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Detection of OXA-Carbapenemase in various species of Acinetobacter in adult ICU patients at a tertiary care hospital

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A B S T R A C T

Background: *Acinetobacter* has emerged as a significant nosocomial pathogen due to the increased spectrum of diseases caused by this organism as well as because of global spread of strains with multi-drug resistance. **Materials & methods:** A prospective observational study was done (January2023-December 2023) in the Department of Microbiology at GMC, Kota.100 samples were received from adult patients admitted in various ICUs. Isolation & speciation of *Acinetobacter* was done with the help of conventional biochemical tests. Antimicrobial sensitivity testing was done by Kirby-Bauer Disc diffusion method as per CLSI(M100ED32) guidelines. Modified Hodge test(MHT) was done to detect OXA-Carbapenemase production. **Results:** Out of 100 samples received from adult ICUs, 31 isolates of *Acinetobacter* were identified. Among these 31 isolates , 14 (45%) were found to be Carbapenem resistant which were further confirmed for class D OXA carbapenemase production by Modified Hodge test. Overall, 13 (42%) were positive for MHT , among which 11 were *A.baumannii* and 2 were *A.lwoffii*. *A.haemolyticus* did not show Carbapenem resistance. **Conclusion:** Detection of OXA-Carbapenemase producing isolates pointed towards highly drug-resistant strains and their timely detection would be very helpful in early and appropriate treatment of patients as well in forming antibiogram for the hospital.

Keywords: *Acinetobacter*, OXA-carbapenemase , Modified Hodge test, ICUs, nosocomial.

INTRODUCTION

Acinetobacter is a genus of non-motile, Gram-negative coccobacilli that are widely distributed in the environment. They are oxidase-negative, aerobic organisms and can survive in both dry & moist environments. It belongs to a deadly group of resistant pathogens, known by acronym "**ESKAPE**" bugs, which includes ***Enterococcus faecium , Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa* & *Enterobacter* species**[1]. *Acinetobacter baumannii* accounts for more than 80% of isolates causing human infections among all sibling species ,particularly in immunocompromised patients[2,3]. A number of acquired mechanisms of resistance including production of extended spectrum beta-lactamase enzymes, modification enzymes against aminoglycosides, altered binding sites for quinolones, and a variety of efflux mechanisms has resulted in significant challenges for the clinician to select an appropriate empirical antimicrobial agent[4]. OXA-carbapenemase production by *Acinetobacter* species is a significant mechanism of resistance to carbapenem antibiotics, which are often used as a last resort in treating multi-drug resistant infections. Keeping this in mind, this study has been conducted to identify OXA-carbapenemase producing isolates in adult ICU patients as early detection will further aid in arresting the evolution and spread of multi drug resistant strains and thus improving the clinical outcome with decreased morbidity and mortality among hospitalized patients.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Microbiology at GMC, Kota from January 2023 to December 2023 which included 100 samples received from adult patients (> 18 yrs) admitted in various ICUs.

Ethical clearance: This study protocol was proceeded after the approval from Institutional Ethical Clearance Committee and Research Review Board [F.3( )NO.62/dated 15/12/22].

Inclusion criteria: Patients above 18 yrs of age, both male & female which were admitted in various ICUs ,with or without prolonged catheterization, prosthesis, shunts etc. were included.

Exclusion criteria: Patients below 18yrs of age and not admitted in ICU were excluded.

Sample processing: The samples collected were processed according to the standard procedures which included direct microscopic examination of Gram-stained smear and inoculation of samples on MacConkey agar and blood agar which were then incubated aerobically at 37°Cfor 18-24hrs.After preliminary identification of growth, isolates were subjected to Gram staining and conventional biochemical tests for further identification. Antimicrobial susceptibility testing was done by Kirby-Bauer Disc diffusion method as per CLSI(M100ED32) guidelines. Carbapenem resistant isolates were further confirmed by Modified Hodge test for OXA-carbapenemase production.

