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STUDY OF MULTIDRUG RESISTANT ACINETOBACTER SPECIES FROM CLINICAL ISOLATES AND ITS RISK FACTORS

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ABSTRACT

Background: Acinetobacter species, particularly A. baumannii, is emerging as significant nosocomial pathogens worldwide. These pathogens have a great propensity to develop multidrug resistance (MDR). These Gram-negative, non-fermenting coccobacilli cause diverse infections particularly in critically ill patients, posing major treatment challenges due to limited antimicrobial options.
Materials and Methods: A hospital-based, observational study was conducted over 18 months (January 2022–June 2023) in a tertiary care center. A total of 3,048 clinical specimens were analysed out of which 82 (2.69%) Acinetobacter isolates were recovered and identified using standard microbiological procedures. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method per CLSI guidelines. Clinical data including demographics, risk factors, and prior antibiotic usage were collected. Risk factor analysis for MDR was performed using Chi-square and logistic regression tests.
Results: Out of 82 isolates 51 (62.19%) were from general wards and 31 (37.80%) belonged to intensive care unit. A. baumannii was the most common species (76.82%) detected. Major infections included septicemia (39.02%), abscesses (24.39%) and urinary tract infections (14.6%). Prolonged hospital stay (>7 days), invasive procedures, prior surgery and diabetes were found to be risk factors associated with MDR infections. High resistance was seen for cephalosporins and fluoroquinolones. MDR was noted in 85.36% of isolates, with A. baumannii showing significantly higher resistance to multiple drug classes (96.77% MDR).
Conclusion: MDR Acinetobacter (Particularly A. baumannii) presents a critical therapeutic challenge in both ICU and general ward settings. Strict infection control, antimicrobial stewardship and antibiotic therapy based on susceptibility patterns are imperative to fight the growing antibiotic resistance threat.
Keywords: Acinetobacter baumannii, Drug Resistance, Multiple, Bacterial, Intensive Care Units, Nosocomial Infections, Anti-Bacterial Agents.

INTRODUCTION

Occurrence of multidrug resistant pathogens in hospital environment is increasing worldwide and limiting the therapeutic options for clinicians. Reason underlying development of resistance among pathogenic organisms against antibiotics may be non judicious and overuse of many antibiotics which has the roots in inherent inclination of clinicians towards prescribing the potent antibiotics.[1]
Acinetobacter spp. is Gram Negative, strictly aerobic, non-fastidious, nonfermenting encapsulated coccobacilli causing mostly nosocomial infections. According to most recent scientific literature, Acinetobacter spp. are the second most common non fermenting Gram negative pathogen isolated from clinical samples after Pseudomonas aeruginosa.[2]
There are many species in this genus, but only three species i.e. A. baumanni, A. caloaceticus and A. lowffii appear to be of clinical importance. These species have been included under the term A. calcoacetius-A.baumanni complex & are usually reported as Acinetobacter. The resistance mechanisms in Acinetobacter are multiple. They

include production of beta-lactamases, alteration in cell wall channels and efflux pumps by which the organism becomes resistant to beta-lactam antibiotics; production of aminoglycoside modifying enzymes and mutations in genes gyrA and parC mediate resistance to aminoglycosides and quinolones respectively.[3]

Acinetobacter spp. are opportunistic pathogens and recently reported to cause a number of outbreaks of nosocomial infections in hospital patients like septicemia, pneumonia, wound sepsis, endocarditis, meningitis, urinary tract infections and peritonitis.[4] Predisposing factors for Acinetobacter infections include presence of prosthesis, endotracheal intubation, intravenous catheters and prior antibiotic therapy in a seriously ill patient in hospital.[5]

Resistance to all known antibiotics has now emerged in Acinetobacter spp. with the majority of strains still being susceptible to carbapenem and colistin. Treatment options are severely limited;More research and greater emphasis on the prevention of health-care associated transmission of MDR Acinetobacter infection are very essential.[6]

Rapid, accurate analysis of antimicrobial susceptibility will be useful in determining the precise use of antimicrobial agents. Hence, clinical input from a microbiologist is necessary to keep one step ahead in controlling nosocomial infections. Periodic surveillance by molecular typing of isolates from patients is recommended for early detection of an epidemic strain, which consequently serves as an effective control measure.[7]

The present study was undertaken to focus on antimicrobial resistance pattern of Acinetobacter species isolated from various clinical specimens of patients admitted and attending the various clinical departments of a tertiary care institute and evaluation of associated risk factors for acquisition of these pathogens, in the advent of rapidly emerging multi drug resistant isolates of Acinetobacter species worldwide.

