



Surveillance of *Acinetobacter* Spp. and Drug Sensitivity Pattern in an Indian Tertiary Care Teaching Hospital

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ABSTRACT

The aim of the present study was to record prevalence & antibiotic resistance in *Acinetobacter* Spp. strains, isolated from clinical samples of a tertiary care teaching hospital by surveillance, over a period of 12 months (April 2015-Mach 2016). Clinical samples from nosocomial sources, i.e. wards, cabins and intensive care unit (ICU) and community (outpatient department, OPD) sources of the hospital were used for isolation of strains of *Acinetobacter* Spp. Among the 12,345 clinical samples 7,499 were culture positive and 227 were identified as *Acinetobacter* Spp & sensitivity pattern of eleven antibiotics representing various group were recorded. This study on surveillance of a hospital revealed the daunting state of occurrence of drug resistant *Acinetobacter* Spp.

Keywords: *Acinetobacter* spp., Antibiotic resistance, intensive care units.

INTRODUCTION

A *cinetobacter* 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 surfaces¹. 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}.

Geographical variation of Antibiotic susceptibility pattern of *Acinetobacter* is wide and also wide between various units of the same hospital at various time points. The variations in 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 this species from various clinical samples by a simplified phenotypic identification protocol and to determine the antibiotic susceptibility pattern of these isolates.

MATERIALS AND METHODS

A retrospective, hospital record-based, cross-sectional study was carried out from April 2015 to March 2016 in the Department of Microbiology at a tertiary care teaching hospital in Odisha. According to the documentation, a total of 12,345 clinical samples like wound (swab, pus, blood, urine, sputum, central line tip, body fluids, tracheal aspirate, and endotracheal tube were collected from the patients and transferred to the

laboratory without delay for further processing. Patients from whom *Acinetobacter* spp were isolated, they were only included in this study. Patient from whom *Acinetobacter* spp was isolated in the absence of a clinical disease suggesting colonization were not included in this study.

Sample Processing and Antibigram

In the laboratory, all the collected samples excepting urine, were cultured aerobically on blood agar and MacConkeyagar. Blood specimen was cultured in Brain-Heart infusion (BHI) broth and subcultured on blood agar, chocolate agar and MacConkeyagar. Urine sample was cultured in CLED agar. The isolation and identification, were done according to the standard procedure⁶.

All isolates were tested for antimicrobial susceptibility testing by the standard Kirby-Bauer disk diffusion method according to Bauer⁷. The test organism was picked up with a sterile loop, suspended in normal saline. The turbidity of the suspension was adjusted to 0.5 McFarland's standard [1.5×10^8 colony forming units (CFU)/ml]. It was then spread on the surface of a Mueller-Hinton agar (MHA) plate using sterile cotton swab.

The following standard antibiotic disks were placed on the MHA plate: ampicillin-sulbactam (10/10 mcg), amikacin (30 mcg), tobramycin (10 mcg), piperacillin/tazobactam (100/10 mcg), imipenem/cilastin (10/10 mcg), meropenem (10 mcg), Levofloxacin (5 mcg), ceftriaxone-tazobactam (30/100mcg), cefepime-tazobactam (30/100 mcg), tigecycline (15mcg)and colistin (10 mcg). The plate was incubated at 37°C overnight. The zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines⁸. All dehydrated media and

antibiotic disks were procured from Himedia Labs (Mumbai, India). In addition, the antibiotic potency of the disks was standardized against the reference strains of *Escherichia coli* ATCC 25922 as the negative control and *A. baumannii* ATCC 19606 as the positive control. Multidrug resistant (MDR) *Acinetobacter* spp isolates are defined as those resistant to \geq one agent of \geq three classes of antibiotics. An isolate is classified as pan-resistant if it was resistant to all antibiotics^{9,10}.

RESULTS

Of the total 12,345 samples, 7,499 (60.74%) were found to be culture positive. Out of total culture positive samples, 227 (3.03%) infections were found to be due to *Acinetobacter* (Fig 1). They were predominantly isolated from wound swab/pus (48.46%) followed by tracheal aspirate (16.74%), urine (15.86%), sputum (9.25%), blood (3.96%). (Table 1)



Figure 1: Colony morphology of *Acinetobacter* spp. on MacConkey agar plate



Figure 2: Antibiotic sensitivity on Muller-Hinton agar plate
Maximum isolates were from Intensive Care Units (ICUs) (47.14%) followed by surgical departments (27.75%), medicinal departments (14.54%), OPDs (10.57%) (Table 2). In 31 samples (all are wound swab/pus), in addition to *Acinetobacter* isolate, other bacterial etiology was there in significant count. Majority were *Escherichia coli*,

Klebsiella pneumoniae, followed by *Klebsiella oxytoca*, *Citrobacter freundii*, *Enterobacter* spp, *Staphylococcus aureus* & *Enterococcus* spp.

