Section: Microbiology

Original Research Article

ISOLATION OF ACINETOBACTER SPECIES FROM VARIOUS CLINICAL SAMPLES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN AT TERTIARY CARE HOSPITAL, LUCKNOW

Ana Rizvi[1], Sarah Hassan[2], Sana Siddique[2], Shadma Yaqoob[3], Syed Abid Asghar[4]

[1]P.G. Student, Department of Microbiology, Era's Lucknow Medical College & Hospital, Lucknow, Uttar Pradesh, India.
[2]Assistant Professor, Department of Microbiology, Era's Lucknow Medical College & Hospital, Lucknow, Uttar Pradesh, India.
[3]Professor, Department of Microbiology Era's Lucknow Medical College & Hospital, Lucknow, Uttar Pradesh, India.
[4]Assistant Professor, Department of Community Medicine, Era's Lucknow Medical College & Hospital, Lucknow, Uttar Pradesh, India.

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Corresponding Author:
Dr. Syed Abid Asghar,
Email: drsabid@gmail.com

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Abstract

Background: The aim is to isolate Acinetobacter species from various clinical samples and analyze their antimicrobial susceptibility patterns at a tertiary care hospital in Lucknow. Acinetobacter species are associated with healthcare-related infections, particularly in patients using respiratory therapy equipment and indwelling catheters. These pathogens can cause a range of infections, including pneumonia, septicemia, wound sepsis, urinary tract infections, endocarditis, and meningitis. Infections caused by pathogenic Acinetobacter species are becoming a significant threat to human health due to their opportunistic nature. The emergence of resistance to nearly all antibiotics has made Acinetobacter species increasingly important. Once considered a silent bystander, its role in nosocomial infections has now been recognized. **Materials and Methods:** A cross-sectional study was conducted in the Department of Microbiology at Era's Lucknow Medical College and Hospital, Lucknow, from November 2023 to April 2024. Various clinical samples were collected from patients for culture and identification. Antimicrobial susceptibility testing was performed using the Kirby-Bauer Disk Diffusion Method. **Result:** Out of 345 clinical samples from both inpatient and outpatient departments, 61 isolates tested positive for Acinetobacter species. The isolates were predominantly recovered from pus (34%), followed by endotracheal aspirate (31%). The majority of Acinetobacter species were isolated from the ICU (46%), with lower isolation rates from medicine wards (7%) and obstetrics& gynecology (3%). The most predominant species isolated was Acinetobacter baumannii (87%), followed by Acinetobacter lowffii (13%). All Acinetobacter isolates were 100% sensitive to Tigecycline and Colistin. High levels of resistance were observed for Ciprofloxacin, Amoxiclav, Levofloxacin (100%), followed by Amikacin (98.36%), Tobramycin Sulfate (98.36%), Doripenem (98.36%), Imipenem (98.36%), Meropenem (98.36%), Piperacillin-Tazobactam (98.36%), Cefepime Hydrochloride (98.36%), and Ceftriaxone (98.36%). **Conclusion:** Acinetobacter strains exhibited a pattern of multidrug resistance, predominantly among hospitalized patients. Colistin and Tigecycline remain effective treatment options for multidrug-resistant Acinetobacter infections.

INTRODUCTION

Acinetobacter species are small, aerobic, free-living bacteria commonly found in moist environments, and they can easily be isolated from sources such as sewage, water, food, and soil.[1,2] These species are catalase-positive, oxidase-negative, aerobic, non-fermenting, non-fastidious, and non-motile coccobacilli that thrive in damp environments.[3] The most prevalent and clinically significant member of this genus is Acinetobacter baumannii ; other species, such as Acinetobacter lwoffii and Acinetobacter haemolyticus , are less frequently isolated from patients.[1] In intensive care units, Acinetobacter

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species are responsible for nearly 20% of infections, making them one of the most significant opportunistic non-fermenting bacteria associated with hospital-acquired infections.[4] They have emerged as the second most common gram-negative bacteria found in clinical samples, following Pseudomonas aeruginosa.[5]

