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Incidence and Susceptibility Pattern of *Acinetobacter* Species among Clinical Isolates in a Tertiary Care Hospital

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A B S T R A C T

Acinetobacter can survive for a long time in hospital environments, and colonizes almost all patients on prolonged hospitalization. Additionally, they are often multi-drug resistant. The present study aims at assessing the incidence and susceptibility pattern of *Acinetobacter* species in a tertiary care center. Retrospective study analyzing1169clinical isolates identified as *Acinetobacter* using Gram's-stained smears, colony characteristics and biochemical reactions. Antibiotic Susceptibility Testing was performed by Kirby - Bauer Disc Diffusion Method, using antibiotics as per latest CLSI guidelines. Most isolates occurred in pus and wound swabs (506), followed by tracheal aspirates and sputum (271) and Bacteremia (249). Additionally, 56.48% isolates of *Acinetobacter* were from patients admitted for over two weeks; these findings were statistically significant These were generally resistant to Cephalosporins (24.70% Sensitive), and relatively susceptible to β-Lactam + β- Lactamase Inhibitor combinations (41.47& Sensitive), and generally susceptible to Tetracyclines (55.87% Sensitive). The present study confirms the multi-drug resistant nature of *Acinetobacter* species, and highlights the correlation between its incidence and duration of hospitalization. Rigorous use of standard precautions and minimizing hospital stay would reduce infection, and consequently, antibiotic overuse, which contributes further to drug resistance. In addition, a robust antibiotic policy and antibiotic stewardship in the hospital can minimize its incidence.

Introduction

Bacteria belonging to *Acinetobacter* species are widely distributed in the environment. They are often commensals on the skin, mucous membranes and in various secretions of the body (Carrol *et al.*, 2016). It is documented that *Acinetobacter* species plays a significant role in the colonization and infection of patients admitted to hospitals (Sastry *et al.*, 2018). In addition, certain *Acinetobacter* species persist in the environment for several days, even in dry conditions, on particles and dust (Jawad *et al.*, 1998). Consequently, the carriage rate has been found to be higher among hospital staff than the community at large. It is therefore unsurprising that though some rare cases of community-acquired infections have been reported, these bacteria primarily are

nosocomial pathogens. Studies have also shown that *Acinetobacter* can survive on dry surfaces for durations even longer than *Staphylococcus aureus* (Joly-Gillou, 2005). The marked virulence of *Acinetobacter* species is due to its alarming ability to rapidly develop resistance against antibiotics commonly used in intensive care settings, which is further enhanced by its ability to form biofilms (Sastry *et al*, 2018; Bergogne-Berezin E *et al.,* 1996).

Materials and Methods

The present study entailed retrospective analysis of clinical isolates of *Acinetobacter* species, arising from any type of sample received in the Bacteriology Laboratory, for a period of 36 months from January 2018 to December 2020. The data was collected using WHONET 2020 software.

Clinical isolates were inoculated on Blood Agar and MacConkey's Agar; culture plates were incubated overnight at 37ºC. Biochemical tests like Indole, Methyl Red, Voges-Proskauer, Citrate utilization, Urease production, Triple Sugar Iron test and Hugh Leifson's Oxidative - Fermentative Test were put up along with Lysine and Ornithine Decarboxylase, and Arginine Dehydrolase.

Antibiotic Susceptibility Testing was performed by Modified Kirby-Bauer Disk Diffusion Method using 0.5 McFarland turbidity standard. Drugs tested were Amikacin (AMK), Gentamicin (GEN), Tobramycin (TOB), Imipenem (IMP), Meropenem (MEM), Cefepime (FEP), Ceftazidime (CAZ), Ciprofloxacin (CIP), Ampicillin – Sulbactam (SAM), Piperacillin – Tazobactam (TZP), Cotrimoxazole (SXT), Minocycline (MNO), Tetracycline (TCY); conforming to Clinical Laboratory Standards Institute (CLSI) M-100 guidelines published in 2020 (Weinstein *et al.*, 2020).

Results and Discussion

The isolates producing small, circular, smooth, pale yellow to greyish-white non hemolytic colonies on Blood Agar (Photo 2), and pinkish non-lactose fermenting smooth circular colonies on MacConkey's Agar (Photo 3) were considered for further evaluation. Organisms visualized as Gram-negative coccobacilli arranged in pairs and sometimes clusters (Photo 1), which were non-motile with occasional twitching motility, Oxidase negative and Catalase positive, were presumptively identified as *Acinetobacter* species (Bergogne-Berezin *et al.*, 1996) (Fig. 1–3).

