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KEY WORDS: *Acinetobacter, A.baumannii, A.lwoffii*, Nosocomial infections, carbapenamases, metallo-β-lactamases

PROFILE OF *ACINETOBACTER* SPECIES IN A TERTIARY CARE HOSPITAL IN MEERUT CITY

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ABSTRACT

Background: *Acinetobacter* are the "superbugs" of the hospital environment causing nosocomial infections with high mortality rates. *Acinetobacter* infections are often extremely difficult to treat because of their widespread resistance to antibiotics.
Aims & Objectives: i) To study the frequency of isolation of *Acinetobacter* species from clinical samples. ii) To identify the isolates of *Acinetobacter* upto species level. iii) To determine the in-vitro antimicrobial susceptibility of the isolates.
Methods: A total of 11955 clinical samples were processed and isolates were identified as per standard bacteriological techniques. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per CLSI guidelines 2017.
Result: The frequency of isolation of *Acinetobacter* species was 4.16% (498/11955), out of which 87.75% isolates were of *Acinetobacter baumannii* and the remaining 12.25% isolates were of *Acinetobacter lwoffii.* Isolation of *Acinetobacter* species was most common from pus samples (25.30%) and majority of the isolates were from ICUs (46%) indicating increased hospital acquired infections.
Conclusions: *Acinetobacter* infections has become a common drug resistant threat in hospital acquired infections. A combined effort of continuous surveillance and infection control protocols have to be implemented to control the increasing incidence of highly resistant *Acinetobacter* species.

INTRODUCTION

The genus *Acinetobacter* is classified in the family Moraxellaceae and consists of bacteria that are non- motile, oxidase-negative, Gram-negative coccobacilli.[1] They are strictly aerobic, free living saprophytes in soil and water and are capable of growing at a wide range of temperatures.[2] Despite their low pathogenic potential *Acinetobacter* species survive in the hospital environment for a long time and there by getting an opportunity to cause hospital acquired infections.[3] *A.baumannii* as well as its close relatives, belong to 2 genomic species "*A.baumannii* complex" and "*A.lwoffii*" .Most frequently encountered species is *A. baumannii* and it is commonly associated with infections, such as bacteremia, urinary tract infection, meningitis, skin and soft tissue infections and pneumonia with high mortality rate of 30- 75% in hospital patients.[4,5] The spread of multidrug resistant *Acinetobacter* strains among hospitalized patients has become an increasing cause of concern.[6,7]

MATERIALS AND METHOD:

The prospective study was carried out in the Department of Microbiology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut, over a period of one year (from May 2017- April 2018).

Ethics approval:

Approval by the institutional ethics committee was taken before conducting the study.

The study group included all the patients from various inpatient units and outpatient departments, irrespective of age and gender.

Sample Processing:-

Clinical samples such as urine, blood, endotracheal aspirate, pus, sputum, pleural fluid, cerebrospinal fluid and other body fluids, received in the laboratory,were processed as per standard bacteriological techniques.[1,2] Samples were first subjected to direct microscopy by Gram staining and were inoculated on to Blood agar, Chocolate agar, MacConkey agar. Plates were incubated at 37ş C for 24 hours. If there was no growth, culture plates were re-incubated for another 24 hours. The isolates were identified by colony characteristics, culture smears, motility and the biochemical tests such as Catalase, Oxidase, Nitrate reduction test, Indole, Methyl red, Citrate utilization, Urease, Triple sugar iron agar, Phenylalanine deaminase, Sugar fermentation, Amino acid decarboxylases tests and Hugh and Leifson's Oxidation fermentation (glucose) test and Oxidation fermentation (lactose) test.[1,2] Genus *Acinetobacter* was identified by non-lactosefermenting, non-motile, oxidase negative coccobacilli. Speciation of *Acinetobacter* was done on the basis of biochemical reactions[1,2,8] and *A. baumanni* was identified on the basis of oxidative pattern in OF glucose and OF lactose, growth at 42°C and positive arginine dihydrolase. Antibiotic susceptibility test was performed as per CLSI guidelines[9] 2017 by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar using 0.5 McFarland turbidity standard and comparing zone sizes with the control strain *Pseudomonasaeruginosa* ATCC 27853. Piperacillin (100µg), Cefepime (30µg), Ceftazidime (30µg), Piperacillin – Tazobactam (100/10µg), Ciprofloxacin (5µg), Aztreonam (30µg), Amikacin (30µg), Gentamicin (10µg), Tobramycin (10µg), Meropenem (10µg), Imipenem (10µg) and Colistin (10µg).