These organisms can be isolated from a variety of sources, including sputum, pus, wound swabs, tissue, bronchial washings, blood, cerebrospinal fluid (CSF), endotracheal aspirate, and urine.[6] Acinetobacter species have been linked to nosocomial infections, including wound infections, urinary tract infections, septicemia, and ventilator-associated pneumonia (VAP).[7] Risk factors for infections caused by Acinetobacter include prolonged hospital stays, immunodeficiency, surgery, burns, aging, the use of antibacterial agents, and invasive devices.[8] As one of the six pathogens in the "ESKAPE" group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species), Acinetobacter has become increasingly important as an opportunistic pathogen that is difficult to treat.[9] The resistance of Acinetobacter species to nearly all commonly prescribed antimicrobial drugs, such as aminoglycosides, fluoroquinolones, and broad-spectrum beta-lactams, is on the rise. Although most strains are resistant to the cephalosporin class of antibiotics, reports of resistance to carbapenems are becoming more frequent.[10] The antibiotic susceptibility patterns of Acinetobacter can vary significantly across different geographic regions and even within different hospital units over time. Due to these variations, it is essential to regularly monitor these pathogens to select the most appropriate treatment.[11] Acinetobacter infections contribute to increased mortality in critically ill patients, with a significant impact on morbidity and fatality rates. While generally considered a low-pathogenicity bacterium, Acinetobacter poses a serious threat when isolated from severely ill or immunocompromised patients. These organisms are typically associated with nosocomial infections rather than community-acquired diseases.[12]

This study aims to identify the source of Acinetobacter infections and determine the most effective treatment approach.

MATERIALS AND METHODS

A hospital-based cross-sectional study was conducted in the Department of Microbiology at Era's Lucknow Medical College and Hospital, Lucknow, U.P., from November 2023 to April 2024. The study aimed to isolate Acinetobacter species from various clinical samples and analyze their antimicrobial susceptibility patterns. The sample size was calculated using the formula provided by Gupta N et al.[11]

$n = \{Z2\ 1 - \alpha/2\ p\ (1-p)\} / \{d2\}$

$n = 345$

(The calculated sample size was 345)

Data Collection: The study included samples such as pus, urine, blood, sputum, endotracheal aspirates, body fluids, bronchoalveolar lavage (BAL) fluid, and cerebrospinal fluid (CSF). Samples were collected from patients of all age groups and sexes attending both OPDs and IPDs at ELMCH. Stool samples and samples of inadequate volume were excluded.

Sample Collection and Processing: Written informed consent was obtained from each patient prior to the start of the study. Patient information, including age, sex, and ward, was also recorded.

Study Procedure

All samples were collected in sterile, properly labeled, leak-proof, and capped containers using aseptic techniques. Samples were immediately transported to the laboratory and processed within 2 hours. Upon arrival in the bacteriology lab, all samples were subjected to Gram staining. All samples were inoculated using a calibrated wire loop onto Blood agar, MacConkey agar, and CLED agar (for urine samples) and incubated at 37°C for 24-48 hours.

Further identification was based on colony morphology (size, shape, pigmentation, and hemolytic properties) and Gram staining. Speciation was conducted using biochemical tests, including Catalase, Oxidase, Indole, Methyl Red, Urease, Citrate, Triple Sugar Iron (TSI) test, Oxidative-Fermentative (OF) test, and sugar fermentation tests (Glucose, Galactose, Sucrose, Lactose, Maltose, Mannitol, and Mannose). Antimicrobial susceptibility testing of various isolates was performed using the Kirby-Bauer Disk Diffusion Method on Muller Hinton Agar (HiMedia), following the Clinical Laboratory Standards Institute (CLSI) 2023 guidelines.

Ethical Considerations: The study was approved by the Ethical Committee of Era's Lucknow Medical College and Hospital, Lucknow. Participation was voluntary, and written consent was obtained from each patient prior to sample collection. The confidentiality of all patient information and clinical histories was strictly upheld.

RESULTS

The present study was conducted in department of Microbiology in Era's Lucknow Medical College and Hospital. In our study we enrolled 345 clinical samples. Samples were collected from OPD and IPD and sent for bacteriological culture to Microbiology lab.

Table 1: total clinical samples.