MODIFIED HODGE TEST [5]**:** Originally described by CDC (Centre for Disease Control, Atlanta) for Carbapenemase detection in Enterobacteriaceae, MHT was performed for OXA-Carbapenemase detection in all *Acinetobacter* isolates.

Procedure :- 0.5 McFarland's standard of E.coli ATCC 25922 was prepared using 5ml of peptone water. Then 1:10 dilution was obtained by adding 0.5ml of the 0.5 McFarland's standard to 4.5ml of peptone water. A lawn culture of E.coli ATCC 25922 suspension on Muller Hinton agar(MHA) plate was made and allowed to dry for about 3-5 minutes. A 10µg Meropenem disc was then placed in the centre of the MHA plate. Carbapenem resistant *Acinetobacter* isolates were streaked from edge of the disc to the periphery of the plate in a straight line using a loop in four different directions like a cross. Positive and negative controls were streaked on the same plate at 90º angulations from centre to periphery for quality control. The plate was then incubated for 18-24 hours at 37°C. Zone of inhibition was then read with the help of a scale/callipers.

Interpretation :-
MHT Positive test: A clover leaf like indentation was seen at the intersection of the organism tested and the E.coli 25922. It was also seen within the inhibition zone of the Meropenem disc. A positive MHT indicated OXA-Carbapenemase production by that particular isolate.

MHT Negative test: No growth of E.coli 25922 within the zone of inhibition of Meropenem disc as well as along the test organism.

Statistical analysis: Descriptive statistical methods like percentage distribution and graphical presentation were used for the analysis of categorical variables in the study. The Chi-square test was used to test the association between ESBL production and biofilm formation.

RESULTS
A total of 100 samples were collected from adult ICU patients and processed. Among 83 culture positive samples , 86 isolates were identified. *Acinetobacter*(36%) and *Pseudomonas*(28%) were the most common isolates followed by *Escherichia coli*(14%) and *Klebsiella*(12%) species. *Proteus mirabilis*(1.16%) and *Citrobacter*(1.16%) species were the least common among the Gram-negative bacilli identified[Table 1]. Most of the patients infected with *Acinetobacter* species belong to the age group of 41-60 yrs. Patients above 80 yrs. and below 40 yrs. were least affected[Table 2]. Among the affected individuals , males overnumbered the females. Among all the *Acinetobacter* isolates (total 31),

Acinetobacter baumannii (68%) was the most common species isolated followed by *Acinetobacter lowffii* (29%).*Acinetobacter haemolyticus* was the least common species (3.22%) identified in the present study. Carbapenem resistance was shown by 12(57%) out of 21isolates of *A.baumannii*, 2(22%) out of 9 isolates of *A.lwoffii*. *A.haemolyticus* did not show carbapenem resistance[Table 3]. In other words, out of total 31 isolates , 14 (45%) were Carbapenem resistant. Modified Hodge test was performed for confirmation of class D OXA carbapenemase production. Overall, 13 (42%) were positive for MHT , among which 11 were *A.baumannii* and 2 were *A.lwoffii*[Table 4].

TABLE 1: DISTRIBUTION OF VARIOUS ISOLATES (n=86)

Name of the organism	Number of isolates	Percentage(Prevalence)
Acinetobacter species	31	36.04%
Pseudomonas aeruginosa	24	27.90%
Escherichia coli	12	13.95%
Klebsiella species	10	11.62%
Proteus mirabilis	01	1.16%
Citrobacter species	01	1.16%
Staphylococcus aureus	02	2.33%
CoNS	02	2.33%
Enterococcus	02	2.33%
Yeast bodies	01	1.16%
Total	86	100%