MATERIALS AND METHODS

This was a hospital-based, observational study conducted in the Department of Microbiology. The duration of study was 18 months from January 2022 to June 2023. The study was conducted after obtaining approval from the Institutional Ethics Committee (IEC). The study included both inpatient and outpatient departments of a tertiary care hospital. The sample size was calculated on the basis of expected prevalence of multidrug-resistant Acinetobacter species , considering a 95% confidence interval. Using the formula for sample size estimation for proportion studies, $n = Z^2 \times p \times (1 - p) / d^2$, where $Z = 1.96$ for 95% confidence, $p = 0.5$, and $d = 0.062$, the minimum required sample size was estimated to be 70. During study period 82 (2.69%) Acinetobacter strains were isolated. The sample size was more than the minimum sample size required for study.

A total of 3048 clinical specimens including sputum, blood, pus, urine, cerebrospinal fluid (CSF), and peritoneal fluid were collected as per standard specimen collection protocols. 82 Samples with culture positivity for Acinetobacter spp. were further processed. All samples were transported and processed immediately in the microbiology laboratory.

Specimens were cultured on 10% sheep blood agar and MacConkey agar and incubated at 37°C for 18-24 hours. Colonies suspected to be Acinetobacter were further analyzed. Colonies on blood agar were cream to white, 0.5–2 mm in diameter, smooth, convex with entire margins. On MacConkey agar, colonies were non-lactose fermenting and often had a faint pink tint.

Presumptive identification of Acinetobacter spp. was based on Gram staining and standard biochemical tests. Isolates that appeared as Gram-negative coccobacilli resembling tiny diplococci were subjected to confirmatory testing. If the isolates were found to be non-motile by hanging drop method, catalase positive, oxidase negative, and showed negative results for nitrate reduction, indole, and methyl red tests, with positive citrate utilization and variable urease activity, they were considered to be Acinetobacter spp.

Antimicrobial Susceptibility Testing (AST) was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included aminoglycosides, fluoroquinolones, cephalosporins, carbapenems, and polymyxins. Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Data on patient demographics, clinical history, comorbidities, prior antibiotic usage, ICU stay, mechanical ventilation, invasive procedures, and catheterization were collected using a structured proforma. Risk factors associated with the isolation of multidrug-resistant (MDR) Acinetobacter spp. were analyzed.

All data were compiled and analyzed using SPSS version 23.0. Descriptive statistics were applied to summarize demographic and clinical data. Chi-square test and logistic regression analysis were used to identify significant risk factors for MDR Acinetobacter spp. A p-value less than 0.05 was considered statistically significant.

Inclusion Criteria

- All culture-positive specimens for Acinetobacter spp.

Exclusion Criteria

- Specimens with incomplete clinical data.

RESULTS

Out of the total 3,048 samples processed, 82 (2.69%) Acinetobacter strains were isolated. Out of the 82

isolates, 51(1.88%) isolates were from general wards and 31 (9.19%) were from intensive care units. Significantly higher percentage of Acinetobacter strains were found in ICU compared to general wards.(P <0.05) [Table 1].

Table 1: Distribution of specimens and Acinetobacter isolates

	No. of Specimens	No. of Acinetobacter isolates (%)
General wards	2711	51(1.88)*
ICU	337	31(9.19)*
Total	3048	82(2.69)

Acinetobacter strains were predominantly isolated from blood 32 (39.02%), followed by pus 20 (24.39), urine 12(14.6%), endotracheal tube 9 (10.97%), wound swab 6(7.31%).singe acinetobacter strains (1.21%) were isolates from pleural fluid, pleural fluid, CSF and sputum [Table 2].

