

## Frequency and antibiogram of *Acinetobacter* species isolated from various clinical samples in a tertiary care hospital

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### ABSTRACT

**Background:** *Acinetobacter* species have emerged as opportunistic pathogens and can cause a wide range of healthcare associated infections like ventilator associated pneumonia, meningitis, and bacteremia, urinary tract infections, peritonitis, etc. *Acinetobacter* spp, most often multi-drug resistant, are difficult to treat pathogens and can lead to treatment failure. **Methods:** Our study was conducted to determine the frequency and antimicrobial resistance pattern of *Acinetobacter* species from various clinical samples. The isolates were identified by standard protocols and further tested for antimicrobial resistance by Kirby-Bauer disk diffusion method as per CLSI guidelines. **Results:** From 261 *Acinetobacter* isolates, maximum (39.5%) were obtained from pus/swab, followed by blood (31%), urine (19.9%) and other samples (9.6%). *Acinetobacter* species were resistant to ciprofloxacin (73.4%), amikacin (57.9%), gentamicin (70.4%), ceftazidime (82.9%), cefoperazone (82.4%), ampicillin/sulbactam (58%). The low resistant pattern of sulbactam-ceftazidime (18.4%), piperacillin/tazobactam (15.1%), imipenem (23.6%), indicate that they are effective drugs. All the isolates were found to be sensitive to colistin. **Conclusion:** Multi drug resistant isolates are increasing day by day, due to indiscriminate use of these antibiotics in healthcare settings. 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. This hospital-based epidemiological data will help to implement better infection control strategies and improve the knowledge of resistance pattern in our region.

**Keywords:** *Acinetobacter* species, nosocomial infection, antimicrobial resistance

### Introduction

*Acinetobacter* species account for 1 to 3% of hospital-acquired infections and primarily affect immunocompromised hosts and patients with comorbid diseases.[1]

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*Acinetobacter* species are the second most common non-fermenting bacteria after *Pseudomonas* species that are isolated from human specimens, especially among nosocomial infections[2]. The genus *Acinetobacter* is classified in the family *Moraxellaceae*. Based on DNA hybridization and studies on homology groups, there are currently 41 genome species within the genus *Acinetobacter*. *A. baumannii* is responsible for the majority of *Acinetobacter* infections; a minority are due to *A. calcoaceticus*, *A. lwoffii*, *A. hemolyticus*, *A. johnsonii*, *A. junni*, *A. radioresistens* and other genospecies.[3] *Acinetobacter* species are opportunistic pathogens and

cause wide-spread hospital infections such as-ventilator associated pneumonia, central line associated bloodstream infections, post-neurosurgical meningitis, catheter associated urinary tract infections, wound and soft tissue infections and infections in burn patients. Predisposing factors include the presence of a prosthesis, endotracheal intubation, intravenous catheter, peritoneal dialysis, underlying severe illness, long term hospitalization, stays in intensive care units (ICUs) and selective antimicrobial pressure.[4] The potential source of contamination with *Acinetobacter* in hospital environment is the medical equipments used for therapy or from contamination in the environment by airborne route or by contact with the patients. Its great capacity to survive in low- moist environment coupled with its ability to develop resistance to antimicrobial agents can also increase the spreading in hospitals.[5]The spread of multidrug resistant *Acinetobacter* strains among hospitalized patients has become an increasing cause of concern.[6] Therefore, treatment of infections caused by these organism should be based on antimicrobial susceptibility testing. Efficient infection control strategies and strict isolation procedure of colonized or infected patients prevent the dissemination of these strains to the environment.The

present study was carried out to know the frequency of *Acinetobacter spp.* in our hospital setting isolated from various clinical specimens and to determine their antimicrobial susceptibility.