Detection of Carbapenemase production.

A) **Screening for Carbapenem resistance by disc diffusion[9] using Meropenem disc(10µg):**Isolates with zone diameter <19mmwere considered to be Meropenem screen positive.
B) **All the Meropenem resistant isolates were further subjected to Modified Hodge Test (MHT) and Combined Disk test (CDT).[9]**

Production of carbapenemase was confirmed by MHT[9,10,11] and Metallo beta lactamase(MBL) production was detected by performing CDT[9,12,13].

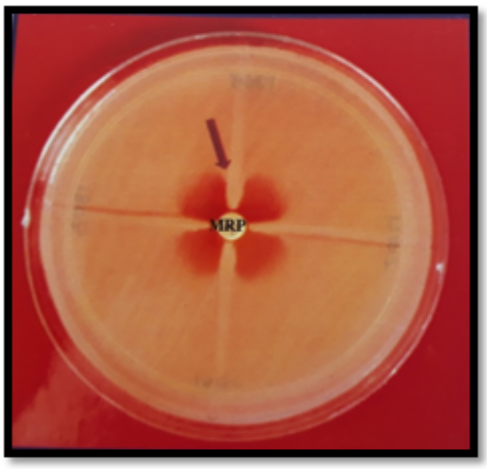
Modified Hodge test:

The Modified Hodge test (MHT) performed as recommended using 10 µg Meropenem disc and *Escherichia coli* ATCC 25922.

A clover leaf like indentation of *Escherichia coli* 25922 growing along the test organism growth streak within the disc diffusion zone were considered as MHT positive (Figure 1). No indentation of Escherichia coli 25922 growing along the test organism growth streak within the disc diffusion zone were MHT negative.

Combined disc test (CDT):
CDT was peformed using Imipenem (IPM) & ethylene diamine tetra acetic acid (EDTA) was used for the detection of MBL producers.

An increase in zone diameter of ≥7 mm around the IPM – EDTA disc as compared to that of the IPM disk alone was considered positive for an MBL production. If the difference in zone diameter of IPM – EDTA disc and IPM disk alone is less than 7 mm, it was considered negative for an MBL. (Figure 2



CLOVER LEAF LIKE INDENTATION
Fig.1: Modified Hodge Test Positive



Fig.2: Combined disc test positive:
INCREASE IN ZONE DIAMETER OF ≥ 7 MM

RESULTS:
Out of total of 11955 clinical samples, 498 (4.16%) were culture positive for *Acinetobacter* species. Among the 498 *Acinetobacter* isolates, 437 (87.75%) were of *Acinetobacter baumannii* and the remaining 61 (12.25%) were of *Acinetobacter lwoffii.*(Figure3). *Acinetobacter* species were isolated predominantly from pus sample (25. 30%) followed by endotracheal aspirate (19.28%), blood (15.48%), sputum (15.66%), urine (12.66%) and pleural fluid (6.62%).(Table 1).

Acinetobacter infections were more common in patients of age group 41-50 years, followed by 51-60 years. Our data showed male preponderance. Males outnumbered females with the ratio of 4:1. (Figure 4). Highest isolates were recovered from ICUs (46%) followed by wards and OPD.(Figure 5).

