-clavulanic acid (95.31%), Amikacin (93.75%), Cefoxitin (93.75%), Ciprofloxacin (90.62%), Cefepime (90.62%), Cefotaxime (90.62%), Co-trimaxazole (90.62%) and Gentamicin (78.12%). Isolates showed minimum resistance of 37.5% against Imipenem. In the present study 12% *Acinetobacter* were found to be MBL producer and 8% were found to be ESBL producer.

Conclusion: In the present study, Out of 64 *Acinetobacter* spp. 53.12% were from medical wards including ICU. While surgical wards contributed for 20.31% rest from other wards. Most common infective site for *Acinetobacter* infection was respiratory followed by operative and urinary tract. However maximum *Acinetobacter* isolated from Pus sample. The incidence of isolates possessing MBL activity in the present study represents an emerging threat of higher resistance to carbapenems and other commonly prescribed drugs among *Acinetobacter* spp. in India.

Key words

Acinetobacter, A. baumannii, A. lwoffii, Nosocomial infections, ESBL, MBL.

Introduction

Acinetobacter is a ubiquitous and has a wide distribution in the nature. Members of the genus *Acinetobacter* are involved in wide spectrum of infections. *Acinetobacter* is involved with hospital acquired as well as community acquired infections. Infectious *Acinetobacter* has emerged as a serious threat due to its multidrug resistance moving towards complete resistance. At least 32 different DNA groups have been reported, but only 17 have been describing properly [1, 2]. At the recent time, this organism is considered about 9 to 10 % of all hospital acquired infections.

This organism transmits through hands of hospital staff is being the major risk factor of patient colonization. From the different species of *Acinetobacter*, *A. baumannii* is important species causing human infections. *Acinetobacter* frequently colonize in patients' respiratory tract and skin. The pathogenicity of the bacterium depends on the patient's immune system as well as site of infection. It may cause mild to severe illness and it can be fatal. Some strains of Acinetobacter showing pan resistant, emerged in an outbreak in a clinical unit. In tropical environment with humidity and hotness frequent infection of Acinetobacter are seen [3].

It was many years before clinical microbiologists realized that the aerobic gram-negative diplobacilli isolated originally only from rare cases of bacteremia, pneumonia or urinary tract infection (UTI) were true pathogenic organisms. The identification of these oxidase-negative, non-motile, coccobacilli was difficult, because of its morphology and coccobacilli often mimicked Neisseria spp [4].

Most *Acinetobacter* species produce beta-lactamases capable of inactivating carbepenems, cephalosporins and penicillins. Some strains also

produce metallo-beta-lactamases which inactivate beta lactamase inhibitors like clavulanate or tazobactam and also carbapenems. Therapy for carbapenem-resistant *Acinetobacter* is particularly problematic. Neither option is perfect as of today.

Members of the genus *Acinetobacter*, particularly multidrug resistant strains of *A. baumannii*, are implicated in a wide spectrum of nosocomial infections, including bacteremia, secondary meningitis and urinary tract infection, but have now assumed a particularly important role as agents of nosocomial pneumonia in intensive care units (ICUs) [5]. Health care – associated infections tend to occur in long-term care facilities like Ventilator. Additional risk factors include recent surgery, central vascular catheterization, tracheostomy, mechanical ventilation etc. [6-8].

Species isolation is important in the epidemiology of *Acinetobacter* infections. There is, therefore, a need to isolate different species of *Acinetobacter* from various clinical samples. Some strains can survive in environment of wars and natural disasters for a long time. They can be transmitted by fomites in hospitals [9-16]. *Acinetobacter* is a frequently isolated, multidrug resistant organism, at our institute. There is a necessity to know the species prevalent at our hospital and also to know their resistance pattern for antibiotics. The information generated will help us to create a baseline data for early and intensive management of the patients and to take preventative measures to control the spread of these pathogens in our hospital.

Aim

To study the presence and antimicrobial resistance pattern of *Acinetobacter* spp. from the specimen of the patients admitted in the Dhiraj General Hospital.

Objectives

- To evaluate various clinical specimens received for bacteriological assessment in the microbiology laboratory for the presence of *Acinetobacter* spp.
- To evaluate the rate of *Acinetobacter* infections in our hospital.
- To identify the common *Acinetobacter* species prevalent in this area and to correlate with different clinical condition.
- To study the antibiotic susceptibility pattern and assess the extent of drug-resistance in clinical isolates.

Materials and methods

This study was conducted in Department of Microbiology associated with Dhiraj General Hospital, SBKS Medical Institute and Research Centre, Sumandeep Vidyapeeth University; Piparia, Vadodara; Gujarat. Various clinical specimens were received in Clinical microbiology Laboratory of Dhiraj General Hospital and were included in this study during period of one year.

Samples were processed immediately in the laboratory. Each sample was subjected to Gram staining [17] and inoculated on Blood agar, MacConkey agar and Nutrient agar for aerobic culture as per standard guidelines [18]. Isolates were identified and confirmed by biochemical reaction.