The disc diffusion susceptibility testing shows the high degree of resistance of *Acinetobacter* spp to all common antibiotics. We have recorded the result of 11 antibiotics. Maximum sensitivity was shown to tigecycline (99.56%) and colistin (96.47%) followed by amikacin (30.39%), Levofloxacin (29.95%), meropenem (25.55%). Maximum resistance was seen in ampicillin-sulbactam (95.15%) followed by cefepime-tazobactam (94.27 %), tobramycin (92.51 %), ceftriaxone-tazobactam (89.43%) (Table 3). Of 227 isolates of *Acinetobacter* species, 71 (31.28%) were found to be ESBL producing strains identified by the combination disc method with ceftizidime (30 mcg) and ceftizidime-clavulanic acid (30/10 mcg). 144 (63.44%) isolates were multidrug resistant strains. Colistin resistance was reported in 8 cases (3.52%). One isolate was resistant to all the antibiotics.

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 speciality. *Acinetobacter* isolated from normal skin and mucous membranes are reported to cause serious and sometimes fatal infections¹¹. These species are the second most common non-fermenting bacteria after *Pseudomonas* species that are isolated from human specimens, especially among nosocomial infections¹². They normally inhabit soil and water and have also been isolated from foods and animals. In humans, they can colonize skin, wounds, respiratory and gastrointestinal tracts¹³. It is a pathogen of tropical and humid environment, but some species can survive environmental desiccation for weeks, a characteristic that promotes transmission through fomite contamination in hospitals¹⁴.

In our study, *Acinetobacter* spp accounted for 3.03% of total aerobic bacterial isolates. Previously, published studies from different parts of India have accounted 12.9%, 4.5%, 2.9%, 2.9%, 3.36%^{15,6,5} of *Acinetobacter* isolates from total culture positive samples, respectively. Only 10.57% of isolates were from OPDs (community acquired) & majority rest were from in-patient departments (nosocomial). Lahiri KK reported 82.9% of nosocomial source¹⁵. *Acinetobacter* is ubiquitous in the hospital setting. Its ability to survive for long periods coupled with its ability to demonstrate a number of antimicrobial resistance genes has made *Acinetobacter* a successful hospital pathogen¹⁶. Isolation rate from ICUs was very high (47.14%) in this study. Similar findings were obtained by other researchers also, like 45.2%, 38%^{6,5} respectively.

We have isolated maximum species from pus/wound swab i.e, 48.46%. Similar findings were obtained by Dash M (56.9%) and Rynga D (53%).^{6,17} However, studies by

Gupta N and Rit K showed lower isolation from pus/swab i.e, 22.5% & 31.2% respectively^{5,18}. In various studies have shown predominant isolation 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 departments¹⁹. Only 3.96% of total isolates of *Acinetobacter* was from blood which is in contrary to the other studies from various part of India where percentage of isolation is significantly high like 36.9%, 13.1%, 14.3%^{5,6,18}. 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 antimicrobials²⁰. In our study, all isolates of blood sample were from ICUs & critical care unit, which is consistent with previous reports^{5,6,17,18}.

Table 1: Isolation of *Acinetobacter* Spp from Different Clinical Samples

Clinical Samples	No. of <i>Acinetobacter</i> isolates (n=227)	Percentage
Wound swab/Pus	110	48.46
Tracheal aspirate	38	16.74
Urine	36	15.86
Sputum	21	9.25
Blood	9	3.96
Others(IV catheter tip, Endotracheal tube)	13	5.73

Table 2: Source of *Acinetobacter* isolates

Sources	No. of isolates (n=227)	Percentage (%)
ICUs	107	47.14
Surgical departments	63	27.75
Medicinal departments	33	14.54
Out Patient Department	24	10.57

Table 3: Antibiotic sensitivity pattern of *Acinetobacter* spp.