Among the isolates of*Acinetobacter* species, 65.07% were resistant to meropenem and 60.02% were resistant toimipenem. Out of meropenem resistant isolates of *Acinetobacter* species(324/498,65.07%), *A. baumannii* were 94.45% (306/324) and *A. lwoffii* were 5.55% (18/324). Among the isolates of *A. baumannii and A. lwoffii,* carbapenamase production was seen in 39.21% (120/306) and 33.33% (6/18) respectively. Among all the meropenem resistant isolates of *A. baumannii* and *A. lwoffii*, metallo -lactamases (MBL) production was seen in 50.98%(156/306) and 66.66%(12/18) respectively (Table 2).

Co-production of carbapenamase and metallo β-lactamases was seen in 18.51% (60/324) of *Acinetobacter* species. Among *A. baumannii* and *A. lwoffii* isolates co – producers were 17.64% and 1.96% respectively (Table 3). *Acinetobacter* species showed 100% susceptibility to colistin.

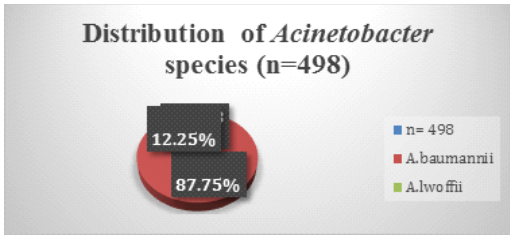


Figure 3:Distribution of *Acinetobacter* species (n=498)

Table 1: Isolation of *Acinetobacterspecies*(n=498) from clinical samples.

Clinical samples	No. of isolates (n)	Percentage %
Pus	126	25.30%
Endotracheal Aspirate	96	19.28%
Blood	77	15.48%
Sputum	78	15.66%
Urine	63	12.66%
Pleural fluid	33	6.62%
Broncho alveolar lavage	5	1%
Menstrual blood	4	0.80%
Placental membrane	6	1.20%
Ascitic fluid	7	1.40%
Synovial fluid	3	0.60%

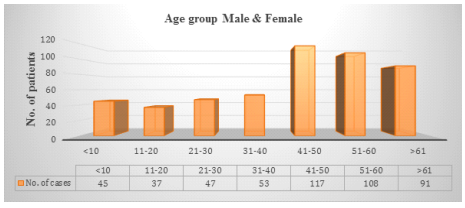


Figure 4: Age wise distribution *Acinetobacter* Isolates (n=-498).

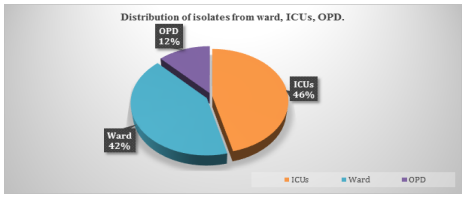


Figure 5: Distribution of isolates from ward, ICUs, OPD (n=498).

Isolate	MHT Positive		Imipenem EDTA disc test Positive	
	Number	**Percentage**	**Number**	**Percentage**
Acinetobacter baumannii (n=306)	120	39.21%	156	50.98%
Acinetobacter lwoffii(n=18)	6	33.33%	12	66.66%

Table 3: Co-production of carbapenamases&metallo lactamase in *Acinetobacter* isolates.

Both MHT & Imepenem EDTA disc test Positive (Co – producers)		
Organism	**Number**	**Percentage**
Acinetobacter baumannii(n=306)	54	17.64%
Acinetobacter lwoffii (n=18)	6	1.96%
Total (n=324)	**60**	**18.51%**

DISCUSSION:

Acinetobacter species has emerged as a cause of infection in hospital settings. Multidrug resistant *Acinetobacter* spp. have become established as "alert" pathogens, particularly in ICUs and are associated with outbreaks of nosocomial infection.[14] Their ubiquitous nature in the ICU environment and inadequate infection control practice have continuously raised the incidence of *Acinetobacter* infection over the past two decades.[15]

In our study, the isolation rate of *Acinetobacter* species was 4.16 %. Our findings were similar to the study carried out by Riet *et al.*[16] and Dash *et al.*[17] who had reported the isolation of *Acinetobacter* species in 4.5% and 3% cases respectively. However the recovery rate of 12.6% was observed by Huidrom S *et al.* reported in Bengaluru.[18]

