

# Frequency, risk factors, and antibiogram of *Acinetobacter* species isolated from various clinical samples in a tertiary care hospital in Odisha, India

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## ABSTRACT

**Background:** For the past two decades, *Acinetobacter* spp. have emerged as an important pathogen globally in various infections. **Objectives:** This study was conducted to determine the frequency, risk factors, and antibiotic resistance pattern of *Acinetobacter* spp. from various clinical samples. **Materials and Methods:** This retrospective, hospital record-based, cross-sectional study included a total of 8749 clinical samples collected from patients at a tertiary care hospital in Odisha, India from July 2010 to December 2012. The samples were processed and identified by standard protocol. The *Acinetobacter* isolates were tested for antibiotic resistance by Kirby-Bauer disk diffusion method [according to the Clinical and Laboratory Standards Institute (CLSI) guidelines]. **Results:** From 8749 clinical samples, 4589 (52.5%) yielded significant growth and only 137 (3%, 137/4589) *Acinetobacter* spp. were isolated. Maximum (56.9%) isolates were obtained from pus/swab, followed by blood (13.1%) and urine (12.4%). Elderly age, being inpatients, longer duration of stay in the hospital, associated co-morbidity, and invasive procedure were found to be significant risk factors in the setup investigated ( $P$  is less than 0.05). Out of 137 isolates, 75 (54.7%) were resistant to more than three classes of antibiotics (multidrug resistant) and 8 (5.8%) were resistant to all commonly used antibiotics (pan-drug resistant). Majority of the isolates were sensitive to imipenem, meropenem, and piperacillin/tazobactam, and showed resistance rates of 19%, 22%, and 23%, respectively. All eight pan-drug resistant isolates were 100% sensitive to colistin. **Conclusion:** This hospital-based epidemiological data will help to implement better infection control strategies and improve the knowledge of antibiotic resistance patterns in our region.

**Key words:** *Acinetobacter* species, antibiotics, frequency, resistance, risk factors

## INTRODUCTION

Members of the genus *Acinetobacter* are ubiquitous, free-living, and saprophytic bacilli that can be obtained easily from soil, water, food, and sewage.<sup>[1]</sup> These are aerobic, gram-negative, non-fermenter of glucose, and opportunistic pathogens that emerge as an important cause of hospital-acquired infections and intermittent outbreaks globally. *Acinetobacter* has undergone significant taxonomic modification over the last 30 years. Its most common and important representative is *Acinetobacter baumannii*, and the other species such as *Acinetobacter lwoffii*, *Acinetobacter*

*johnsonii*, and *Acinetobacter haemolyticus* are rarely isolated from patients.<sup>[2]</sup> Its great capacity to survive in low-moist environment coupled with its ability to develop resistance to antimicrobial agents can increase the possibility of spreading in hospitals.<sup>[3]</sup>

The risk of colonization and subsequent infection are associated with factors such as the presence of underlying severe illnesses, long-term hospitalization, stays in intensive care units (ICUs), selective antimicrobial pressure, and invasive interventions such as use of mechanical ventilation or catheters.<sup>[4,5]</sup> The nosocomial infections

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caused by *Acinetobacter* include pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis, and meningitis.<sup>[6]</sup> In addition to infection among hospitalized patients, community-acquired *Acinetobacter* infection is increasingly reported.<sup>[7]</sup>

There is a significant difference in the behavior and spread of multi-drug resistant *Acinetobacter* spp. recovered various geographic locations.<sup>[8]</sup> Since several factors cause resistance in *Acinetobacter* spp., treatment of infections caused by this organism should be based on antibiotic susceptibility tests. Therefore, having information regarding the prevalence and pattern of bacterial resistance to these drugs is important.<sup>[9,10]</sup>

Keeping these above facts in view and due to the paucity of reports from Odisha, India, we analyzed the frequency, risk factors, and resistance pattern of *Acinetobacter* spp. that were isolated from different clinical samples in a tertiary care hospital in Odisha.