Table 2: Sample-wise Distribution of Acinetobacter isolates from ward and ICU.

Sr. No	Specimens	No. of Acinetobacter isolates (%)	WARD	ICU
1	Blood	32 (39.02)	26(50.98)	06(19.36)
2	Pus	20 (24.39)	09(17.64)	11(35.48)
3	Urine	12 (14.6)	09(17.64)	03(9.67)
4	Endotracheal tube	09 (10.97)	01(1.96)	08(25.80)
5	Wound swab	06 (7.31)	05(9.8)	01(3.22)
6	Pleural fluid	01 (1.21)	0	01(3.22)
7	Ascitic fluid	01(1.21)	01(1.96)	0
8	Csf	01 (1.21)	0	01(3.22)
9	Sputum	01 (1.2)	01(1.96)	0
		82 (100)	51(62.19)	31(37.80)

21 (25.66%) Acinetobacter strains were isolated from paediatric ward, 15(18.29%) from general medicine, followed by intensive care unit which included MICU 13(15.85%), SICU 11(13.41%),NICU 7(8.53%) whereas lowest number of isolates from respiratory unit 1(1.21%). In general ward Septicemia was the commonest infection (50.98%), followed by abscess (17.64%), urinary tract infection (17.64%) and wound infection (9.80%). In intensive care unit maximum number of Acinetobacter strains caused abscess (35.48%), followed by ventilator associated pneumonia (25.80%) Septicemia (19.35%) and urinary tract infection (9.67%) [Table 3].

Table 3: Distribution of Acinetobacter infections in general ward and ICU (n=82)

Sr. No	Specimens	No. of Acinetobacter isolates (%)	No. of Acinetobacter isolates from ward (%)	No. of Acinetobacter isolates from ICU (%)
1	Septicemia	32 (39.02)	26(50.98)	06(19.35)
2	Abscess	20 (24.39)	09(17.64)	11(35.48)
3	Urinary tract infection	12 (14.6)	09(17.64)	03(9.67)
4	Pneumonia	09 (10.97)	01(1.96)	08(25.80)
5	Wound infection	06 (7.31)	05(9.8)	01(3.22)
6	Pleural effusion	01 (1.2)	-	01(3.22)
7	Peritonitis	01(1.2)	01(1.96)	-
8	Meningitis	01 (1.2)	-	01(3.22)
	Total	82	51	31

Acinetobacter isolates was more common in males (54.87%) as compared to females (45.13%).It was more common in the age group 51 to 60(21.95%) ,more than 60 age group (17.07%) and 0 to 1 age group (14.63%) [Figure 1].

Out of the total 32 patients with septicemia were associated with risk factors such as extended hospital stay (46.87%), IV catheterization (18.75%). Cases with abscess and wound infection the most common associated risk factor was postsurgical (30.76%), followed by diabetes mellitus (15.38%). Out of all the cases of urinary tract infection, 41.66% were associated with catheterization. In pneumonia cases, mechanical ventilation was a major risk factor [Table 4].

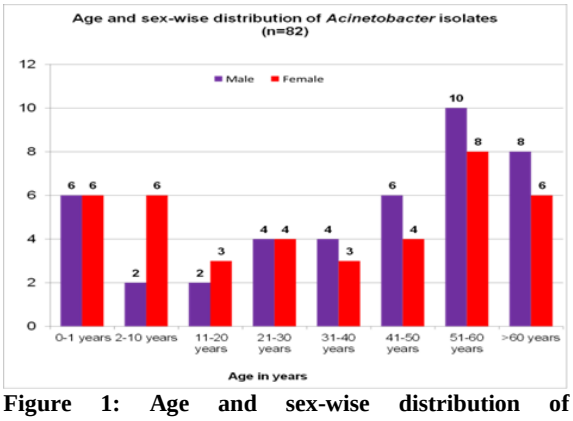

Figure 1: Age and sex-wise distribution of Acinetobacter isolates (n=82)

