Original Article

Prevalence and antimicrobial resistance pattern of *Acinetobacter baumannii* Complex: An under-estimated bacteria roaring to be considered

Kumar H, Mir RF, Kaur N, Bala R, Chauhan J, Chauhan S

Abstract:

Introduction: *Acinetobacter baumanii* complex have been known to cause hospital acquired infection along with community-acquired infection. Moreover with inherited (intrinsic) or acquired anti-microbial resistance, it becomes hard for the physician to treat the patient.

Aim: To know the prevalence and anti-microbial resistance pattern of *A. baumanii* complex in various clinical samples.

Material and Methods: A total of 4123 various clinical specimens received from OPD and IPD of various specialties were processed during a period of 1 year (May, 2022 to April, 2023).

Results: Out of 4123 samples, 44.96% samples showed growth among which 6.74% were from OPD and 93.28% were from IPD. A total of 183 strains of *A. baumanii* complex were isolated and the total prevalence was 4.44%. The isolated strains of *A. baumanii* complex showed increased resistance to fluoroqunilones and cephalosporins. The most effective antibiotic was colistin followed by Minocycline. Among all *A. baumanii* complex, 53.55% were MDR, 27.32% were XDR while 1.64% were PDR.

Conclusion: *A. baumanii* complex cause hospital acquired as well as community acquired infections. It is therefore advised to establish high-quality recommendations for the "rational use of antibiotics" that must be properly followed and implemented.

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INTRODUCTION

Nonfermenting gram-negative bacteria (NFGNB) are a varied taxonomically group of aerobic, nonsporing bacteria that either do not use glucose as a source of energy or use it oxidatively. [1] About 15% of all bacterial isolates from a clinical microbiology laboratory are known to be NFGNB. *Pseudomonas* species, *Acinetobacter* species, *Burkholderia* species, *Stenotrophomonas maltophilia* etc. are among the diverse types of bacteria that make up non-fermentative gram-negative bacteria (NFGNB).

The *Acinetobacter baumannii* named after scientist Paul Baumann was used to be thought of being a low-virulence commensal bacteria. It has nonetheless established itself as a powerful pathogen, and the majority of infections linked to healthcare have affected very sick patients in the ICU.[2] In recent years, *A. baumannii* infections have afflicted patients with co-morbid conditions outside of the ICU, in trauma patients following natural catastrophes, and even in community-dwelling patients. It is responsible for several diseases, including meningitis, pneumonia, urinary tract infections, bacteremia, wound infections, and pneumonia.[3]

In four major classes of antimicrobials—fluoroquinolones (50-73% non-susceptible), aminoglycosides (19-31% non-susceptible), β-lactams (39-66% non-susceptible), and carbapenems (9-39% non-susceptible)—an analysis of susceptibility data for *Acinetobacter baumannii* Complex between 1995 and 2004 revealed an increase in the percentage of resistance to all antimicrobial agents.[4] *A. baumannii* infections that were multidrug resistant (MDR) were traditionally treated with carbapenems, but recent usage of these drugs has increased the prevalence of carbapenem resistance. Although previously avoided due to systemic toxicities (nephrotoxicity and neurotoxicity), polymyxins are now commonly utilised as the antibiotic of choice for MDR A.

Author Affiliations

Harit Kumar, Ph.D Scholar, **Rumana Farooq Mir**, Assistant Professor, **Narinder Kaur**, Professor and Head, **Rosy Bala**, Associate Professor, **Jyoti Chauhan,** Assistant Professor, **Shubham Chauhan,** Tutor, Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Maharishi Markandeshwar (Deemed to be) University, Mullana, Haryana, India

Correspondence

Dr Narinder Kaur, Professor and Head, Department of Microbiology, Maharishi Markandeshwar Institute of Medical Sciences and Research, Maharishi Markandeshwar (Deemed to be) University, Mullana, Haryana, India

Email: docnarinder@gmail.com,

Mobile: +91-9467806959

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Keywords

Nonfermenting gram-negative bacteria**,** Multi-drug resistant, Colistin resistant.

baumannii infections. However, colistin-resistant strains have been reported in many parts of the world.[5]

Therefore in view of the above, this cross-sectional study was planned to know about the prevalence of *A. baumanii* complex and their antibiotic resistance pattern at a tertiary care institute.

MATERIAL AND METHODS

A cross-sectional study was conducted (May, 2022 to April, 2023) on clinical samples received from Out-patient department (OPD) and in-patient department (IPD) of various specialties in the Department of Microbiology, MMIMSR, Mullana. The Ethical clearance was taken from institutional ethical committee.