Preliminary identification of *Acinetobacter* is made by the colony morphology, Gram negativity, Coccobacillary shape, absence of motility, negative oxidase and positive catalase reactions. Identification will be confirmed by subjecting it to standard biochemical reactions [9].

Differentiation of species done by

- Citrate Utilization test
- Growth at 37°C, 41°C and 44°C
- Oxidation/Fermentation (OF) Medium
- Gelatin hydrolysis

The antibiotic susceptibility testing of the *Acinetobacter* was done on Muller Hinton agar

using standard Modified Kirby- Bauer disc diffusion method as per CLSI guidelines [19].

Extended spectrum B-lactamase detection in Acinetobacter isolates was done by Phenotypic confirmatory disk diffusion test, using disks Ceftazidime (30 µg) and Cefixime (5 µg) alone and in combination with Clavulanic acid (30/10 µg) (5/10 µg) respectively.

Screening of MBL production was done by Combined Disc Synergy Test (CDST), using disks Imipenem (10 µg) in combination with Imipenem– EDTA.

Results

A total of 64 *Acinetobacter* were identified from 360 non-lactose fermenting bacteria isolated from various specimens received during the study period. Out of 64 isolates, 61 were *A. baumannii*, 2 were *A. lwoffii* and 1 was *A. calcoaceticus* (**Table – 1**).

In present study, polymicrobial infection was seen in 7 (11%) cases. Three cases showed Gram Negative Bacilli isolation associated with *Acinetobacter* spp. Three cases associated with Gram Positive cocci. However one case showed *Candida spp.* isolation with *Acinetobacter* infection. (**Table - 2**)

Out of the total 64 strains isolated, 38 were from males and 26 were from females. It was observed that patient's age varied between 1 day and 80 years. Twenty four patients were above 50 years of age. Three were between 0-10 years of age, out of which 2 were neonate. (**Table - 3**)

Maximum (53%) *Acinetobacter* isolated from the Medicine wards (ICU, ICCU, MMW, FMW, Special room), 20% from Surgical wards (OTR, MSW, FSW, Special room) and 27% from others. Maximum *Acinetobacter* were isolated from the Respiratory site 27 (42%) followed by Operative site 23 (35.93%), Urinary tract 6 (9.37%), Blood stream 4 (6.25%) and Meninges 4 (6.25%). (**Table - 4**)

Table - 1: Species wise distribution of various *Acinetobacter* isolates under present study.

***Acinetobacter* spp. Isolated**	**No. of *Acinetobacter* spp. (n=64)**	**Percentage**
A. baumannii	61	95.31%
A. calcoaceticus	1	1.56%
A. lwoffii	2	3.12%
Total	64	100%

Table - 2: *Acinetobacter* associated with polymicrobial infection.

Name of Organism	**Total (n=7)**
***Acinetobacter spp.* + GNB**	**3**
Acinetobacter spp. + Pseudomonas spp.	2
Acinetobacter spp. + Klebsiella spp.	1
***Acinetobacter spp.* + GPC**	**3**
Acinetobacter spp. + CONS	2
Acinetobacter spp. + Staphylococcus aureus	1
Acinetobacter spp. + Candida spp.	**1**
Acinetobacter spp. + Candida glabarata	1

Most of the isolates were resistant to Cefuroxime (96.87%) followed by Amoxicillin-clavulanic acid (95.31%), Amikacin (93.75%), Cefoxitin (93.75%), Ciprofloxacin (90.62%), Cefepime (90.62%), Cefotaxime (90.62%), Co-trimaxazole (90.62%) and Gentamicin (78.12%). Isolates showed minimum resistance of 37.5% against Imipenem. Among 64 isolates of *Acinetobacter*

spp. 5 (7.81%) were ESBL producer and 8 (12.5%) were MBL producer. A total number of 23 *Acinetobacter* were Multi drug resistant which does not showed susceptibility to any drug used in the present study. While 22 isolates showed sensitivity against only Imipenem single drug. (**Table - 5**)

Maximum strains of *Acinetobacter* spp. were isolated from different ICU (43.75%). Out of 64 patients from whom *Acinetobacter* were isolated, 27 had hospital stay of 8 days and more. On an average hospital stay of 8 days in ICU is only in 20-30% of ICU patients and here it was 42.18% of patients who had *Acinetobacter* infection. Hence it is one of the important risk factor. High level of resistance was recorded for Cefuroxime (100%), Cefoxitin (100%), Cefotaxime (96.42%), Cefepime (96.42%), Amoxicillin-clavulanic acid (96.42%), Amikacin (96.42%), Co-trimaxazol (96.42%), Ciprofloxacin (92.85%) and Gentamicin (85.71%). Imipenem showed maximum activity with an overall low resistance of (28.57%). (**Table - 6**)

Discussion

Comparison of various antibiotics in different studies showing M. Sinha, et al. [20] and A. Nahar, et al. [6] maximum sensitivity for Imipenem, which was resulted in our study also.

