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Research Article

Acinetobacter spp

A Study on Acinetobacter spp isolated from various clinical samples and analysis of their susceptibility pattern at a tertiary care centre

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Background: Among the gram-negative bacterial infections, Acinetobacter spp infections are of critical importance given the severity of infections, intrinsic resistance to most antibiotics, and also capability to acquire new drug resistance. **Aim:** To study the prevalence of Acinetobacter spp and its antibiotic resistance patterns in a tertiary care hospital. **Materials and methods:** A retrospective study, including samples from various departments, submitted to the microbiology laboratory from January 2020 to December 2020. All the samples were processed according to standard conventional methods, and the Acinetobacter isolates were studied in detail about the demographic characters, speciation, and antibiotic susceptibility pattern. **Results:** Out of the 1242 positive isolates, 7.24 % were identified as Acinetobacter species; 90% were A. baumannii. Males were most common, most commonly belonging to 45-65 yrs. Out of all the recovered Acinetobacter spp, 30% were from ICU. 40% of the isolates were MDR, 74.07% from ICU and 26.98% from non-ICU settings were statistically significant ($p < 0.005$). Cephalosporins showed the highest resistance to the isolates Ceftazidime 82.5%, followed by Gentamicin 73.3%. **Conclusion:** The present study showed that Acinetobacter spp prevalence and antibiotic resistance (MDR) are more common in ICUs. The emergence of carbapenem resistance in more than half the isolates is worrisome. The study suggests susceptibility testing to be done to help select the best antimicrobial drugs for therapy, thus helping in judicial use of antibiotics is necessary to prevent the emergence of antimicrobial resistance.

Keywords: Non-fermenters, ICU (Intensive care units), Drug resistance

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Introduction

Gram-negative bacterial (GNB) infections are one of the most crucial health problems not only in the community but also in hospitalized patients. Due to the Lipopolysaccharide layer (LPS), GNB's, are known to cause sepsis at a higher rate and hence increased morbidity and mortality of patients [1].

Two large groups, Enterobacteriaceae and the non-fermenters, are responsible for most clinical isolates from cases of gram-negative infections [2].

Though the proportion of infection with non-fermenters is less when compared to that of Enterobacteriaceae, non-fermenters are of critical importance given the severity of infections they can cause and intrinsic resistance to most antibiotics [3].

Among the non-fermenters, the *Acinetobacter* and *Pseudomonas* are the two most important clinically significant pathogens. *Acinetobacter spp* is intrinsically resistant to several commonly used antibiotics, including aminopenicillins, first, second-generation cephalosporins and chloramphenicol [4].

Apart from the intrinsic property, they have a high capacity to acquire resistance to broad-spectrum β-lactams, aminoglycosides, fluoroquinolones and tetracyclines. They have emerged as a highly troublesome pathogen for many institutions especially in intensive care units (ICUs) globally, due to their immense ability to acquire or up-regulate antibiotic drug resistance determinants [5,6]. Their ubiquitous nature in the ICU environment and inadequate infection control practice has continuously raised.

There is an increase in the incidence of not only *Acinetobacter* infection over the past two decades [7] but also the emergence of MDR *Acinetobacter* and, in a few studies, even pan drug resistance. However, many studies are available giving the prevalence of *Acinetobacter* and also antimicrobial resistance patterns. Resistance rates can vary from person to person, geographic area and hospital setting. This study focuses on studying the prevalence of *Acinetobacter spp* isolated from various clinical specimens and also on identifying the portion of MDR Acinetobacter and comparison of their prevalence in ICU (Intensive care units) to Non-ICU (Non-Intensive care units) in a tertiary care hospital located in Hyderabad.

Materials and methods

Study setting: The study was carried out in a 400 bedded hospital, tertiary care teaching hospital with 4 ICUs (medical, surgical, pediatric and neonatal), located in Hyderabad, Telangana.

Type of study & methodology: This is a retrospective study carried out over one year from January 2020 to December 2020. All the samples from various departments submitted to the microbiology laboratory for culture and antibiotic susceptibility during this one year were included in the study. All samples were subjected to routine microscopy, Gram staining and inoculated onto Blood agar and MacConkey agar for primary isolation and incubated aerobically at 37°C for 18-24 hours. Identification of isolates was performed by standard conventional methods based on the colony morphology, preliminaries like gram staining, catalase, oxidase, motility. Various biochemical tests were used to identify genus *Acinetobacter* like indole, citrate utilization test, urease test, triple sugar iron agar test, phenylalanine deaminase test. Identification of *Acinetobacter baumanni* species was made conventionally using specific tests like oxidative/fermentation glucose test, Arginine decarboxylation, and growth at 42°C [8]. Antibiotic susceptibility testing was performed by the Kirby Bauer disc diffusion method on Mueller-Hinton agar plates and interpreted according to the CLSI guidelines [9].