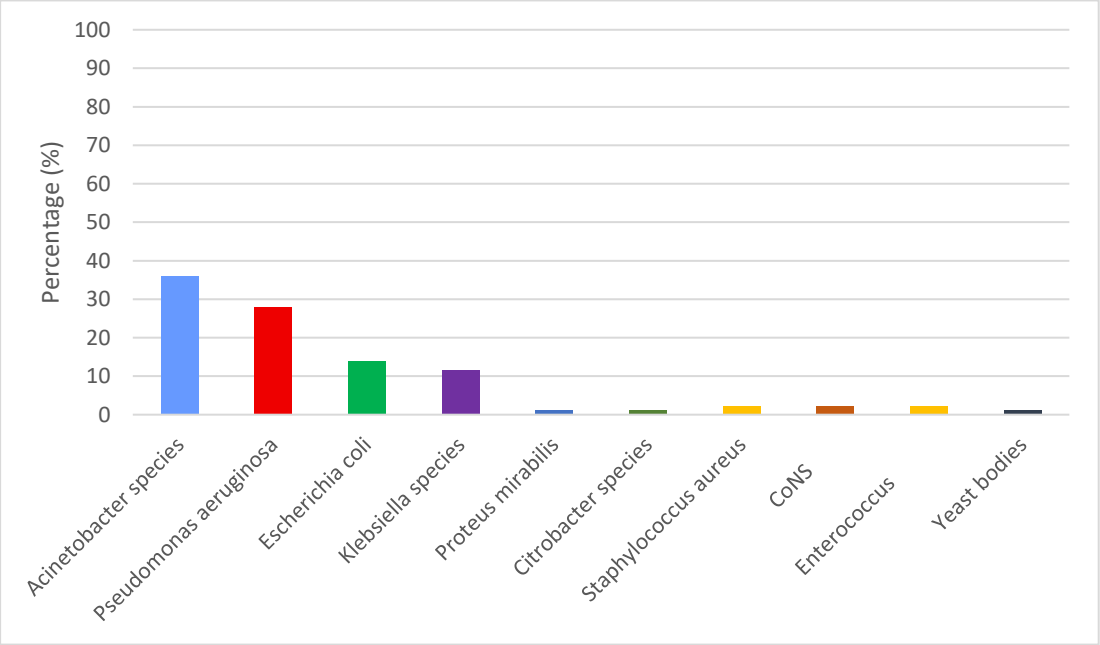


TABLE 2: AGE & SEX-WISE DISTRIBUTION OF *ACINETOBACTER* SPECIES (n=31)

Age (years)	Male No.(%)	Female No.(%)	Total No.(%)
18-40	04(20%)	02(18%)	06(19%)
41-60	10(50%)	05(45%)	15(48%)
61-80	05(25%)	03(27%)	08(26%)
>80	01(5%)	01(9%)	02(6.5%)
Total	20(100%)	11(100%)	31(100%)

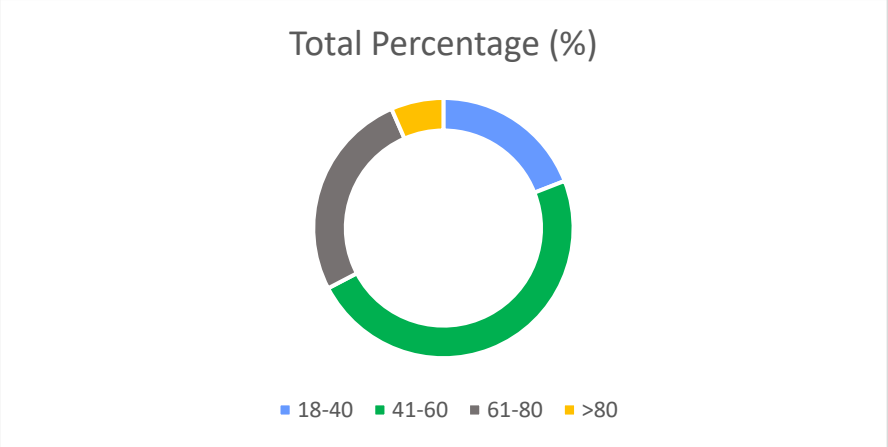


TABLE 3: CARBAPENEM RESISTANCE IN ACINETOBACTER ISOLATES

Antibiotic	***A.baumannii* (n=21)**			***A.lwoffii* (n=9)**			***A.haemolyticus* (n=1)**		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Imipenem(IPM)	8(38%)	1(5%)	12(57%)	7(78%)	0	2(22%)	1(100%)	0	0