Resistance patterns among bacterial pathogens particularly those which cause nosocomial infections may vary widely from country to country at any given point and within the same country over time [21]. ESBL production in various studies shows different results like M. Sinha, et al. [20] shows 28%, Owlia P., et al. [13] shows 21%, Safar F., et al. [22] shows 70%, while present study showed low rate of ESBL production i.e. 8%.

Various studies carried out for MBL production. Higher prevalence of MBL production was noted in South Indian study of S. M. Amudhan, et al. [23] and Shanthi M., et al. [11] showed 81% and 60% respectively. Low rate of MBL production was detected by a study of Kalidas Rit, et al. [24] i.e. 22% and also in present study i.e. 12%.

Table - 3: Age and gender wise distribution of patients in whom *acinetobacter* was isolated.

Age group (Years)	Male	Female	Total no. of cases	Percentage (n=64)
0--10	0	3	3	4.68%
11--20	3	4	7	10.93%
21--30	5	2	7	10.93%
31--40	9	4	13	20.31%
41--50	5	5	10	15.62%
51--60	5	3	8	12.5%
61--70	8	2	10	15.62%
71--80	3	3	6	9.37%
TOTAL	38	26	64	100%

Table - 4: Distribution of *Acinetobacter* isolated from various sites of infection.

Site of infection	No. of Isolate (n=64)	Percentage
Respiratory	27	42.18%
Operative site	23	35.93%
Urinary Tract	6	9.37%
Blood stream	4	6.25%
Meninges leading to Meningitis	4	6.25%

Table - 5: General antibiotic sensitivity pattern shown by *Acinetobacter* isolates.

Antibiotic name	Total sensitivity (n=64)	Percentage
Imipenem	40	62.5%
Amikacin	4	6.25%
Gentamicin	14	21.87%
Ciprofloxacin	6	9.37%
Cefuroxime	2	3.12%
Cefepime	6	9.37%
Cefotaxime	6	9.37%
Cefoxitin	4	6.25%
Amoxicillin-clavulanic acid	3	4.68%
Co-trimaxazole	6	9.37%

Table - 6: Significant risk factors and prognostic factors associated with isolated *Acinetobacter* spp.

FACTORS		TOTAL NO.	PERCENTAGE (n=64)
Age			
	< 40 Years	30	46.87%
	> 40 Years	34	53.12%
Gender			
	Male	38	59.37%
	Female	26	40.62%
Hospital Stay			
	≥ 8 days	27	42.18%
	< 8 days	37	57.81%
Antibiotype			
	Complete Resistance Type	23	35.93%
	Susceptible type	41	64.06%
Antibiotic pattern of *Acinetobacter*			
	Resistant to Ceftazidime	58	90.62%
	Sensitive to Ceftazidime	6	9.37%
	Resistant to Ciprofloxacin	58	90.62%
	Sensitive to Ciprofloxacin	6	9.37%
	Resistant to Imipenem	24	37.5%
	Sensitive to Imipenem	40	62.5%
	Resistant to Amikacin	60	93.75%
	Sensitive to Amikacin	4	6.25%

The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation antibiotics. Moreover, the treatment alternatives are unavailable, or expensive/toxic with poor outcome [12].

Conclusion

Acinetobacter is emerging as multi-drug resistant nosocomial pathogen mainly affecting the

patients with impaired host defences. Its prevalence is much more in ICU, where the selective pressure of antibiotics is already high, showing a need for rational use of antimicrobials. Strict infection-control measures may prevent nosocomial infection and reduce mortality.

In the present study, Out of 64 *Acinetobacter* spp. 53.12% were from medical wards including ICU. While surgical wards contributed for 20.31% rest from other wards. Most common infective site for *Acinetobacter* infection was respiratory followed by operative and urinary tract. However maximum *Acinetobacter* isolated from Pus sample.

Younger age (0-10 years) and age above 40 years contributed for 57.8% *Acinetobacter* infections.
Most active group of antibiotics which are employed in treatment of infections caused by *Acinetobacter* is carbapenems, which showed 37.5% resistance in our study. Risk factor analyses will be useful for further hospital epidemiology studies of *Acinetobacter*. Further research related to mechanisms of resistance and metallo-beta-lactamase patterns should be needed.

This study confirms the magnitude of the emergence of MDR *Acinetobacter* spp. as potential pathogens causing infections in our ICU, the best approach to manage this problem seems to be adaptation of preventive strategies.

Utilization of this surveillance data will help formulate antimicrobial treatment plan of *Acinetobacter* infections in our set up. Active surveillance combined with education of the Health Care Worker, hand hygiene, environmental cleaning, contact precautions, and antimicrobial stewardship will help reduce the rates of antimicrobial resistance among *Acinetobacter* spp.

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