Pus samples included Swabs taken from the wound, ear discharge and pus secretions. Blood and Body fluids included all the sterile fluids from the body while respiratory samples included sputum, endotracheal secretions and Broncho-alveolar lavage. Urine samples were not included in the study. Only *A. baumanii* complex was further included in the study while other bacterial isolates were excluded from the study.

Sample Processing:

Pus sample and Respiratory samples: All the samples were subjected to the gram staining followed by culture on Blood agar and MacConkey agar (HiMedia, India).

Blood and body fluids: For Blood culture pair of blood was taken from two different peripheral veins and inoculated into two blood culture bottles. In case of other sterile fluids around 3-5 mL was inoculated in blood culture bottles and these bottles were incubated in automated blood culture system (BD BACTEC FX40). For blood culture, blood culture bottles were incubated for 5 days while for other sterile body fluids were incubated for 48 hours before declaring them negative. The flagged positive blood culture bottles were processed by subculturing on Blood agar and MacConkey agar (HiMedia, India), and pure bacterial isolates were subjected to species identification and antibiotic susceptibility testing.

Note: Only blood cultures with both bottles identified as positive were judged to be positive. The blood culture bottles that only had one bottle flagged positive or that had not been flagged positive for up to five days were regarded as contaminated and negative, respectively.

Bacterial Identification and Antibiotic sensitivity testing:

Identification of all bacterial isolates was performed by putting GN I.D and GP I.D card in Vitek-2 Compact (Biomeurix). Antibiotic sensitivity testing for *A. baumanii* complex was performed by using N406 AST card in Vitek-2 Compact (Biomeurix) as per manufacturer standard operating procedures. Results of antibiotic sensitivity testing was evaluated using CLSI 2022 guidelines. [6]

Colistin Antibiotic sensitivity testing:

The Colistin MIC was evaluated on uncoated 96-well polystyrene microtitre plates using the standard BMD method (MIC range: 0.25-16 mg/L) and by CLSI standards.[6] *A. baumannii* has colistin MICs that fall between the EUCAST MIC breakpoints of >2 mg/L for resistance and 2 mg/L for susceptibility.[7] Proteus mirabilis (colistin MIC >16 mg/L) was used as Colistin-resistant quality-control strain for each run.

Identification of Multi-drug resistant, Extensively drug resistant and Pan drug resistant isolates of *A. baumannii* complex: As suggested by US Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC), isolates that are resistant to at least one antibiotic from three or more antimicrobial group will be considered as multidrug-resistant (MDR), isolates those are resistant to at least one antibacterial agent in all categories except two or fewer will be considered as Extensively drug-resistant (XDR). Pan drug resistance (PDR) will be those isolates which will be resistant to all antibiotics tested with all agents in all antimicrobial categories listed in the Clinical and Laboratory Standards Institute (CLSI) guidelines.[6,8]

Quality Control:

Acinetobacter baumanii (ATCC 19606), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) strains were used as reference strain for quality control in culture and antimicrobial susceptibility assays.

RESULTS

Among total of 4123 samples, 1606 were pus samples, 1630 were blood and body fluids while 887 were respiratory samples. Out of 4123 samples, 44.96% were culture positive while 55.04% samples showed no growth. (Table 1 and Table 2) Among Pus samples, *Escherichia coli* was (27.63%) the most predominant bacteria followed by *Staphylococcus aureus* (22.69%). In Blood and body fluids, *Escherichia coli* (30.33%) was the most predominant bacteria followed by *Klebsiella pneumoniae* (24.43%). Among Respiratory samples, *Klebsiella pneumoniae* (32.31%) was the most predominant bacteria followed by *Pseudomonas aeruginosa* (32.06%). (Table-3) Due to less number, isolates of *Enterococcus* spp, *Stenotrophomonas maltophila* and *Burkholderia cepacia* were excluded from the study. The overall prevalence of *A. baumanii* complex was 4.44%. The prevalence of *Acinetobacter baumannii* Complex in pus samples was 4.42%, in blood and body fluids was 3.19% while in respiratory samples was 6.76%. Isolates of *A. baumanii* complex were found highly resistant to Fluoroquinolones and 3rd and 4th generation cephalosporins. The most effective antibiotic against *A. baumanii* complex was Colistin and Minocycline. (Table 4) Among 183 isolates of *A. baumannii* complex, 53.55% were MDR, 27.32% were XDR and 1.64% were PDR. (Table 5)

DISCUSSION

The current high risk posed by antibiotic-resistant pathogenic bacteria makes treating patients in hospitals a significant issue.[9] The abbreviation "ESKAPE" (Enterococcus faecium, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomona aeruginosa, and Enterobacter cloacae* refers to the most prevalent MDR pathogens.[2]