### Material and Methods

This retrospective study was conducted in the Microbiology department of tertiary care hospital from July 2015 to Dec 2016. A total of 261 *Acinetobacter* isolates were recovered from various clinical samples like pus/swab, urine, sputum, blood, body fluids, tracheal aspirates. The samples received in the laboratory were processed according to standard procedures and the isolates were identified on the basis of colony characteristics, Gram' staining, motility test, oxidase test and Triple Sugar Iron agar.[7] *Acinetobacter spp.* were identified by characteristics colony appearance, non lactose-fermenting, gram negative coccobacilli, non-motile, oxidase negative, catalase positive and alkaline reaction on Triple Sugar Iron Agar Test. Further species differentiation was done on the basis of Glucose oxidation, citrate test, growth at 42°C, fermentation of 10% lactose.(Table1)

**Table 1: Identification of isolates**

	Glucose oxidation	Citrate Test	Growth at 42°C	Fermentation of 10% Lactose
<i>A.baumannii</i>	+	+	+	+
<i>A.lwoffii</i>	-	-	-	-

After identification by phenotypic methods antimicrobial susceptibility testing was performed for each isolate by Kirby- Bauer disc diffusion method as recommended by CLSI guidelines.[8]Multidrug-resistant (MDR) *Acinetobacter spp.*are defined as those isolates resistant to more than three classes of antibiotics, An isolate was classified as pan resistant when it was resistant to all of the commonly used antibiotics. [9]

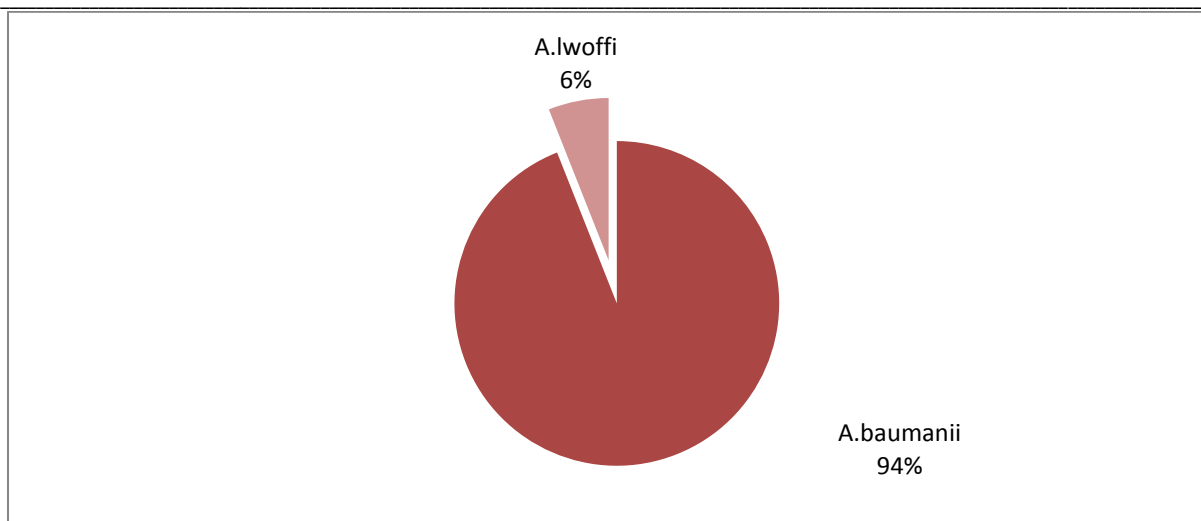
### Results

From 261 *Acinetobacter* isolates recovered from very various clinical specimens, majority (78%) were detected from patients admitted in various wards of hospital and the rest (22%) were isolated from the OPD cases. Majority of the *Acinetobacter* species were isolated from pus/swabs, followed by blood , urine and body fluids (4.6%). (Table2).

**Table 2: Distribution of *Acinetobacter* isolates in various clinical specimens**

Specimen	Percentage of isolates
Pus/Swab	39.5%
Blood	31%
Urine	19.9%
Body fluids	4.6%
Tracheal aspirate	2.6%
Sputum	2.4%

*A.baumannii* was the main species responsible for (94%) of the infection, followed by *A.lwoffii* (6%). (Figure1)



**Fig 1: Pie diagram showing percentage of *Acinetobacter* species isolated in various clinical specimens**

In the present study, most of the *Acinetobacter* species were resistant to ciprofloxacin (73.4%), amikacin (57.9%), gentamicin (70.4%), ceftazidime (82.9%), cefoperazone (82.4%), ampicillin/sulbactam (58%). The low resistant pattern of imipenem (23.6%),

sulbactam-ceftazidime (18.4%) piperacillin/ tazobactam (15.1%) indicate that they are effective drugs. All the isolates were found to be sensitive to colistin. (Table 3).