## MATERIALS AND METHODS

### Study area, population, and methodology

A retrospective, hospital record-based, cross-sectional study was carried out from July 2010 to December 2012 in the Department of Clinical Microbiology at a tertiary care hospital in Odisha. This 600-bedded hospital has three ICUs, one emergency ward, six medical and surgical wards, and outpatient departments (OPDs). A total of 8749 clinical samples like pus/swab, urine, sputum, blood, body fluid, tracheal aspirate, endotracheal tube, and intravenous (IV) catheter tips were collected from the patients and transferred to the laboratory without delay for further processing. A retrospective evaluation of patient's age, sex, co-morbidity (including diabetes mellitus, chronic obstructive pulmonary disease, asthma, neurologic impairment, congestive cardiac failure, end-stage renal disease, cancer, hepatitis and human immunodeficiency virus), admission into the hospital, duration of stay, and special invasive procedure conducted was carried out on the basis of the case record histories. A healthcare-associated infection or nosocomial infection is defined as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent (s) or its toxin (s) that was not present on admission to the hospital. An infection is considered as nosocomial if all the elements of a site-specific infection criterion of Center of Disease Control and Prevention (CDC) were first present together on or after the 3<sup>rd</sup> hospital day (day of hospital admission is day 1).<sup>[11]</sup> Patient from whom *Acinetobacter* was isolated in the absence of a clinical disease suggesting colonization were not included in this study. The study was conducted

after due approval was obtained from the institutional ethical committee.

The differences in the risk factors among patients infected with *Acinetobacter* spp. and patients suspected with bacterial infections were compared and investigated for significant risk factors in patients with these infections.

### Sample processing and antibiogram

In the laboratory, all the collected samples were cultured aerobically on blood agar and MacConkey agar. Blood specimen was cultured in trypticase soy broth (TSB) and subcultured in blood agar and chocolate agar. The isolation, identification, and speciation were done according to the standard procedure.<sup>[12]</sup>

All isolates were tested for antimicrobial susceptibility testing by the standard Kirby-Bauer disk diffusion method according to Bauer *et al.*<sup>[13]</sup> The test organism was picked up with a sterile loop, suspended in peptone water, and incubated at 37°C for 2 h. The turbidity of the suspension was adjusted to 0.5 McFarland's standard [ $1.5 \times 10^8$  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), ciprofloxacin (5 mcg), gentamicin (10 mcg), amikacin (30 mcg), tobramycin (10 mcg), ceftazidime (30 mcg), piperacillin/tazobactam (100/10 mcg), imipenem (10 mcg), meropenem (10 mcg), ofloxacin (5 mcg), cefepime (30 mcg), and colistin (10 mcg). The plate was incubated at 37°C overnight. The zone of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>[14]</sup> 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.

The isolate was considered as highly resistant when it was resistant to imipenem, amikacin, and ampicillin/sulbactam. Multidrug-resistant (MDR) *Acinetobacter* spp. are defined as those isolates resistant to more than three classes of antibiotics.<sup>[15]</sup> An isolate was classified as pan-resistant when it was resistant to all the commonly used antibiotics.<sup>[15]</sup>

### Statistical analysis

The data were analyzed for mean, median and standard deviation, and *P* value (Chi-square with Yates' correction and Fisher's exact test) by using GraphPad QuickCalcs (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was defined when the *P* value was less than 0.05.

## RESULTS

During the study period from July 2010 to December 2012, a total of 8749 clinical samples were aerobically cultured, of which 4589 (52.5%) yielded significant growth and rest of the samples [4160 (47.5%)] were either sterile or showed non-significant growth. From the 4589 growth-positive samples, a total of 137 (3%) *Acinetobacter* spp. were isolated. From the 137 isolates, majority [124 (90.5%)] were detected from inpatients (ICUs and emergency ward 45.2% and surgical ward 26.3%, followed by medicine ward 19%) and the rest [13 (9.5%)] were isolated from outpatients (community-acquired infection). Majority of the *Acinetobacter* spp. were isolated from pus/swab samples (56.9%), followed by blood (13.1%), urine (12.4%), sputum (8.1%), and body fluids (5.1%) [Table 1].