All the *Acinetobacter* isolates were tested for their antibiotic susceptibilities for various classes of antimicrobials using the following antibiotic discs: Cephalosporins (ceftazidime, ceftriaxone), Aminoglycosides, (Gentamicin, Amikacin), Fluoroquinolones (Levofloxacin, Ciprofloxacin), beta-lactam and beta-lactamase inhibitor combination drugs (Ampicillin + Sulbactam, Piperacillin + Tazobactum), carbapenems (imipenem, meropenem). Isolates showing resistance to three or more classes of antibiotics were categorized as (multidrug-resistant) MDR *Acinetobacter spp*. Isolates resistant to all commonly used antibiotics were pan-resistant [10].

Inclusion criteria: The study included all *Acinetobacter species* isolated from various specimens of all ages and different wards, including ICU and non-ICU settings.

Exclusion criteria: specimens with incomplete

Patient demographics, antimicrobial susceptibility testing reports that did not comply with Clinical and Laboratory Standards Institute guidelines (CLSI) were excluded.

All the Data was extracted from WHONET and was statistically analyzed using Epi-info version 7. Chi-square test and descriptive statistics were used to calculate the prevalence of *Acinetobacter species* and also the prevalence of MDR *Acinetobacter* in ICU and non-ICU settings. *P-values* less than 0.05 were considered statistically significant.

Ethical consideration: Institutional ethical committee approval was taken to conduct this study.

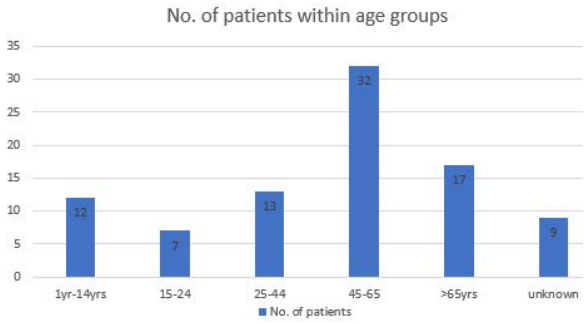
Results

A total of 9,840 non-repetitive samples from various wards and ICU's [medical (MICU), surgical, pediatric, neonatal ICU's) were received in the Department of Microbiology, ESIC Medical College, Hyderabad, from January 2020 to December 2020, for culture and antibiotic susceptibility testing. Out of the 1242 positive isolates, 90 (7.24 %) were identified as *Acinetobacter* species. Among the 90 isolates, 81 (90%) were identified as *A. baumannii* and 9 (16.67%) as other *Acinetobacter* species. The demographic data of the specimens yielding growth of *Acinetobacter spp* showed males as the most common, with a male to female ratio of 2:1. Maximum number 32/90 of *Acinetobacter* isolates were recovered from 45-64 years age group (figure 1).

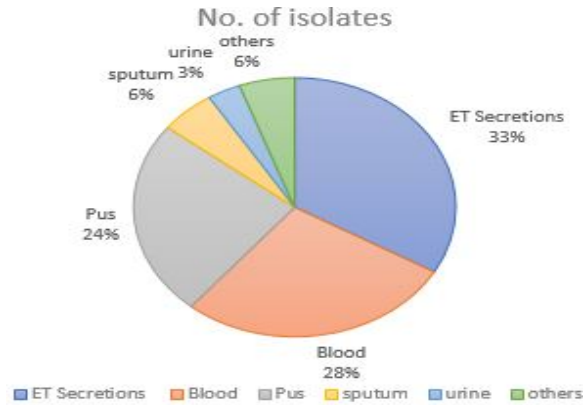
The distribution of various samples which yielded *Acinetobacter* isolates is discussed in fig 2, with endotracheal secretions 33% being the significant proportion. Other specimens are blood 25/90 (28 %), pus (24%), sputum (5) (6%), urine (3), and others 6%. (Graph 2).