In current study, a total of 4123 samples were taken in which 55.04% were sterile or showed no growth and 44.96% showed growth. The positivity rate in IPD was 93.28% as compared to OPD i.e., 6.74%. In another study conducted by Abebe M *et al* (2019), the positivity rate was 46.78% which is concordance with our study.[10]

According to a systematic analysis of the clinical and economic impact of antibiotic resistance, ESKAPE bacteria are associated with the highest risk of mortality, resulting in higher health-care costs.[11]

In our study, *Enterococcus* spp. was not included due to less sample size. In pus samples, the most prevalent organism was *Escherichia coli* (27.63%) followed by *Staphylococcus aureus* (22.63%) and *Pseudomonas aeruginosa* (20.96%). Another study conducted by Roy S *et al* (2017) showed most prevalent organism as *Staphylococcus aureus (42.21%)* followed by *Klebsiella* species (15.86%) and *Pseudomonas* species (15.28%).[12] Among Blood and body fluids, most common isolated organism was *Escherichia coli* (30.33%) followed by *Klebsiella pneumoniae* (24.43%) and *Pseudomonas aeruginosa* (14.32%). In another study conducted by Shume T *et al* (2022) the most prevalent organism was *Klebsiella pneumoniae* (26.5%) and *Escherichia coli* (20.6%).[13]

Among respiratory samples, most commonly isolated organism was *Klebsiella pneumoniae* (32.31%) followed by *Pseudomonas aeruginosa* (32.31%) and *Escherichia coli* (16.79%). In another study by Singh S et al (2020), reported *Pseudomonas* species was the commonest isolate (31%), followed by *Klebsiella pneumoniae* (21.3%).

Acinetobacter baumannii is an opportunistic gram-negative coccobacillus that exhibits exceptional survival under varied environmental circumstances and inherent resistance to commonly recommended antibiotics. [14]*A. baumannii* isolates predominately develop in healthcare facilities and are closely associated with nosocomial infections, especially in patients receiving critical care and in immunocompromised people. [15] The most frequent clinical symptoms include meningitis, urinary tract infections (UTI), central line-associated bloodstream infections (BSI), ventilator-associated pneumonia (VAP), and pneumonia. [16] Unfortunately, the absence of effective treatments has resulted in a high crude death rate for infections arising in sterile locations, which can range from 40% to 80%.[17]

The prevalence of *A. baumannii* complex was 4.44% among total samples. The prevalence of *A. baumannii* complex in pus samples was 4.42%, in blood and body fluids was 3.19% while in respiratory samples was 6.76%. In another study conducted by Sharma RK *et al* (2017) the overall prevalence of *Acinetobacter baumannii* was 6.42% and maximum number of *Acinetobacter baumannii* were isolated from the endotracheal secretions followed by pus samples.[3]

The World Health Organisation (WHO) declared carbapenem-resistant *A. baumannii* to be a critical priority in 2017 and stated that new antibiotics are urgently required to treat it.[18] Clinical isolates of *A. baumannii* throughout the 1970s were susceptible to widely used antibiotics such as ampicillin, gentamicin, chloramphenicol, and nalidixic acid. However, it became a severe nosocomial infection in the late 1970s, primarily due to the introduction of broad-spectrum antibiotics in hospitals.[19]Also, the intrinsic (inherent) or acquired resistance with the help of various mechanisms in *A. baumannii* complex make situation more drastic.[20]

In current study, among *A. baumannii* complex maximum resistance was reported to Fluoroquinolones and Cephalosporins (3^{rd} & 4^{th} generation). However increased resistance was seen in Gentamicin and Piperacillin-tazobactam. The strains of *A. baumannii* complex was most susceptible to Colistin followed by Minocycline and Meropenem. In another study conducted by Kaur N *et al* (2021) higher resistance was seen to Fluoroquinolones and Cephalosporins (3^{rd} & 4^{th} generation).[21] In another study conducted by Chauhan S *et al* (2022) the maximum strains of *A. baumannii* complex were multi-drug resistant.[22]

Today, the majority of first-line antibiotics are no longer effective. When treating multidrug-resistant (MDR) *A. baumannii*, tigecycline, and colistin are the only antibiotics still effective against it. However, colistin-resistant strains have been reported in many parts of the world.[23]

In current study, 3.64% strains of *A. baumannii* complex were resistant to colistin. In another study conducted by Chauhan S *et al* (2022), total number of colistin resistant *Acinetobacter baumannii* were 6.36%.