Total clinical sample	**345**
Isolated Acinetobacter species	61

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Percentage (%)	18%

Table 2: ward-wise distribution of acinetobacter species

S.NO.	Wards	Isolation (n=61)	Percentage (%)
1.	IPD	57	93%
2.	OPD	4	7%

Table 3: gender-wise distribution of acinetobacter species

S.NO.	Gender	Number (n=61)	Percentage
1.	Male	42	69%
2.	Female	19	31%

Table 4: age-wise distribution of acinetobacter species

S.NO.	Age(year)	Number (n=61)	Percentage
1	0 – 19	8	13%
2	20-39	15	24%
3	40-59	23	38%
4	60-79	14	23%
5	80-99	1	2%

Table 5: distribution of acinetobacter species isolated from various clinical specimens

Specimens	Number (n=61)	Percentage
PUS	21	34%
ENDO-TRACHEAL ASPIRATE	19	31%
BLOOD	3	5%
BAL FLUID	2	3%
URINE	4	7%
SPUTUM	4	7%
TISSUE	2	3%
TIP	4	7%
PLEURAL FLUID	2	3%

Table 6: distribution of isolates in various wards

S.NO.	Ward	Number of isolates (n=57)	(%)
1	ICU	26	46%
2	HIGH DEPENDENCY UNIT (HDU)	15	26%
3	SURGERY WARD	10	18%
4	OBSTETRICS AND GYNAECOLOGY	2	3%
5	MEDICINE WARD	4	7%

Table 7: speciation of acinetobacter isolates

S.no.	Acinetobacter species	No. Of isolates (n=61)	Percentage (%)
1.	Acinetobacter baumannii	53	87%
2.	Acinetobacter lowffii	8	13%

Table 8: AST pattern of acinetobacter isolates

S.no.	Antibiotics	Sensitive		Intermediate		Resistance	
		No.	%	No.	%	No.	%
1	TIGECYCLINE	61	100%	0	0	0	0
2	COLISTIN	61	100%	0	0	0	0
3	NETILMICIN	2	3.27%	0	0	59	96.73%
4	AMIKACIN	0	0	1	1.64%	60	98.36%
5	TOBRAMYCIN SULPHATE	1	1.64%	0	0	60	98.36%
6	DORIPENEM	0	0	1	1.64%	60	98.36%
7	CEFOPERAZONE+SUBLACTUM	5	8.19%	3	4.91%	53	86.88%
8	GENTAMICIN	2	3.27%	0	0.00%	59	96.73%
9	CIPROFLOXACIN	0	0	0	0.00%	61	100.00%
10	IMIPENEM	0	0	1	1.64%	60	98.36%
11	MEROPENEM	0	0	1	1.64%	60	98.36%
12	AMOXICLAV	0	0	0	0	61	100.00%
13	PIPERCILLIN+ TAZOBACTUM	1	1.64%	0	0	60	98.36%
14	CEFEPIME HYDROCHLORIDE	1	1.64%	0	0	60	98.36%
15	DOXYCYCLIN	13	21.32%	0	0	48	78.68%
16	NORFLOXACIN (U)*	0	0	0	0	4	100.00%
17	NITROFURANTON (U)*	0	0	0	0	4	100.00%
18	CEFTRIXONE	1	1.64%	0	0	60	98.36%
19	LEVOFLOXACIN	0	0	0	0	61	100%
*Used only in urine samples							

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DISCUSSION

The present study aimed to isolate Acinetobacter species from various clinical samples and analyze their antimicrobial susceptibility patterns at a tertiary care hospital in Lucknow. Globally, infections caused by Acinetobacter spp. are becoming a significant concern in many healthcare facilities.[13] These bacteria can survive on both dry and moist surfaces and resist common disinfectants, enabling some Acinetobacter spp. to persist in hospital environments.[14]

In this study, 61 (15%) Acinetobacter spp. were isolated from 345 clinical samples [Table 1]. Similar prevalences of 12.9% and 11.49% were reported by Lahiri KK et al,[15] (2015) in Pune, India, and Rajkumari S et al,[16] (2020) at a tertiary care hospital in Chitwan, Nepal, respectively.