Out of all the *Acinetobacter Spp* that were recovered, 30% (27/90) were from ICU.

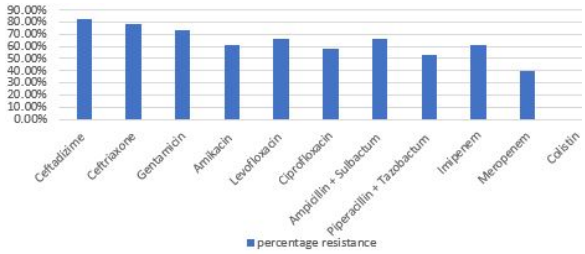
Antibiotic susceptibilities for the isolates are as per CLSI depicted in **figure 3.** Overall resistance percentages of *Acinetobacter Spp* for various classes of antibiotics the resistance patterns are as described. Cephalosporins had the highest resistance to the isolates (ceftazidime 82.5% is more resistant than ceftriaxone 78%), Aminoglycosides, (Gentamicin 73.3% slightly higher Resistance than Amikacin (61.4%) Fluoroquinolones (Levofloxacin 66.4% somewhat more resistant than Ciprofloxacin 57.9 %. When tested for beta-lactam and beta-lactamase inhibitor combination drugs, isolates showed slightly more resistance to Ampicillin + Sulbactam (66.7%) than Piperacillin + Tazobactum (52.8%). Nearly more than half of the isolates showed resistance to carbapenems. More number of isolates were resistant to imipenem (61.4%) as compared to meropenem (40%). Almost all the isolates showed resistance to any one of the classes of antibiotics mentioned above. Isolates showing resistance to three or more classes of antibiotics were categorized as MDR *Acinetobacter spp.* Isolates resistant to all commonly used classes of antibiotics except Polymyxin B were classified as pan-resistant. Out of 90 isolates, 36 (40%) were MDR. Because of MDR *Acinetobacter*, ICU isolates (74.07% %) and (26.98%) in non-ICU settings were MDR and were statistically significant ($p < 0.005$). Among all the isolates, 6 were pan-drug resistance from ICU and one from non-ICU. 18.5% of isolates from ICU are found to pan drug-resistant. (Fig 4). Resistance to colistin was not identified in the present study.



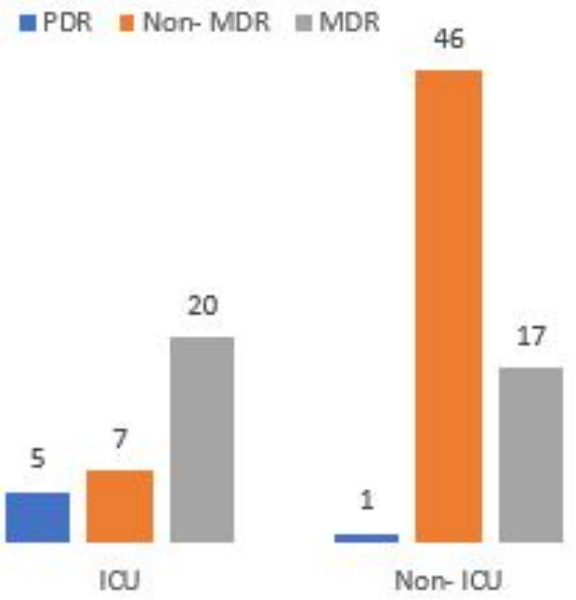
Graph 1: Age-wise distribution of study population.



Graph 2: Percentage distribution of various clinical specimens which yielded Acinetobacter.



Graph 3: Resistance percentage of *Acinetobacter sps* to various antibiotics.



Graph 4: Distribution of MDR & PDR *Acinetobacter* among ICU and Non-ICU.

Discussion

Acinetobacter spp accounts for 7.24 % of all the positive cultures obtained from various specimens, similar to studies from Morocco [11] showing 6.94%, an international study compiling data of 75 countries [12]. showing (8.8%), studies from Rajasthan [13]. (6.42%) and Nepal [14] showing 11.4%, whereas studies from Punjab [15]. 9.6%. Among the 90 isolates, 81 (90%) were identified as A. baumannii and 9 (16.67%) as other Acinetobacter species similar to other studies where Acinetobacter baumanni was the most common species [16,17]. Kaur et al (91.6%) [15]. whereas W. Nageeb et al. [18] proved that A.baumannii was the only species isolated in clinical samples.