**Table 1: Distribution of *Acinetobacter* spp. based on mode of acquisition of infection and in various clinical samples (N=137)**

Type of sample	Nosocomial infection			CA infection	Total (%)
	ICUs and emergency unit	Surgical unit	Medical unit		
Pus/swab	31	23	13	11	78 (56.9)
Blood	09	03	06	-	18 (13.1)
Urine	07	05	03	02	17 (12.4)
Sputum	08	02	01	-	11 (8.1)
Body fluids*	04	02	01	-	07 (5.1)
Others*	03	01	02	-	06 (4.4)
Total (%)	62 (45.2)	36 (26.3)	26 (19)	13 (9.5)	137 (100)

\*Body fluids include cerebrospinal fluid, pleural fluid, and peritoneal fluid; others include tracheal aspirate, endotracheal tube, intravenous catheters; ICUs: Intensive care units, CA: Community-acquired

*A. baumannii* was the main species responsible for 109 (79.6%) of the infections, followed by *A. lwoffii* [17 (12.4%)], and the remaining 11 (8%) were caused by other *Acinetobacter* species. The mean age of patients infected with *Acinetobacter* spp. was 38.58 years (median 39, standard deviation  $\pm$  16.44, 95% confidence intervals 35.37-41.8, range 3-85 years). The gender (male:female) ratio was 1.08:1. *Acinetobacter* infection was significantly observed among inpatients and the elderly ( $\geq$ 55 years), was associated with co-morbidity and longer duration of stay in the hospital ( $\geq$ 7 days), and was found in those who had undergone any invasive procedure ( $P$  was less than 0.05) [Table 2].

Table 3 shows the frequency of multidrug resistance among *Acinetobacter* spp. Out of the 137 isolates, 75 (54.7%) were MDR and only 8 (5.8%) were pan-drug resistant (PDR).

In the present study, most of the *Acinetobacter* spp. were highly resistant to ceftazidime (93%), cefepime (89%), ciprofloxacin (86%), ofloxacin (81%), ampicillin/sulbactam (79%), gentamicin (76%), amikacin (61%), and tobramycin (55%). The low resistant patterns of imipenem (19%), meropenem (22%), and piperacillin/tazobactam (23%) indicate that they are effective drugs. All eight (5.8%) PDR isolates were 100% sensitive to colistin [Table 4].

## DISCUSSION

*Acinetobacter* spp. are the second most common non-fermenting bacteria after *Pseudomonas* species that are isolated from human specimens, especially among

**Table 2: Risk factors for *Acinetobacter* spp. infection among patients attended a tertiary care hospital, South Odisha, India (N=8749)**

Risk factors	Total no. of patients (N=8749)	No. of <i>Acinetobacter</i> spp. isolated (N=137)	Chi-square ( $\chi^2$ )	P value
Attended hospital as				
Inpatients	4492	124	81.338	<0.0001 (HS)
Outpatients	4257	13		
Age (years)				
$\geq$ 55	1283	36	13.487	0.0002 (HS)
<55	7466	101		
Co-morbidity*				
Present	1937	78	91.162	<0.0001 (HS)
Absent	6812	59		
Duration of stay				
<7 days or no stay	7374	104	6.475	0.0109 (S)
$\geq$ 7 days	1375	33		
Invasive procedure				
Conducted (catheterization, intubation, ventilation)	1237	53	63.534	<0.0001 (HS)
None	7512	84		
Gender				
Male	4713	71	0.152	0.6966 (NS)
Female	4016	66		

\*Co-morbidity includes diabetes mellitus, chronic obstructive pulmonary disease, asthma, neurologic impairment, congestive cardiac failure, end-stage renal disease, cancer, hepatitis and human immunodeficiency virus; HS: Highly significant, S: Significant, NS: Not significant

**Table 3: Frequency of multidrug resistance in *Acinetobacter* spp. (N=137)**

Parameter	Resistance to one or several classes of antibiotics (%)					Total
	1	2	3	4	>4	
No. of classes of antibiotics	1	2	3	4	>4	
No. of isolates of <i>Acinetobacter</i> spp.	09 (6.6)	17 (12.4)	36 (26.3)	48 (35)	27 (19.7)	137 (100)