Among the isolates of *Acinetobacter* species, 87.76%were *A. baumannii* and the remaining 12.24% were *A. lwoffii.* A study in Odisha by Dash M *et al.*[17] found that the predominant isolates were of *A. baumannii*(76.9%), followed by*A. lwoffii*(12.4%) and the remaining 8% were caused by other *Acinetobacter* spp. Various other studies had also reported the popularity of *Acinetobacter baumannii* in nosocomial infections.[19,20,21]

In the present study, maximum number of *Acinetobacter* isolates were recovered from pus (25.30%) followed by endotracheal aspirate (19.28%), blood (15.48%), sputum (15.66%), urine (12.66%) and pleural fluid (6.62%). In a study done by Tripathi A.*et al.*[22] *Acinetobacter* spp. were recovered most commonly from respiratory samples (35.78%) followed by pus (32.84%), blood (23.52%), body fluids (03.92%) and urine (03.92%). However, Dash M *et al.*[17] recovered majority of *Acinetobacter* isolates from pus/swab samples (56.9%), followed by blood (13.1%), urine (12.4%), sputum (8.1%), and body fluids (5.1%).

In our study, out of total isolates, 46% isolates were from ICUs, 42% were from general wards and 12% were from OPD. Tripathi A.*et al*[22] reported 61.27% isolates from ICUs and 38.2% from general wards.This variation could be due to the varying prevalence rates of *Acinetobacter* species in the hospital environment and the community in different geographical areas.We observed that the infection was more common in middle-elderly patients. Similarly, in the study of Mindolli *et al.*[19]isolates of *Acinetobacter* were in age group >45 years possibly due to weakened immune system and associated chronic disease in this age groups.

Our data shows that among the isolates of *Acinetobacter* species, 65.07% were resistant to meropenem and 60.02% were resistant to Imipenem. Study done by Dash M*et al.*[17] found that 19% of *Acinetobacter* isolates were resistant to imipenem and 22% to meropenem. But Mostofi*et al.*[23] observed meropenem resistance in 31% and imipenem resistance in 76% of *Acinetobacter* isolates. In the present study, out of meropenem resistant isolates of *Acinetobacter* species(65.07%), *A. baumannii* were 94.45% and *A. lwoffii* were 5.55%. Among the isolates of *A. baumannii and A. lwoffii,* carbapenamase production was seen in 39.21% and 33.33% respectively. Among all the meropenem resistant isolates of *A. baumannii* and *A. lwoffii*, metallo β lactamases (MBL) production was seen in 50.98% and 66.66% respectively. Co-production of carbapenamase and metallo- β -lactamases was seen in 18.51% of *Acinetobacter* species. Among *A. baumannii* and *A. lwoffii* isolates co – producers were 17.64% and 1.96% respectively. *Acinetobacter* species showed 100% susceptibility to colistin.

MHT detects class D β lactamases like OXA type carbapenemases, which are predominant cause of carbapenem resistance in Acinetobacter species. CDT (Combined Disc Test), using Imipenem and Imipenem-EDTA, is used for detection of class B/ Metallo-β-lactamase.[24,25]

However, a study done in Lucknow by Sinha N. *et al.*[26] found that among the meropenem resistant *Acinetobacter* isolates, MBL production was seen in 57.14% of isolates whereas MHT was negative for all MBL producers.Gupta N. detected MBL producing *Acinetobacter spp.* in 14.4% of isolates in Pune.[27]

Limitations of the study:

Identification of molecular mechanism of resistance in the isolates would have made the study more conclusive.

CONCLUSION:

Acinetobacter baumannii is recognised as species of great clinical significance, causing various nosocomial infections. Emergence of multidrug resistance *Acinetobacter* strains has led to the limitation of therapeutic options. Hence combined effort of surveillance and strict infection control practices should be implemented to decrease the burden of multidrug resistant bugs.

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