Out of the specimens that yielded Acinetobacter, 64.4% were from males, similar to studies by Uwingabaye et al. [11] and Ayenew Z et al. [19], showing 64.4 % & 60% respectively

And is in contrast to a study by Kaur et al. [15] where there is a female preponderance. The predominance of male patients infected with Acinetobacter has also been shown in other studies [20, 21]. The maximum number of isolates, 35.5%, were recovered from the 45-64 years age group, similar to a study by Uwingabaye et al. [11] with a similar age group of 42-68 years. In contrast, a study by Ayenew Z et al. [19] had a slightly younger population with a mean age of 30.79 as the most affected group. Samples that yielded Acinetobacter isolates were mostly 33% endotracheal secretions followed by blood 28%, similar to a study done by Uwingabaye et al. [11] and Rajesh et al. [13], where all the studies showed ET secretion as the major proportion. On the other hand, Kaur et al. [15] had shown urinary isolates to be the most common, in contrast to the present study. In the present study, 30% of the isolates were recovered from ICU patients. In contrast, a lower percentage, 11.4%, were isolated from ICU, as reported by Kaur et al. [15], and a higher percentage was reported by Rajesh et al. [13] Xia et al. [22]. and Tahseen and Talib [23]. The variation in these percentages could be due to the varying prevalence rates of Acinetobacter spp in different hospital settings and geographical areas. In the present study higher percentage of 82.5% of resistance was shown to the cephalosporin group of drugs which is similar to Kaur et al. [15]. and Saha S et al. [24]. About 73.3% of isolates showed resistance to aminoglycosides which is similar to studies by Raj kumari et al. [14]. 74.6%, and Taneja et al (72.8%) [25]. Levofloxacin is slightly more resistant than Ciprofloxacin 57.9 %, which is similar to Kaur et al. [15]. Though carbapenem is considered the treatment of choice, more than half of the isolates, 55.6%, were resistant to carbapenems in the present study, which is less when compared to studies showing (80%) by De Francesco MA et al. [26]. And (75%) by Shareek et al. [27]. The lower percentage could be due to the lesser proportion of patients from ICU in our study.

Out of 90 isolates, 36 (40%) were MDR similar to Dash et al. [28]. showing 54 %, De Francesco MA et al. [26] showing (54%), whereas studies from Kaur et al. [15]. 73.24%, Raj kumari et al [14]. 78. 6%, Saudi Arabia (74%) (29), from USA (30) 71. 6% and Bosnia and Herzegovina (78.4%) [19]. More isolates from ICU (74.07% %) were MDR than from non-ICU 26.98%. A similar trend

Of a higher percentage of MDR Acinetobacter in ICU was seen in a study by Uwingabiye J et al., showing 92.6% in ICUs and 75.3% and non ICU [11]. Among all the isolates, 6.6 % were pan-drug resistance, in the present study similar to Kaur et al. (8.5%) [15]. On the other hand, pan-resistant A. baumannii were not isolated in a study by Rajesh et al. [13]. Resistance to colistin was not identified in the present study, similar to Kaur et al. [15], Raj kumari et al. [14], Raina et al. [31], whereas Rajesh et al. [13]. identified (0.87%) colistin resistance.

Conclusion

Various studies have indicated the role of Acinetobacter spp as a nosocomial pathogen, especially in ICUs, and have intrinsic resistance to many antibiotics apart from the capability to acquire them. Their prevalence varies depending on the geographical area and hospital setting. The present study has also shown that Acinetobacter spp is more common in the ICUs compared to the wards, and also the prevalence of MDR Acinetobacter spp. In the present study, Acinetobacter spp was most commonly resistant to cephalosporins and aminoglycosides. The emergence of carbapenem resistance in more than half the isolates is worrisome. In our study, colistin was the most sensitive antibiotic against Acinetobacter spp with a resistance rate of zero percent. The study suggests susceptibility testing to be done to help select the best antimicrobial drugs for therapy, according to the hospital setting. Also, judicial use of antibiotics is necessary to prevent antimicrobial resistance. Of course, not to forget the importance of adhering to hospital infection control practices both go hand in hand.

What this study adds to the existing knowledge?

Author's contribution: Wajid: Supervision, correction of the manuscript, **Saranya**: data analysis, **Prasanna**: Collection of data. **Shazia**: Preparation of the manuscript.

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