Table 4: Major risk factors associated with Acinetobacter infections

Acinetobacter infections	Associated risk factors	No. of cases (%)
Septicemia (n=32)	Hospital stay (>7 days)	15(46.87)
	IV catheter	6(18.75)

	Surgery	5 (15.62)
	Diabetes mellitus	2 (9.37)
Abscess and wound infection(n=26)	Postsurgical	12(30.76)
	Trauma	5 (19.23)
	Diabetes mellitus	4 (15.38)
	Previous infection	3 (11.53)
Urinary tract infection (n=12)	Catheterization	8 (41.66)
	Hospital stay (>7 days)	4(33.33)
	Prolonged antibiotic use* (>7 days)	3(25)
Pneumonia (n=09)	Mechanical ventilation	7(77.00)
	Chronic obstructive pulmonary disease	1 (22.22)
Pleural effusion (n=1)	-	-
Ascitis(n=1)	-	-
Meningitis (n=1)	-	-

A. baumannii (76.82%) was the commonest species in Acinetobacter infection in ICUs (90.32%) as well as general wards (68.62%). In ICUs only five Acinetobacter spp. were isolated and in general ward six Acinetobacter spp. were isolated. A. baumannii was the most common species responsible for septicemia(81.25%), abscess (85%), urinary tract infection (83.33%), pneumonia (44.44%) and wound infection (50.00%) [Table 5].

Table 5: Distribution of Acinetobacter species in general ward and ICU.

S. No.	Acinetobacter species	No. of cases		
		General wards (%)	ICU (%)	Total (%)
1	A. baumannii	35(68.62)	27(87.09)	62 (76.82)
2	A. calcoaceticus	6 (11.76)	1 (3.22)	7 (8.53)
3	A. junii	4(7.8)	1(3.22)	5 (6.09)
4	A. lwoffii	3(5.8)	1(3.22)	4(4.87)
5	A. haemolyticus	2 (3.92)	1(3.22)	3(2.43)
6	A. johnsonii	1 (1.9)	-	1(1.21)
Total		51	31	82

A. baumannii (76.92%) was the commonest species to cause septicemia, followed by A. calcoaceticus (11.53%) and A. haemolyticus (3.8%). Abscess was mainly due to A. baumannii (77.77%), followed by A. calcoaceticus and A. junii (11.11% each). Urinary tract infection was mainly due to A. baumannii (66.66%), followed by A. calcoaceticus and A. junii (11.11% each) wound infection was most commonly due to A. baumannii (40.00%) and. Ascites was due to A.haemolyticus. Pneumonia found to be caused by A.lwoffii. Moreover A. baumannii (100%) was the commonest species to cause abscess. ventilator associated pneumonia was mainly due to A. baumannii (50.00%), followed by A. calcoaceticus and A. junii, A. lowffii, A haemolyticus (12.5%). Urinary tract infection were most commonly due to A. baumannii. Wound infection, pleural effusion and meningitis found to be caused by A. baumannii (100% each).

Maximum sensitivity of Acinetobacter was seen to Polymyxin B (91.14%), tigecycline (95.12%), Colistin (87.80%), imipenem (59.00%) and amikacin (63.41%), followed by tobramycin (40.24%). Maximum resistance was observed ceftazidime (100%), Cefotaxime (100%), cefepime (100%) and trimethoprim sulfamethoxazole (86.58%). Imepenam resistance was seen in 56 (78.87%) [Table 6].

Table 6: Antimicrobial sensitivity pattern of Acinetobacter isolates (n=82)