**Table 3: Frequency of Antimicrobial resistance in *Acinetobacter* spp. in various clinical samples**

Antimicrobial	Resistant (%)	Sensitive (%)
Ciprofloxacin (5mcg)	73.4	26.6
Amikacin (30mcg)	57.9	42.1
Gentamicin (10mcg)	70.4	29.6
Ceftazidime (30mcg)	82.9	17.1
Cefoperazone (75mcg)	82.4	17.6
Ampicillin/Sulbactam (10/10mcg)	58	42
Sulbactam/ceftazidime (30/10mcg)	18.4	81.6
Piperacillin/tazobactam (100/10mcg)	15.1	84.9
Imipenem (10mcg)	23.6	76.4
Colistin (300units)	0	100

Out of 261 isolates, 57.9% were multidrug-resistant (MDR) and 15.1% were pan-drug resistant (PDR).

## Discussion

In this current study the frequency and antibiogram of *Acinetobacter* species isolated from various clinical samples in our hospital was studied. *Acinetobacter* spp has emerged as the causative agent of nosocomial infection due to increased use of invasive procedures, overuse of broad spectrum antimicrobials 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.[10]

*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.[11] 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.[12] Hospital environment is heavily contaminated with these

organisms. The carriage rate is much higher among hospital staff than community. Unhygienic practices in hospital (contaminated hands of staff) and warm hospital environment (summers) promote colonization. *Acinetobacter* persist on inanimate surfaces for prolonged periods of time ranging from 3 days to 5 months and can be detected on various equipment including bedrails, curtains, ventilation equipments(e.g. AMBU bags, ventilator filter).[13] Patients with underlying diseases or immunosuppression are predisposed to invasion and pathogenesis. Virulence factors attributed to pathogenesis are Outer membrane protein (OmpA), lipopolysaccharide, lipases ability to form biofilm and siderophores. [14] Out of 261 isolates of *Acinetobacter* species, 78% of *Acinetobacter* isolates were obtained from patients admitted to various wards, whereas only 22% were obtained from OPD cases. We isolated majority of the *Acinetobacter* species from pus/swabs (39.5%), followed by blood (31%), urine (19.9%), body fluids (4.6%) tracheal aspirates (2.6%) and sputum (2.4%). Similar findings of majority of *Acinetobacter spp* isolation from pus/swab were also obtained by Chakraborty et al. in West Bengal.[15] 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%).[16] In the present study *A.baumannii* was found to be the frequent cause of infections. Like our study, W. Nageeb et al., also proved that *A.baumannii* was the only *Acinetobacter spp.* encountered in clinical specimens and this supported the finding that infections by other *Acinetobacter spp.* are infrequent.[17] There are some other studies which also found that among different *Acinetobacter spp.*, *A.baumannii* was the most prevalent in clinical specimens and the most often responsible for nosocomial infections.[16,18,19] Hospital outbreaks caused by problematic microorganisms, like multidrug-resistant *Acinetobacter baumannii*, resulting in increased morbidity and mortality, especially in intensive care units, surgical wards in a big hospital complexes, have been reported worldwide.[20] Also there are many reports, showing that persistent hospital environmental contamination with *A. baumannii* strains may play an important role in nosocomial dissemination of these organisms.[21,22] Management of *Acinetobacter* infections is huge challenge because of the broad array of antimicrobial resistance. The resistance patterns of *Acinetobacter* isolates towards various antimicrobial agents were determined by disc diffusion method as recommended by CLSI guidelines. In the present study, *Acinetobacter* isolates exhibited the highest resistance (82.9%) against ceftazidime followed by cefoperazone (82.4%), ciprofloxacin (73.4%), gentamicin (70.4%),