Multidrug-resistant Gram-negative bacteria are known to cause substantial morbidity and death. *Acinetobacter* spp. which is multidrug-resistant has been identified as one of the most difficult illnesses linked with healthcare to manage and treat. The primary targets of this organism are patients hospitalized in the burn unit, intensive care unit, and wards with central intravenous catheters and respiratory devices.[2,24] In current study, 53.55% of *A. baumannii* complex isolates were MDR, 27.32% were XDR while 1.64% were PDR which is in concordance with study conducted by Chauhan S *et al* (2022) which showed 30.99% isolates as MDR.

CONCLUSION

The overall prevalence of *A. baumannii* complex was 4.44%. The prevalence in pus samples, blood and body fluids while in respiratory samples was 4.92%, 3.19% and 6.76% respectively. Isolates of *A. baumannii* complex were found highly resistant to fluoroquinolones and 3rd and 4th generation cephalosporins. The most effective against *A. baumannii* complex was colistin and minocycline. Among the isolates of *A. baumannii* complex MDR, XDR and PDR were found to be 53.55%, 27.32% and 1.64% respectively. This study has revealed that maximum isolated strains of *A. baumannii* complex are multi-drug resistant and only a few antibiotics are effective for treating the infections caused by this pathogen.

LIMITATION

In this study there was lack of additional clinical information and risk factors. Also it was not possible for the molecular characterization of antibiotic resistant determinants and multifactorial phenotypes related to pathogenesis such as biofilm formation which would suggest the ability of this pathogen to persist and survive in the hospital settings.

Table 1: Sample-wise distribution

S.No	Type of Specimen	Number of specimen (n=4123)
1.	Pus	1606
2.	Blood and Body fluids	1630
3.	Respiratory samples	887

Table 2: Culture positivity rate in OPD and IPD among various clinical samples

Total no. of samples	Culture Positive (n=1854)		No growth/Sterile (n=2269)	
4123	OPD	IPD	OPD	IPD
	125 (6.74%)	1729 (93.28%)	321 (14.15%)	1948 (85.85%)

Table 3: Distribution of isolates among various clinical samples

Type of sample (n=1854)	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii* Complex	*Staphylococcus aureus*	*Enterobacter cloaceae*
Pus (n= 749)	207 (27.63%)	121 (16.15%)	157 (20.96%)	71 (9.47%)	170 (22.69%)	23 (3.07%)
Blood and Body fluids (n=712)	216 (30.33%)	174 (24.43%)	102 (14.32%)	52 (7.30%)	94 (13.20%)	74 (10.39%)
Respiratory samples (n= 393)	66 (16.79%)	127 (32.31%)	126 (32.06 %)	60 (15.27%)	12 (3.05%)	02 (0.50%)

Table-4: Sample-wise antibiotic resistance pattern of *Acinetobacter baumannii* Complex

Antibiotic	Pus Samples (n=71)	Body and Body Fluids (n=52)	Respiratory Samples (n=60)	Total Resistance rate (n=183)
Ciprofloxacin	64 (90.14%)	46 (88.46%)	51 (85%)	87.97%
Levofloxacin	62 (87.33%)	44 (84.61%)	49 (81.67%)	84.69%
Gentamicin	60 (84.50%)	41 (78.84%)	48 (80 %)	81.42%
Amikacin	55 (77.46%)	40 (76.92%)	43 (71.66%)	75.40%
Cefepime	61 (85.91%)	45 (86.53%)	49 (81.66%)	84.69%

Ceftazidime	64 (85.91%)	47 (90.38%)	53 (88.33%)	89.62%
Pipercillin tazobactam	61 (85.91%)	43 (82.69%)	42 (70%)	79.78%
Co-trimoxazole	54 (76.05%)	39 (75 %)	41 (68.33%)	73.22%
Minocycline	28 (39.43%)	21 (40.38%)	20 (33.33%)	37.70%
Imipenem	54 (76.05%)	41(78.84%)	41 (68.33%)	74.31%
Meropenem	51 (71.83%)	40 (76.92 %)	40 (66.66%)	71.58%
Colistin	02 (1.41%)	01 (1.92%)	0 (0%)	1.64%

Table 5: Distribution of MDR, XDR and PDR *Acinetobacter baumannii* Complex in various clinical samples

Type of Sample	Non-MDR	MDR	XDR	PDR
Pus (n=66)	10 (15.15%)	35 (53.03%)	19 (28.79%)	02 (3.03%)
Blood and Body fluids (n=36)	08 (22.22%)	19 (52.78%)	09 (25%)	00
Respiratory samples (80)	13 (16.25 %)	44 (55%)	22 (27.5%)	01 (1.25%)
Total no. of *Acinetobacter baumannii* Complex (n=183)	31 (16.94%)	98 (53.55%)	50 (27.32%)	03 (1.64%)

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