Biochemical confirmation was obtained by a Negative Indole, Methyl Red and Voges-Proskauer tests, ability to utilize Citrate and K/NC reaction without Gas or H_2S production on Triple Sugar Iron Test, along with variable production of Urease, and Oxidative pattern of glucose utilization in Hugh-Leifson's Oxidative-Fermentative Test, and positive Lysine Decarboxylase and Arginine Dehydrolase tests (Figure 4).

Of the 37915 samples received in the period from January 2018 to December 2020, a total of 1169isolates had of *Acinetobacter* species, either singly or as a part of polymicrobial infection. The incidence in the present hospital came out to be 0.308‰.

Fisher exact test was applied to study whether the location of admitted patient affected the isolation of *Acinetobacter* species; a p–value of < 0.05 was considered significant. Most of the samples (n=573 of 1169; 49.03%; $p<0.0000001$) were obtained from wards were from those which had housed post-operative patients, like Surgery, ENT, Orthopedics, and Post Natal wards of Obstetrics and Gynecology. Various intensive care units, like Medical, Surgical, Pediatric, and Anesthetic

showed a significant number as well (n = 339 of 1169; 28.99%). Non-operative and non-intensive care medical wards showed comparatively fewer isolates (257 of 1169; 21.98%). The location-wise distribution of isolates is demonstrated in Chart 1.

Fisher exact test was also applied to study whether the site of sample collection affected the isolation of *Acinetobacter* species; a p–value of < 0.05 was considered significant. Most samples (n=506 of 1169; 42.28%; p<0.0000001) were pus and wound swabs, suggesting a post-operative state. This was followed by samples from the respiratory tract – tracheal aspirates and sputum, (n=271 of 1169; 23.18%; p<0.0000001) indicating prolonged hospital stay and possible invasive procedures like endotracheal intubation. This was then followed by samples for blood culture (n = 249 of 1169; 21.30%), indicating *Acinetobacter* bacteremia. Urine, especially from catheterized patients, and other fluids formed a small component (n = 143 of 1169; 12.23%). The sample-wise distribution is provided in Chart 2.

As this study trails over a period of three separate years, the guidelines as to the antibiotics to be tested varied from edition to edition of the CLSI manual. The sensitivity pattern of antibiotics mentioned in the CLSI-2020M-100 manual has been represented graphically (Weinstein *et al*, 2020). The isolates were generally resistant to Cephalosporins (24.70% Sensitive), and relatively susceptible to β-Lactam + β-Lactamase Inhibitor combinations (41.47& Sensitive), and generally susceptible to Tetracyclines (55.87% Sensitive). The detailed Sensitivity pattern of these isolates is given in Table 1 and Chart 3.

Table.1 Resistance Pattern of Isolates

Class of Drug	Drug	Resistance (%)
Aminoglycosides	Amikacin	62.03
	Gentamicin	61.77
	Tobramycin	58.60
Carbapenems	Imipenem	66.90
	Meropenem	57.90
Cephalosporins	Cefepime	72.73
	Ceftazidime	77.87
Fluoroquinolones	Ciprofloxacin	65.70
Penicillin + Inhibitors	Ampicillin – Sulbactam	58.93
	Piperacillin – Tazobactam	58.13
Sulfonamides	Cotrimoxazole	70.93
Tetracyclines	Minocycline	41.23
	Tetracycline	47.03
(Source: WHONET 2020 Database, Department of Microbiology, GMC, Aurangabad)		
Table 1: Showing the resistance pattern among Acinetobacter isolates. The isolates were most resistant to Cephalosporins (%R = 75.30), relatively susceptible to β-Lactam + β - Lactamase Inhibitor combinations (%R = 58.53), and most susceptible to Tetracyclines (%R = 44.13).		