In contrast, Madhavi RB et al,[7] (2022) reported a lower prevalence of 8.9%. This variation could be due to differences in study settings, design, isolation methods, sampling techniques, and patient profiles. Different species of Acinetobacter are typically associated with various habitats, including soil, water, sewage, humans, food, and animals.[17]

Out of the 61 isolates of Acinetobacter species, 57 (93%) were obtained from inpatient departments (IPD), while 4 (7%) were from outpatient departments (OPD) [Table 2]. A similar distribution was observed by Yadav MV et al,[17] (2023) where 93% of the isolates were from IPD and 7% from OPD. Various operational risk factors in hospital settings may facilitate the persistence and spread of Acinetobacter spp. Key risk factors identified include mechanical ventilation, ICU admission, underlying chronic debilitating illnesses, and prolonged hospital stays, all of which contribute to the persistence and spread of Acinetobacter spp. in hospitals.

In this study, males (69%) were more frequently affected than females (31%) [Table 3], consistent with the findings of Ahmad S et al,[18] (2023), who reported a male predominance of 67.55%. Similarly, Murugesh K et al,[9] found a male predominance of 54.45% in their study. However, our findings contrast with those of Rebic V et al. (2018) [19], who observed a higher prevalence in females (54.20%). This difference might be due to the higher frequency of hospital visits among women.

In our study, the age group of 40–59 years had the highest number of isolates (38%). Gupta et al,[11] (2015) found that infections were most common in individuals over 50 years old, followed by those aged 0 to 10. Similarly, Yadav MV et al,[17] (2023) observed that infections were prevalent in patients aged 41–60 years (38%), likely due to a weakened immune system and associated chronic conditions. The majority of Acinetobacter isolates in this study were recovered from pus (34%), followed by endotracheal aspirates (31%) [Table 5]. This aligns with Yadav MV et al,[17] (2023), who reported the highest number of isolates from pus (25%) and tracheal aspirates (24%). Wankhede SV et al,[20] (2016) in Pune, India, similarly found that most Acinetobacter isolates (20%) were recovered from pus. Murugesh K et al,[9] (2019) also reported that the majority of isolates came from pus samples (46.36%).

In contrast, lower percentages of isolates were found in BAL fluid, tissue, and pleural fluid (3% each), differing from Yadav MV et al,[17] (2023) who reported a lower percentage of isolates from CSF (1%). Other studies, such as those by Rajkumari et al,[16] (2020) and Rani C et al,[5] (2022) found the highest number of isolates in sputum (31.88%) and (45.20%), respectively. Dimple et al,[14] (2016) reported the highest number of isolates from tips (43.4%), while Tewari R et al,[3] (2018) found the most isolates in urine samples (38.8%). Gupta et al,[11] (2015) reported the highest number of isolates from blood samples (36.9%), and Guddeti et al,[21] (2023) found the most isolates in endotracheal aspirates (50%). The variation in prevalence of Acinetobacter species can be attributed to differences in geographic distribution and antibiotic policies adopted by different institutions.

In the current study, most isolates were recovered from ICU patients (46%), with lower percentages from medicine wards (7%) and obstetrics (3%) [Table 6]. This finding is consistent with other studies, such as Gupta et al,[11] (2015) which reported that 38% of Acinetobacter species were isolated from ICUs. Wankhede SV et al,[20] (2016) reported that 77% of isolates were from ICUs, and Rajkumari S et al,[16] (2020) found that the majority of isolates were from ICUs (17.39%), followed by medical wards (13.76%), with lower percentages from other wards.

Mechanical ventilation and ICU admission were identified as independent risk factors for Acinetobacter infections in a study by Lone R et al (2009).[22] Resistance to antibiotics may provide certain strains of Acinetobacter baumannii with a selective advantage in environments like modern ICUs, where microorganisms are exposed to extensive antimicrobial treatments.[17] Therefore, in ICUs where the pathogen is endemic, empirical antibiotic therapy should include drugs effective according to the local microbiological ecology.[23]

Acinetobacter baumannii was the most commonly isolated species in this study, accounting for 87% of the isolates, with Acinetobacter lwoffii comprising 13% [Table 7]. This finding is consistent with B Apoorva et al,[24] (2020) where 82.5% of isolates were Acinetobacter baumannii, followed by 5% Acinetobacter lwoffii. Ghoghari CN et al,[25] (2017) similarly found that most isolates (90.18%) were Acinetobacter baumannii, with Acinetobacter lwoffii accounting for 9.8%. Yadav et al,[16] (2023) identified 92% Acinetobacter baumannii, and Osman A et al,[26] (2003) reported that 87.5% of their isolates were Acinetobacter baumannii. The persistence of Acinetobacter baumannii in hospital environments is likely due to its resistance to major antimicrobial drugs, desiccation, and disinfectants.