*S – Susceptible, I- Intermediate, R- Resistant

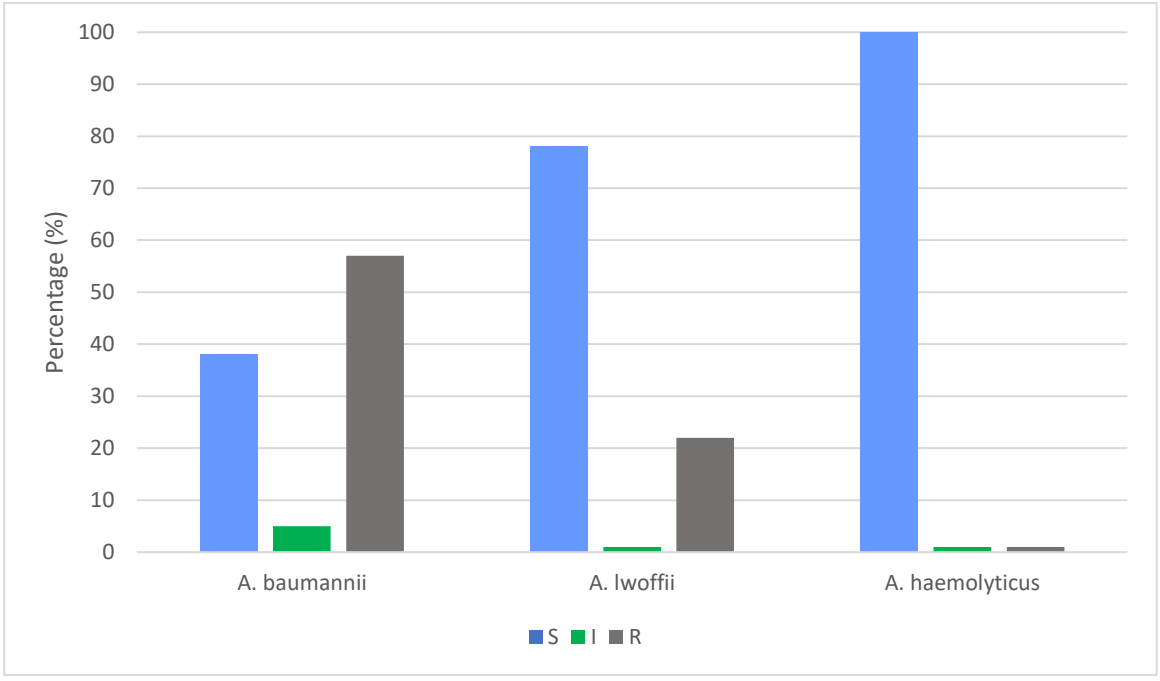
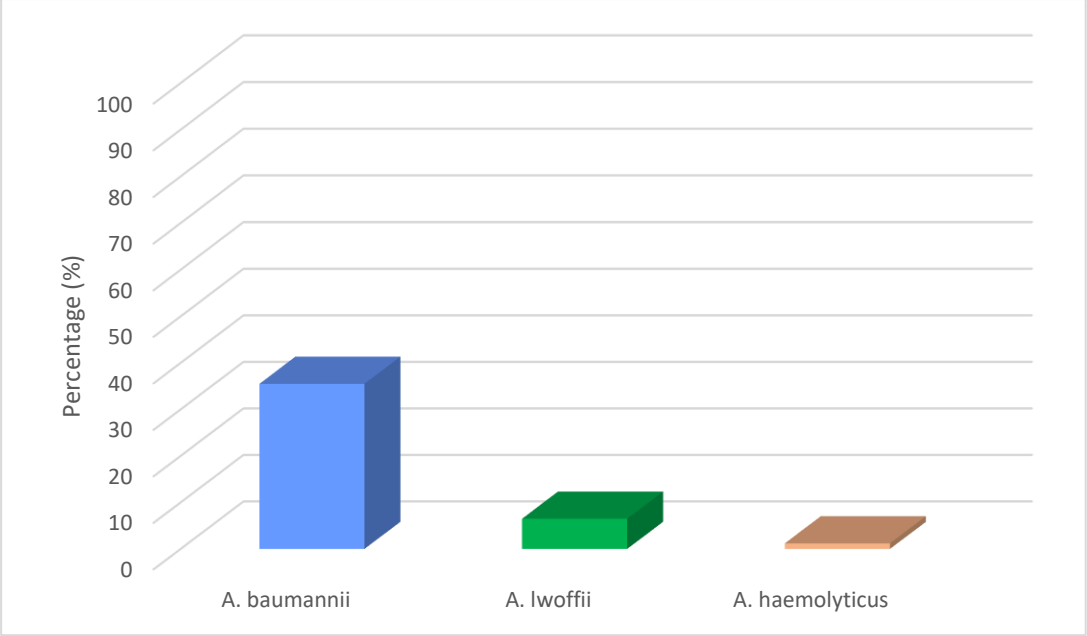