Table.2 Comparison of resistance patterns

Study	Year	Imipenem	Ciprofloxacin	Amikacin	Piperacillin-Tazobactam	Ceftazidime
Quale *et al*	2003	15%	87%	14%	37%	79%
Lee *et al*	2011	51%	67%	47%	Not analyzed	66%
Chattopadhyay *et al*	2013	35%	55%	44.50%	65%	51.40%
Tripathi *et al*	2014	57%	36%	55.40%	86.92%	100%
Kaur *et al*	2018	65.51%	96.55%	86.20%	89.65%	96.55%
Present study	2020	66.90%	65.70%	62.03%	58.13%	77.87%
(Source: References 6, 9, 13, 14, 16)						

Table 2: Showing the comparison between resistance pattern of Acinetobacter isolates in various national and international studies. The resistance pattern of Ciprofloxacin is variable throughout the board, whereas that of Imipenem along with Amikacin and Piperacillin – Tazobactam is on the rise. There is consistently high to Cephalosporins in all studies.

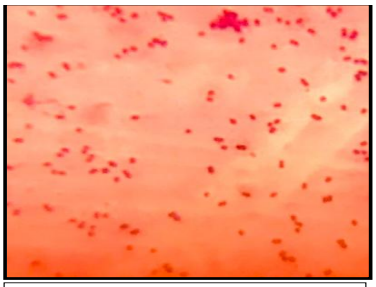


Photo 1 [Original Photo]
Acinetobacter baumannii
(Gram's Stain; 100x)
Gram – Negative Coccobacilli in pairs and clusters

Figure 2 [Original Photo]
Acinetobacter baumannii
(Blood Agar)
Smooth, yellowish, non-hemolytic colonies

Figure3[Original Photo]
Acinetobacter baumannii(MacConkey's Agar)
Pinkish, smooth, non-lactose-fermenting colonies

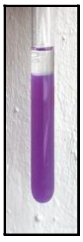
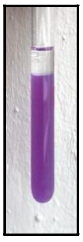
Test	Indole	Methyl Red	Voges Proskauer	Citrate Utilization	Urea Hydrolysis	Triple Sugar Iron	Oxidative Fermentative	Lysine Decarboxylase	Ornithine Decarboxylase	Arginine Dehydrolase
Image										
Result	Negative	Negative	Negative	Positive	Positive	K/NC	Oxidative	Positive	Negative	Positive

Figure 4 [Original Photo]: Showing the biochemical reactions of *Acinetobacter baumannii*

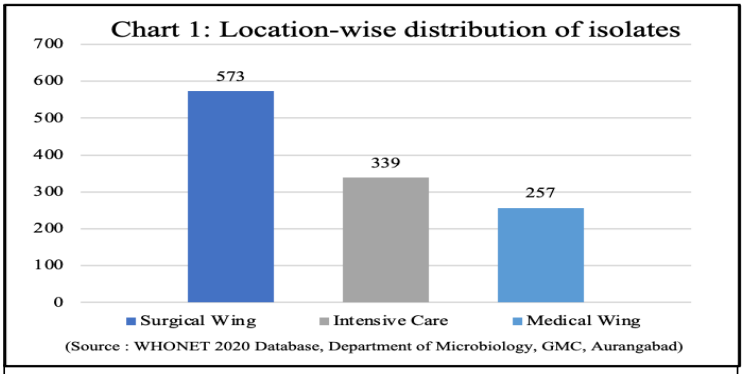


Chart 1: *Showing location-wise distribution of samples received in the Bacteriology Laboratory, which contained Acinetobacter isolates. The maximum number of such samples (573 of 1169) were from surgical wards, whereas non-intensive care medical wards formed a significantly lower proportion (257 of 1169).*

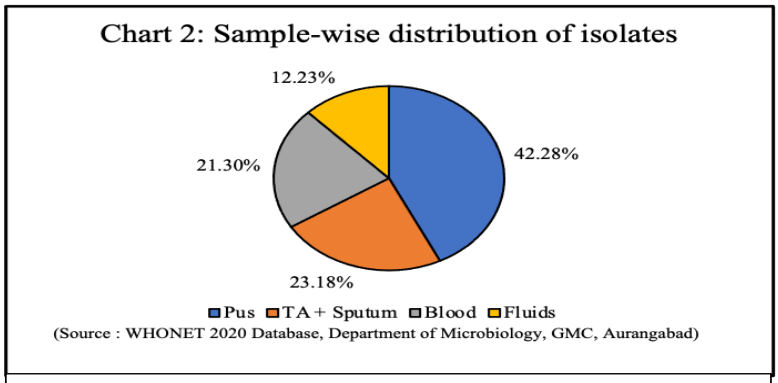


Chart 2: *Showing sample-wise distribution of Acinetobacter isolates. The maximum number of samples were pus and wound swabs (506 of 1169), followed by samples from the respiratory tract (271 of 1169) and blood cultures (249 of 1169).*