ampicillin/sulbactam (58%), amikacin (57.9%). The low resistant pattern of imipenem (23.6%), sulbactam-ceftazidime (18.4%) piperacillin/tazobactam (15.1%) indicate that they are effective drugs. All the isolates were found to be sensitive to colistin. Antimicrobial treatment of the clinical infections caused by *A.baumannii* strains, may be compromised by the multiple-drug resistance of many isolates to beta-lactams, aminoglycosides, and fluoroquinolones.[23] In a study conducted by Bhattacharya et al in West Bengal rates of non- susceptibility are about 80% for ceftazidime, 55.5% for amikacin, 52.17% for ciprofloxacin, 18.2% for piperacillin/tazobactam and 15% for imipenem respectively.[24] We found the corresponding figures for these group of antimicrobials to be 82.9%, 57.9% and 73.4%, 15.1% and 23.6% respectively. Thus in our study higher values were recorded for ceftazidime, amikacin, ciprofloxacin and imipenem respectively. In our study 57.9% of the isolates were Multidrug-resistant (MDR). The other studies conducted by Dash *et al* in Odisha and Mostofi *et al* in Tehran reported MDR isolates to be 54.7% and 54% respectively.[4] In a review comparing hospitals of 10 Asian countries, 1.2-87% of all *Acinetobacter* isolates from patients with Hospital Acquired Pneumonia (HAP) were MDR, with MDR strains most prevalent in India and Thailand.[25] In a study from Pune, about 48% to 68.6% *A.baumannii* isolates were MDR.[26] One report from U.S.A has quoted imipenem resistance in *Acinetobacter baumannii* in the order of 23.1%.[27]

Taneja *et al.* in Chandigarh, India studied 224 *A.baumannii* isolates, out of which 22.3% isolates were resistant to carbapenems.[4] Our results show that about 23.6% were resistant to imipenem which is similar to reports from U.S.A and India.. We found that imipenem and piperacillin/tazobactam were most potent antibiotics against this pathogen. 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. Colistin retains activity against *Acinetobacter spp.* in the face of broad-spectrum antimicrobial resistance and have become the last resort of treatment. A delay in the administration of colistin, has the potential to increase the risk of mortality. However, *A.baumannii* can develop resistance to colistin, and thus extreme vigilance is required to diagnose the development of resistance during treatment. Unfortunately, resistance to colistin has emerged with its increasing use, and the recent observation of heteroresistance to colistin among clinical strains of MDR *A.baumannii* is also a significant cause of concern. Pan drug resistance

typically is the result of the convergence of multiple resistance mechanisms.[28] Pan drug resistant *A.baumannii* isolates, i.e isolates resistant to all antimicrobial agents in-vitro, have been reported from Asia and the Middle-East. In our study 15.1% isolates were pan drug resistant. Colistin retains activity against PDR *Acinetobacter spp.* but side effects like nephrotoxicity and ototoxicity limits its use.[13] Our study did not find any *Acinetobacter* isolate resistant to colistin, which may be due to its selective use only in case of carbapenem- resistant gram-negative bacteria. Colistin activity can be enhanced when combined with some other modes of action such as carbapenems, rifampicin and ceftazidime. Combination therapy of colistin and meropenem has synergistic effect/additive effect. Colistin acts on outer membrane of cell wall and creates pores allowing the other drugs to enter into the bacterial cell. Meropenem has bactericidal activity and binds to PBP of cell wall and inhibits cell wall synthesis.[28] 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.[4] The primary goals for the control of multidrug resistant *Acinetobacter* infection are recognizing its presence in a hospital at an early stage and controlling its spread. Hospital-acquired infections are best controlled by following appropriate sterile techniques and infection control guidelines and by implementing effective protocols for the sterilization and decontamination of medical supplies. Infection control measures such as improved hand hygiene are essential to prevent nosocomial infections due to *Acinetobacter*.

## Conclusion

*A.baumannii* is an important nosocomial pathogen causing significant morbidity and mortality. MDR isolates are increasing day by day, probably due to indiscriminate use of these antibiotics in healthcare settings. 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. Susceptibility testing should be done to help select the best antimicrobial drugs for therapy. A combination of a review of hand-washing practice, education about spread of bacteria via hands and contaminated environment, and the revision of infection control procedures would help in the control of this organism in hospitals.

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