TABLE 4: DETECTION OF OXA-CARBAPENAMASE PRODUCTION IN *ACINETOBACTER* SPP. BY MODIFIED HODGE TEST

No. of Carbapenem resistant isolates	**MHT positive % (n=31)**
A.baumannii = 12	11(35.5%)
A.lwoffii = 2	2(6.5%)
A.haemolyticus = 0	0(0%)
Total = 14(45%)	Total = 13(42%)



DISCUSSION

In Indian set up, incidence of infections in the ICU usually ranges from 2.3% to 49.2% with variations depending on the type of population studied in a particular setting [6]. The prevalence of *Acinetobacter* in the present study in various ICUs was 36.04% followed by *Pseudomonas* (27.90%) , *Escherichia coli* (13.95%) and *Klebsiella* (11.62%). Studies done by Garima Gautam et al[7] and Aarti Sangale et al[8] had almost similar prevalence of *Acinetobacter* i.e. 33.02% and 38.7% respectively , however they differ in prevalence of other organisms such as *Pseudomonas* , *Klebsiella* and *Staphylococcus aureus*. In two other studies conducted by Tuhina Banerjee et al[9] and Tanvir Kaur et al[10] , prevalence of Acinetobacter was little higher (42.9% & 42% respectively) than the present study which could be due to geographical & climatic differences favouring longer survival of organism, inadvertent use of higher antibiotics and larger sample size in their studies. Overall , it was observed that *Acinetobacter* had high prevalence among ICU patients owing to the fact that most of these patients were on mechanical ventilators , had endotracheal tube in situ , longer ICU stay and presence of underlying comorbid illness which made them vulnerable target for this notorious nosocomial pathogen. In the present study , it was evident that there was higher prevalence of *Acinetobacter* species in the age group of 41-60 yrs.(48%) which was similar to the study done by A S Chauhan et al (47.5%)[11].Studies conducted by M V Yadav et al[12] & M Banumathy et al[13] also showed higher preponderance of *Acinetobacter* infections in the same age group, though the prevalence was a little lower (38% and 39% respectively) than the present study which could be due to differences in sample size. Higher prevalence in this age group may indicate that age-related physiological changes and comorbid conditions increase susceptibility to *Acinetobacter* infections. It was not at all surprising to see male predominance in the age group of 21- 40 yrs. & 41-60 yrs. in the present study which showed male/female ratio of 2:1. It was concordant with the studies done by M V Yadav et al (3:1)[12] and M Banumathy et al (3:1)[13].Although study done by A S Chauhan[11] showed higher male/female ratio(4:1). Higher prevalence of *Acinetobacter* infections in males in all these studies could be due to differences in health seeking behaviour and proneness to smoking.

In the present study , percentage of OXA-Carbapenemase producing *Acinetobacter* isolates was 42% which was similar to the studies done by V Sharma et al (48%)[14]. However, it was in contrast with the study conducted by MV Yadav et al [12], which showed OXA-carbapenemase production in only 7% isolates. Higher resistance to Carbapenems might be due to inadvertent use of higher antibiotics and failure to follow local antibiogram in the respective hospital.