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In this study, we observed that the antibiotic sensitivity pattern showed the highest sensitivity to Colistin and Tigecycline (100%) [Table 8]. Rajkumari S et al,[16] (2020) also recorded 100% sensitivity to Colistin. Tewari R et al,[3] (2018) reported 100% sensitivity to Colistin against all Acinetobacter species, and Rani C et al,[5] (2022) similarly found 100% sensitivity to Colistin. Sohail et al,[10] (2016) reported that most Acinetobacter species were sensitive to Colistin (99.9%) and Tigecycline (99.3%). Murugesh K et al,[9] (2019) found that 91.81% of Acinetobacter isolates were sensitive to Colistin. Rani P et al,[27] (2015) reported that the majority of isolates were sensitive to Colistin (80–90%). Yadav et al,[16] (2023) found that most Acinetobacter species were sensitive to Colistin (75.3%) and Tigecycline (71%). Colistin and Tigecycline remain effective treatment options for infections caused by multi-drug resistant Acinetobacter.[13]

In contrast to our study, Ghoghari CN et al,[25] (2017) found that Acinetobacter species were highly sensitive to Meropenem, Piperacillin-Tazobactam, and Ceftriaxone-Sulbactam.

In this study, a high level of resistance was observed for Meropenem, Imipenem, Amikacin, Ceftriaxone, Piperacillin/Tazobactam, Cefepime, Tobramycin, and Doripenem (98.36% each). Similarly, Sohail et al,[10] (2016) reported resistance to Meropenem, Imipenem, and Cefepime at rates of 90.8%, 90.9%, and 98.3%, respectively. Ahmad et al,[18] (2023) also reported resistance to Piperacillin/Tazobactam at 91.2%. Yadav et al,[16] (2023) found resistance rates for Imipenem and Meropenem to be 71% and 75%, respectively, which contrasts with our findings.

In our study, Gentamicin (3.27%) and Cefoperazone-Sulbactam (8.19%) showed the least sensitivity [Table 8]. In contrast, Murugesh et al,[9] (2019) reported relatively higher sensitivity for Gentamicin (34.54%) and Cefoperazone-Sulbactam (46.36%).

We found that Ciprofloxacin, Levofloxacin, and Amoxiclav exhibited the highest levels of resistance (100% each). Norfloxacin and Nitrofurantoin were tested only on urine isolates, and all four isolates were 100% resistant to these antibiotics [Table 8]. Similarly, Sohail et al,[10] (2016) reported resistance rates of 97.3% for Ciprofloxacin and 97.2% for Levofloxacin, while Ahmad et al,[18] (2023) reported 91.2% resistance to Amoxiclav.

The antibiotic susceptibility pattern of Acinetobacter species can vary widely across geographic regions, countries, centers, and even different wards within the same hospital. Therefore, local surveillance studies like this one are crucial for determining the most appropriate therapy for Acinetobacter infections.[28]

CONCLUSION

Based on a six-month investigation involving 345 clinical samples from both IPDs and OPDs, 15% tested positive for Acinetobacter species. Predominantly found in IPDs (93%), males (69%) were more affected than females (31%), with the highest isolation in the 40-59 age group (38%). Pus samples (34%) and endotracheal aspirates (31%) yielded the most isolates. The majority originated from ICUs (46%), while medicine wards (7%) and obstetrics (3%) had lower rates. Acinetobacter baumannii was the most prevalent species. Notably, all isolates were sensitive to Tigecycline and Colistin, contrasting with high resistance to various antibiotics, indicating the need for judicious antibiotic use.

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