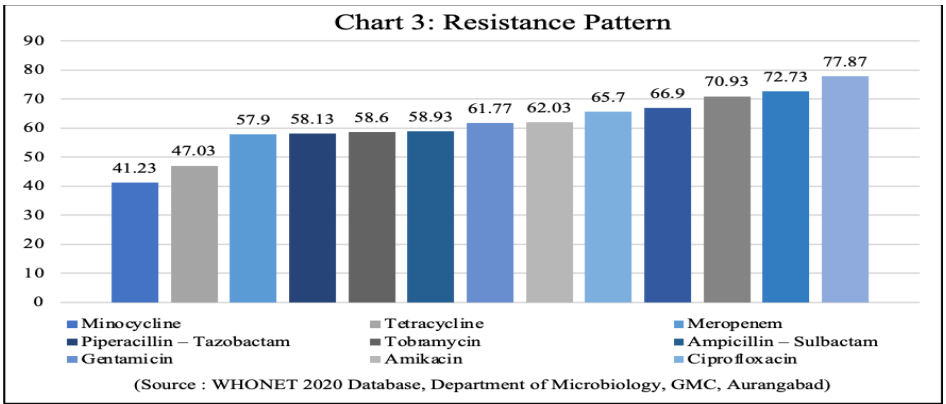
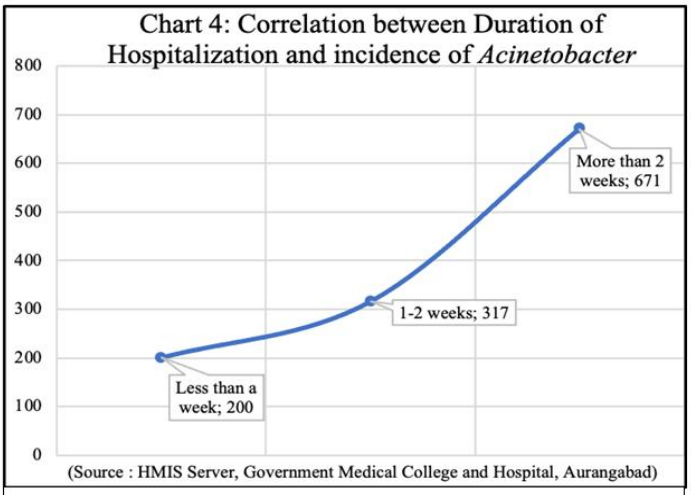


Chart 3: *Showing the resistance pattern of* Acinetobacter *isolates. The isolates were generally resistant to commonly used higher antibiotics, like Cephalosporins (~75% resistant), and showed relatively better sensitivity to Carbapenems (~62% resistant).*

Chart.4 Showing the correlation between duration of hospitalization and isolation of *Acinetobacter* from samples received. Only 200 (16.84%) samples isolated *Acinetobacter* when they were received within one week of admission, while the number rose to 671(56.48%) when samples were sent after two weeks of admission.



Fisher exact test was applied to study whether the duration of hospitalization affected the isolation of *Acinetobacter* species; a p–value of < 0.05 was considered significant. Duration of hospital stay before receiving the first sample which isolated *Acinetobacter* species was also analyzed. It was found that *Acinetobacter* species caused infection as early as the third day of admission. Samples sent within a week of admission begot an isolation of *Acinetobacter* from just 200 samples (16.84%), which increased to 317 samples (26.68%) when samples were sent between 1-2 weeks of admission. The number of samples isolating *Acinetobacter* increased dramatically when samples were sent after two weeks of admission, to 671 (56.48%; $p<0.0000001$). This correlation is represented graphically in Chart 4.

The genus *Acinetobacter* includes Gram-negative coccobacilli that are strictly aerobic, non-motile, Catalase positive, and Oxidase negative. There are seven named species of *Acinetobacter* causing human infections, i.e., *A. baumannii*, *A. lwoffii*, *A. calcoaceticus*, *A. haemolyticus*, *A. junii*, *A. johnsonii*, and *A. radioresistens* (Bergogne-Berezin *et al.*, 1996). Of these *A. baumannii* and *A. lwoffii* are the species isolated most frequently from clinical specimens (Jawad, *et al.*, 1998; Carroll *et al.*, 2016).