CONCLUSION

The present study showed the increasing trend of OXA-carbapenemase producing multi drug-resistant *Acinetobacter* isolates in various ICUs in south-eastern Rajasthan. Timely detection of such isolates would be very helpful in guiding clinicians to start early and appropriate treatment of patients as well in forming antibiogram for the hospital. It will also aid in preventing evolution and spread of more resistant strains.

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Conflicts of interest: There are no conflicts of interest.

BIBLIOGRAPHY

1. Louis B Rice, Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE, *The Journal of Infectious Diseases*, Volume 197, Issue 8, 15 April 2008, Pages 1079–1081.
2. Manchanda V, Sanchaita S, Singh NP. Multidrug resistant Acinetobacter. J. Glob. Infect. Dis. 2(3), 291 (2010).
3. Chusri S, Chongsuvivatwong V, Rivera JI et al. Clinical outcomes of hospital-acquired infection with Acinetobacter nosocomialis and Acinetobacter pittii. Antimicrob. Agents Chemother. 58(7), 4172-4179 (2014).
4. Maragakis LL, Perl TM. *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. *Clin Infect Dis.* 2008;46(8):1254–1263. doi: 10.1086/529198.
5. Rehman U, Dolma KG, Singh TS. Comparative analysis of various phenotypic methods for the detection of beta-lactamase in *Acinetobacter baumannii* isolates from a referral hospital in Sikkim, India. *IJBAMR* 2017;6:226–233. [Google Scholar] [Ref list].
6. Dasgupta S., Das S., Chawan N. S., Hazra A. Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. *Indian Journal of Critical Care Medicine.* 2015;19(1):14–20. doi: 10.4103/0972-5229.148633.
7. Gautam, Garima, et al. "Insight into the Burden of Antimicrobial Resistance among Bacterial Pathogens Isolated from Patients Admitted in ICUs of a Tertiary Care Hospital in India." *Canadian Journal of Infectious Diseases and Medical Microbiology* 2024.1 (2024): 7403044.
8. Sangale A, Vivek B, Kelkar R, Biswas S. Microbiology of Ventilator-associated Pneumonia in a Tertiary Care Cancer Hospital. Indian J Crit Care Med. 2021 Apr;25(4):421-428. doi: 10.5005/jp-journals-10071-23790. PMID: 34045810; PMCID: PMC8138642.
9. Banerjee T,Mishra A, Das A, Sharma S,Barman H,Yadav G.High prevalence and endemicity of Muktidrug resistant Acinetobacter spp. In intensive care unit of tertiary care hospital, Varanasi,India.J Pathog.2018;2018(2):9129083.
10. Kaur, T. A. N. V. I. R., et al. "Prevalence and drug resistance in Acinetobacter sp. isolated from intensive care units patients in Punjab, India." *Asian J Pharm Clin Res* 11.14 (2018): 88-93.
11. Chauhan, Akanksha Singh, R. Sujatha, and Nashra Afaq. "Isolation and identification of Acinetobacter species from various clinical samples with special reference to its antibiotic susceptibility pattern at tertiary care hospital, Kanpur, Uttar Pradesh."
12. MV Yadav, GS Karande, SN Karande, SR Patil, S Sharma. Prevalence of Extended-spectrum Beta-lactamase (ESBL), Metallo Beta-lactamase (MBL) and Carbapenemase Producing *Acinetobacter* Species Isolated from Various Clinical Samples in Tertiary Care Hospital. *J Pure Appl Microbiol.* 2024;18(1):528-541. doi: 10.22207/JPAM.18.1.36.
13. Moolchandani, Kailash, et al. "Antimicrobial resistance surveillance among intensive care units of a tertiary care hospital in Southern India." *Journal of clinical and diagnostic research: JCDR* 11.2 (2017): DC01.
14. Vijeta Sharma, Rajni Sharma et al. "Phenotypic Characterization of Acinetobacter spp. Isolated from Ventilator-Associated Pneumonia in an Intensive Care Unit of Tertiary Care Hospital." *International journal of medical research and health sciences,*2021,10(4):156-163.