**Table 4: Frequency of antibiotic resistance in *Acinetobacter* spp. (N = 137)**

Antibiotic	Disk content (mcg)	Sensitivity patterns in percentage		
		Resistant	Intermediate	Sensitive
Ceftazidime	30	93	-	07
Cefepime	30	89	01	10
Ciprofloxacin	5	86	02	12
Ofloxacin	5	81	01	18
Gentamicin	10	76	04	20
Amikacin	30	61	02	37
Tobramycin	10	55	03	42
Ampicillin/sulbactam	10/10	79	02	19
Piperacillin/tazobactam	100/10	23	04	73
Imipenem	10	19	04	77
Meropenem	10	22	05	73
Colistin*	10	-	-	100

\*Colistin was tested only on eight isolates which were resistant to all common antibiotics and found to be 100% sensitive

nosocomial infections.<sup>[16]</sup> In recent years, this species has emerged as the causative agent of important nosocomial infections in the ICUs and emergency unit, which is probably related to the increasingly invasive diagnostic procedures used, the greater quantity of broad-spectrum antimicrobials used, and prolonged duration of stay in the hospital. Development of resistance against antimicrobials is a major problem in the treatment of *Acinetobacter* infections. Although they are considered as pathogen of mild virulence, they can rapidly acquire resistance.<sup>[17]</sup>

In our study, from 4589 clinical isolates, only 137 (3%) *Acinetobacter* spp. were obtained. Similar prevalence of 4.5% of the total organisms isolated was reported by Rit *et al.* in a tertiary care hospital in West Bengal, India.<sup>[18]</sup> In comparison, higher prevalence rates of 14% and 9.6% among hospital isolates were observed by Mostofi *et al.* in Tehran, Iran and Joshi *et al.* in Pune, India, respectively.<sup>[19,20]</sup> *Acinetobacter* normally inhabits soil and water and has also been isolated from foods and animals. In humans, *Acinetobacter* can colonize skin, wounds, respiratory and gastrointestinal tracts.<sup>[21]</sup> 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 hospitals.<sup>[22]</sup>

We isolated *Acinetobacter* from various clinical samples including blood, urine, body fluids, tracheal aspirate, endotracheal tubes, intravenous catheters, and other samples, but most commonly from pus/swab (56.9%). Similar findings were obtained by Chakraborty *et al.* in West Bengal.<sup>[23]</sup> Lone *et al.* in Srinagar, India reported that majority (39.6%) of the *Acinetobacter* isolates were obtained from urine, followed by pus and wound exudates (29.5%).<sup>[24]</sup>

Overall, in the present study, the significant risk factors for *Acinetobacter* infection were age  $\geq 55$  years, admission in the hospital as inpatients, longer ( $\geq 7$  days) duration of stay in the hospital, having undergone any invasive procedures like catheterization, intubation, and mechanical ventilation, and with underlying co-morbid conditions, i.e., diabetes mellitus, chronic obstructive pulmonary disease, asthma, neurologic impairment, congestive cardiac failure, end-stage renal disease, cancer, hepatitis and human immunodeficiency virus ( $P \leq 0.05$ ). A longer hospital stay in a high-risk unit, use of mechanical ventilation, admission as inpatient into the ICUs, and underlying co-morbid conditions have been identified as the risk factors in previous studies as well.<sup>[15,24,25]</sup>

Our study revealed that majority (54.7%) of the isolates were MDR *Acinetobacter* spp., and among them, eight isolates were PDR. The other studies conducted by Bhattacharyya *et al.* in West Bengal and Mostofi *et al.* in Tehran reported the MDR isolates to be 29% and 54%, respectively.<sup>[19,26]</sup> *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 pathogen.<sup>[3]</sup>