Antibiotic	Sensitive (%)		Resistant (%)	
		Intermediate (%)	Resistant (%)	Total (%)
Ciprofloxacin (CIP)	15(21.12)	0(0)	56(78.87)	56(78.87)
Gentamycin (GM)	26(46.42)	2(3.57)	54(65.85)	56(68.29)
Ceftazidime (CAZ)	0(0)	0(0)	82(100)	82(100)
Imipenem (IPM)	59(71.95)	9(10.97)	24(29.26)	33(40.24)
Tobramycin (TOB)	33(40.24)	4(4.87)	45(54.87)	49(59.75)
Cefotaxime (CTX)	0(0)	0(0)	82(100)	82(100)
Amikacin (AK)	52(63.41)	8(9.75)	22(26.82)	30(36.58)
Tigecycline (TGC)	78(95.12)	2(2.43)	2(2.43)	4(4.86)
Piperacillin- tazobactam (P/T)	28(34.14)	6(7.31)	58(70.73)	62(78.04)
Cefepime (CPM)	014(17.07)	10(12.19)	58(70.73)	68(82.92)
Trimethoprim- Sulfamethoxazole (COT)	11(13.41)	2(2.43)	53(64.63)	55(86.58)
Tetracycline	32(39.02)	5(6.097)	45(54.87)	50(60.97)
Polymyxin B (PB)	75(91.14)	5(6.09)	2(2.43)	7(8.53)
Colistin(CL)	72(87.80)	4(4.87)	6(7.31)	10(12.81)

The antimicrobial resistance pattern among 51 Acinetobacter isolates from general wards revealed significant multidrug resistance, particularly in A. baumannii. Complete resistance (100%) to ceftazidime and cefotaxime was observed across all isolates. High resistance rates were noted for ciprofloxacin (64.70%), gentamicin (66.66%), piperacillin-tazobactam (74.50%), and imipenem (45.09%), predominantly driven by A. baumannii isolates. Colistin (17.14%), polymyxin B (14.28%), and tigecycline (5.71%) demonstrated relatively lower resistance, indicating their continued therapeutic potential. Additionally, multidrug resistance (resistance to ≥3 antibiotics) was significantly higher in A. baumannii isolates (96.77%) compared to other Acinetobacter species (57.14%; P<0.05). Resistance intensified further, with 11.29% of A. baumannii isolates resistant to 12 drugs. This high multidrug resistance profile from general wards closely mirrors patterns typically seen in ICU settings, highlighting the critical threat posed by Acinetobacter infections in both hospital wards and intensive care units. Multiple drug resistance was common among Acinetobacter isolates. There were total 70 (85.36%) Acinetobacter isolates that showed resistance to 3 or > 3drugs, of which 60 (96.77%) were A. baumannii and 4(57.14%) were A. calcoaceticus. There were total 63(76.82%) Acinetobacter isolates (93.54% A. baumannii and 42.85% A. calcoaceticus) which showed resistance to 4or more than 4 drugs. 57 (69.51%) isolates of Acinetobacter showed resistance to 5 or more than 5 drugs of which 88.70% were A. baumannii and 28.57% were A. calcoaceticus. All the Acinetobacter isolates showing resistance for 6 or more than 6, 7 or more than 7 and 8 or more than 8, 9 and more than 9 drugs, 10 and more than 10 drugs and 11 and more than 1 drugs were A. baumannii. There were 7 (11.29%) isolates which showed resistance to 12 drugs. Significantly higher percentage of multidrug resistance was found in A. baumannii strains compared to other Acinetobacter spp (P<0.05) [Table 7].

Table 7: Multidrug resistance in Acinetobacter isolates (n=82)

Multidrug resistant (No.of drugs)	No of resistant isolates						
	A. baumannii*	A. calcoaceticus	A. junii	A. lwoffii	A. haemolyticus	A.Johnsonnii	Total
	n=62 (%)	n=7 (%)	n=5 (%)	n=4 (%)	n=3 (%)	n=1 (%)	n=82 (%)
3 & >3	60 (96.77)	4 (57.14)	3 (60.00)	2 (50)	1 (33.33)	0(0)	70 (85.36)
4 & >4	58 (93.54)	3 (42.85)	1 (20.00)	1 (25)	0(0)	0(0)	63 (76.82)
5 & >5	55 (88.70)	2 (28.57)	0 (0)	0 (0)	0 (0)	0(0)	57 (69.51)
6 &> 6	53 (85.48)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	53 (64.63)
7 & >7	51 (82.25)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	51 (62.19)
8 & >8	33 (53.22)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	33 (40.24)
9 & >9	18 (29.03)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	18 (21.95)
10 & >10	10 (16.12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (12.19)
11 & >11	8 (12.90)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (9.75)
12& >12	7 (11.29)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	7 (8.53)

DISCUSSION

Acinetobacter spp. are Gram-negative, aerobic, non-fermenting coccobacilli increasingly recognized as problematic nosocomial pathogens. These organisms are notably capable of colonizing healthy hosts but predominantly cause serious, often life-threatening, infections in healthcare settings. The organism's propensity for developing multi-drug resistance (MDR) poses significant therapeutic challenges globally.