In recent years, nosocomial infections by multi-drug resistant *A. baumannnii* have been demonstrated to be associated with increased morbidity and mortality. Though defined variably throughout literature, organisms belonging to *Acinetobacter* species that areresistant to more than three classes of drugs is defined as multi-drug resistant (Tripathi PC *et al.*, 2014). It has subsequently been proven that prolonged hospitalization, ICU stay, invasive procedures, and prior exposure to broad-spectrum antibiotics, especially carbapenems, fluoroquinolone, and third generation cephalosporins, increases the risk of infection with multi-drug resistant *Acinetobacter* (Perez *et al.*, 2007). It mainly causes wound infections and pulmonary infections in intubated patients, and localized infections are known to progress to bacteremia (Karlowsky *et al.*, 2003).

In the present study, 1169 *Acinetobacter* strains were isolated from processed clinical isolates (Incidence 0.308 ‰). This turned out to be almost thrice when compared nationally with Tripathi *et al.,* (2014) who found the rate to be 0.102‰ and internationally with Houang *et al.,* (2001) who reported a total of 0.132‰.

The location of admitted patient affected the isolation of *Acinetobacter* species significantly, where 49.03% samples ($p<0.0000001$) were received from wards were from those which had housed post-operative patients, like Surgery, ENT, Orthopedics, and Post Natal wards of Obstetrics and Gynecology. Additionally, 42.28% samples isolating *Acinetobacter* were pus and wound swabs, while 23.18% were tracheal aspirates and sputum ($p<0.0000001$). This serves to indicate that a significant correlation between isolation of *Acinetobacter* from post-operative patients, and those having probably undergone invasive procedures like endotracheal intubation. Significant correlation was also found between duration of hospitalization and isolation of *Acinetobacter* in samples received from such patients. Of the total samples isolating *Acinetobacter*, 56.48% were from patients admitted for over two weeks ($p<0.0000001$). This significant confirms the primary role of *Acinetobacter* as a nosocomial pathogen. This correlation is represented graphically in Chart 4.

Tetracyclines form the most effective treatment against this species. Resistance against Imipenem is on the rise. Quale *et al.,* (2003) found it to be only 15%, while Kaur *et al.,* (2018), found it to be 65.51%. In the present study, 66.90% isolates were Imipenem resistant. Response of *Acinetobacter* to Fluoroquinolones is variable. Lee *et al* calculated Ciprofloxacin resistance as 67% in 2011, Tripathi *et al.,* (2014) as 36%, and Kaur *et al.,* (2018) found it to be 96.55%. In the present study, 65.70% isolates were Ciprofloxacin resistant. Resistance to Aminoglycosides and Penicillin derivatives is steadily increasing. Quale *et al.,* (2003) noted the resistance to Amikacin and Piperacillin-Tazobactam to be 14% and 37%, respectively, whereas Kaur *et al.,* (2018) found it to rise to 86.20% and 89.65%, respectively. In the present study the resistance to Amikacin and Piperacillin-Tazobactam was 62.03% and 58.13%, respectively. *Acinetobacter* has historically shown high degree of resistance to Cephalosporins. Quale *et al.,* (2003) found 79% resistance to Ceftazidime while Tripathi *et al.,* (2014) reported it to be 100%. In the present study, 77.87% isolates were Ceftazidime resistant. Resistance pattern of various studies carried out in India and overseas is summarized in Table 2.

In conclusion, organisms belonging to the *Acinetobacter* species are primarily opportunistic pathogens, though, the last two decades have seen them get established as formidable pathogens in hospital settings (Chattopadhyay R *et al.,* 2013). Their physical toughness, adaptability, and persistence in the hospital environment have further increased their virulence. The present study confirms the multi-drug resistant nature of *Acinetobacter* species. It also highlights the correlation between the duration of hospitalization and incidence of *Acinetobacte r*infection, and the plethora of nosocomial infections caused by it. Stringent safety precautions and minimizing hospital stay would aid in reducing infection and consequently, antibiotic overuse which contributes further to drug resistance. In addition, presence of a robust antibiotic policy and antibiotic stewardship in the hospital is essential to minimize its incidence, and to choose empirical antibiotics in suspected cases.

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