Most of the patients who were admitted in our hospital had previously attended primary and secondary care hospitals and usually received combination of  $\beta$ -lactam antibiotics like third- and fourth-generation cephalosporins (i.e., ceftazidime, ceftriaxone, and cefepime) along with aminoglycosides (i.e., gentamicin and amikacin) or fluoroquinolones (ciprofloxacin, ofloxacin, and levofloxacin). Thus, majority of the isolates in our study were resistant to commonly used antibiotics such as ceftazidime, cefepime, gentamicin, amikacin, tobramycin, ciprofloxacin, ofloxacin, and ampicillin/sulbactam. This means MDR isolates are increasing day by day, probably due to indiscriminate use of these antibiotics in healthcare settings. It is re-emphasized that broad-spectrum antibiotics should be used with caution. We found that imipenem, meropenem, and piperacillin/tazobactam were the most potent antibiotics against this pathogen, although the resistance rates for these drugs were 19%, 22%, and 23%, respectively. The resistance pattern observed by us was similar to those described in previous

studies.<sup>[18,23]</sup> Mostofi *et al.* in their study had reported tobramycin (26%) was the least resistant drug followed by meropenem (31%) and piperacillin/tazobactam (40%), but imipenem (76%) showed high resistance to *Acinetobacter* spp.<sup>[19]</sup> Differences observed between the studies could be due to the methods and the resistance patterns that are influenced by the environmental factors and the antimicrobial patterns used. The lower resistance rate of *Acinetobacter* spp. to carbapenems may be due to their recent introduction, i.e., in the year 2004, for use in our hospital. Higher cost of these drugs is also responsible for their restricted use. Although antibiotic resistance is a worldwide concern, it is first and foremost a local problem – selection for and amplification of resistant members of a species that are occurring in individual hospitals and communities, which can then spread worldwide.<sup>[27]</sup> There are many measures that may impact on antimicrobial resistance; reducing and restricting the use of antimicrobials to only those situations where they are warranted, at proper dose and for the proper duration is the most appropriate solution.<sup>[28]</sup> Thus, hospitals, as the primary incubators of antimicrobial-resistant pathogens, carry the highest responsibility for proper stewardship of our existing antimicrobial resources.

Carbapenems have been the drug of choice for treating *Acinetobacter* infections, but unfortunately, carbapenem-resistant *Acinetobacter* spp. due to carbapenemase enzyme is becoming common worldwide.<sup>[29]</sup> Of the  $\beta$ -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- $\beta$ -lactamases (Ambler class B).<sup>[30]</sup>

Colistin was investigated in eight PDR *Acinetobacter* isolates in our laboratory and all of them were found to be susceptible. Colistin (polymyxin E) and tigecycline are new alternatives in the treatment of *Acinetobacter* species. Similar to our findings, Shareek *et al.* studied 44 isolates of *A. baumannii* and found that all were sensitive to colistin.<sup>[31]</sup> Taneja *et al.* in Chandigarh, India studied 224 *A. baumannii* isolates, out of which 50 (22.3%) isolates were resistant to carbapenems. The significant finding in their study was that eight (3.5%) isolates were resistant to both colistin and tigecycline.<sup>[32]</sup> Various authors have reported the resistance rate to colistin between 1.8% and 2%,<sup>[33,34]</sup> while resistance to tigecycline varies from being nonexistent to 66%.<sup>[35,36]</sup> We did not find any *Acinetobacter* isolate being resistant to colistin, which may be due to its selective use only in case of carbapenem-resistant gram-negative bacteria. In our hospital, tigecycline is not used routinely for treatment of *Acinetobacter* infections; so, we usually do not perform antibiogram using tigecycline disk.

## CONCLUSION

We found 54.7% of *Acinetobacter* isolates were MDR and most of these isolates were sensitive to carbapenems and piperacillin/tazobactam. All eight PDR isolates were sensitive to colistin. Elderly age, being inpatients, longer duration of stay, associated co-morbidity, and invasive procedure were found to be the risk factors in the setup investigated. To avoid resistance, antibiotics should be used judiciously and empirical antibiotic therapy should be determined for each hospital according to the resistance rates of that center. This should be regulated according to antibiogram results. Increasing carbapenem resistance rates in *Acinetobacter* spp. leads to usage of new alternative antibiotics like colistin and tigecycline.

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