The prevalence of Acinetobacter in hospital environments, particularly Intensive Care Units (ICUs), has risen significantly, fueled by invasive medical procedures, extensive antibiotic use, and insufficient infection control practices. Acinetobacter infections range from septicemia and ventilator-associated pneumonia (VAP) to wound infections and urinary tract infections. ICU-acquired Acinetobacter infections notably correlate with increased mortality, prolonged hospital stays, and escalated healthcare costs.

In various studies, Acinetobacter accounted for substantial proportions of nosocomial infections. Oberoi et al,[8] reported Acinetobacter in 8.4% of clinical isolates, while Dash et al,[9] and Shridhar et al,[10] documented much lower prevalences, at 3 isolates and 1.23%, respectively. Acinetobacter was particularly frequent in ICUs, aligning with earlier reports by Patwardhan et al,[11] who found 13.23% of isolates in ICUs and Mindolli et al,[12] who documented 27%.

The organism's ability to develop antibiotic resistance is alarming. Resistance is predominantly mediated by β-lactamases (including carbapenemases), aminoglycoside-modifying enzymes, efflux pumps, and mutations in target enzymes for fluoroquinolones. Carbapenem-resistant Acinetobacter baumannii strains, particularly resistant to imipenem, pose serious treatment challenges, with resistance reaching 40.24% in this study. Mushtaq et al,[13] documented high resistance to trimethoprim-sulfamethoxazole (78.58%) and tetracycline (69%), aligning closely with the present study.

The most frequently isolated species in clinical settings was Acinetobacter baumannii, followed by A. calcoaceticus and A. junii. In terms of clinical syndromes, septicemia was predominant, especially in general wards, supported by Vijaya et al,[14]

reporting 40% Acinetobacter septicemia cases. Abscesses and urinary tract infections were also common. The risk factors identified were prolonged hospital stays, invasive procedures, immunosuppression, and indwelling devices like urinary catheters and mechanical ventilation—factors echoed by Lahiri et al and Lone et al.[15,16]

Antimicrobial susceptibility testing indicated alarming resistance levels to β-lactams, particularly cephalosporins and piperacillin-tazobactam, as reported consistently in global studies. Polymyxins (Polymyxin B, Colistin) and Tigecycline retained the highest susceptibility profiles, highlighting their role as last-resort antibiotics. Notably, resistance to aminoglycosides such as gentamicin and amikacin was also substantial, echoing findings from Swenson et al.[17]

Multidrug resistance (MDR), defined as resistance to three or more antibiotic classes, was significantly high, particularly in A. baumannii isolates (85.36%). The extensive drug resistance (XDR) and pandrug resistance (PDR) phenotypes pose severe challenges, limiting therapeutic options and underscoring the necessity for stringent infection control measures and judicious antimicrobial use.

CONCLUSION

Acinetobacter species have become critical nosocomial pathogens, particularly in ICU settings, causing significant infections among high-risk, critically-ill patients. Their persistence in hospital environments and resistance to multiple antimicrobials, including emerging carbapenem resistance, pose major therapeutic challenges. Multidrug-resistant Acinetobacter infections are often managed with limited antibiotics like colistin, polymyxin B, and tigecycline. Key risk factors include age above 50, prolonged hospital stays, prior antibiotic use and invasive procedures. Effective prevention requires rigorous adherence to antimicrobial stewardship, enhanced microbiological diagnostics, continuous surveillance, stringent infection control practices, environmental hygiene and judicious antibiotic use.

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