Antibiotics	No. of sensitive isolates (n=227)	% of sensitive	% of resistance
Ampicillin-sulbactam	11	4.85	95.15
Amikacin	69	30.39	69.61
Tobramycin	17	7.49	92.51
Imipenem-cilastin	35	15.42	84.58
Meropenem	58	25.55	74.45
Piperacilin-tazobactam	47	20.7	79.3
Levofloxacin	68	29.95	70.05
Cefepime-tazobactam	13	5.73	94.27
Ceftriaxone-tazobactam	24	10.57	89.43
Tigecycline	226	99.56	0.44
Colistin	219	96.47	3.53

As noted by the Infectious Disease Society of America, *Acinetobacter* is “a prime example of mismatch between unmet medical need and the current antimicrobial research and development pipeline.” *Acinetobacter* spp. are notorious for their ability to acquire antibiotic

resistance²¹. Antimicrobial resistance among this species has increased substantially in the past decade and has created a major public health dilemma. The most potent antibiotic drug class currently available are the carbapenems, but resistant strains have emerged²². Most of the patients who were admitted in our hospital had



previously attended primary and secondary care hospitals and usually received combination of β -lactam antibiotics like second- and third-generation cephalosporins along with aminoglycoside or fluoroquinolones. Thus, majority of the isolates in our study were resistant to commonly used antibiotics. We found that tigecycline and colistin were the most potent antibiotics against this pathogen. Amikacin, ofloxacin, meropenem and piperacillin-tazobactam were effective in some cases although the resistance rates for these drugs were very high like 69.61%, 70.05%, 74.45%, 79.3%, respectively. In this study, maximum resistance was observed to ampicillin-sulbactam, cefepime-tazobactam, tobramycin and ceftriaxone-tazobactam. Parween N, Goel N, Dash M & Tripathy reported also high resistance to amikacin i.e., 88.95%, 87.2%, 61% & 55% respectively^{23,20,6}. But low resistance pattern of amikacin was also reported by some studies^{12,21}. Piperacillin-tazobactam was also highly resistant in other studies^{25,24}.

Carbapenems have been the drug of choice for treating *Acinetobacter* infections, but unfortunately, carbapenem-resistant *Acinetobacter* spp. due to carbapenemase enzyme is becoming common worldwide²⁶.

Of the β -lactamases, those with carbapenemase activity are the most concerning for drug resistance and include the serine oxacillinase (belonging to Ambler class D OXA type) and the metallo- β -lactamases (Ambler class B). Our study revealed resistant to imipenem-cilastin was 84.58% & to meropenem was 74.45%. Dash M reported 19% & 22%, Parween N showed 15.21% & 7.22%^{6,23} respectively. Taneja & Tripathy recorded resistance to imipenem 67.4% & 43% in their study respectively^{24,25}. Colistin (polymyxin E) and tigecycline are new alternatives in the treatment of *Acinetobacter* species.

Excepting one case, we did not find any *Acinetobacter* isolate being resistant to tigecycline, which may be due to its selective use only in case of carbapenem-resistant gram-negative bacteria. 3.52% isolates were colistin resistant in our study. Shareek found that all isolates were sensitive to colistin²⁸. The significant finding in their study was that eight (3.5%) isolates were resistant to both colistin and tigecycline²⁴. Various authors have reported the resistance rate to colistin between 1.8% and 2%,^{29,30} while resistance to tigecycline varies from being nonexistent to 66%.^{31,32} In an *in vitro* study, it was revealed that extract of *B. monesperma* can inhibit the growth of *Acinetobacter* spp.³³

CONCLUSION

The occurrence of *Acinetobacter* species among the nonfermenters next to *Pseudomonas* spp. is high in hospital settings.

As ubiquitous organisms (fortunately of low virulence), with few requirements for growth and survival, they are prone to persist indefinitely in the hospital environment and to cause infections periodically when iatrogenic factors are present. This situation, together with the fact

that these isolates have inherent and/or easily acquired mechanisms of resistance against many of the available antimicrobial agents, makes this pathogen one of the most significant microbial challenges of the current era. Rationale use of antibiotics in the form of appropriate indication, dose & duration is very very important and necessary to prevent microbial resistance catastrophe. As the hospitals, as the primary incubators of antimicrobial-resistant pathogens, carry the highest responsibility for proper stewardship of our existing antimicrobial resources. Due to different antimicrobial susceptibility pattern in different hospitals, these surveillance studies are valuable in deciding the most adequate therapy for *Acinetobacter* infections.

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