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ORIGINAL ARTICLE

A Prospective Study on Prevalence and Antibiotic Susceptibility Pattern of *Acinetobacter baumannii* in Clinical Samples obtained from Patients admitted in Various Wards and Intensive Care Units

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ABSTRACT

Introduction: *Acinetobacter baumannii* is a nonfermentative and nonmotile, Gram-negative coccobacillus, which is the most often identified pathogen among *Acinetobacter* species that causes wide range of infection in humans. It has emerged as one of the most troublesome pathogens for health care institutions globally. *Acinetobacter baumannii* strains resistant [multidrug-resistant *A. baumannii* (MDR-Ab)] to all known antibiotics have now been reported.

Aim: To determine the prevalence and antimicrobial susceptibility pattern of *A. baumannii* isolates in the clinical samples obtained from patients admitted in various wards and intensive care units (ICUs).

Materials and methods: A total of 9,540 clinical specimens [from the patients of inpatient department (IPD)] were collected between March 2016 and February 2017 from various wards and ICUs of Mahatma Gandhi Hospital (MGH), Sitapura, Jaipur, Rajasthan, India. All tests were done at microbiology lab of Mahatma Gandhi University of Medical Sciences & Technology, Jaipur, Rajasthan, India, using standard protocol.

Results: Of 9,540 (45%) clinical specimens, 4,293 specimens from various wards and ICUs were culture positive. Out of 4,293 positive cultures, *Acinetobacter* isolates were 276 (6.42%). From 276 *Acinetobacter* isolates, 230 (83.33%) strains were confirmed as *A. baumannii* strains and remaining 46 (16.67%) strains as other *Acinetobacter* species. Maximum frequency of *Acinetobacter* isolates was from respiratory tract intubated patients (endotracheal samples: 59.5%). Maximum frequency of *A. baumannii* isolates was recovered from ICUs (63.04%) compared with wards. In our study, most *Acinetobacter* isolates were resistant (80–99%) to third- and fourth-generation cephalosporins, quinolones, penicillins, aminoglycosides, carbapenems, and macrolides. Drugs of choices are colistin (99.13%), tigecycline (67.83%), cefoperazone/sulbactam (44.78%), minocycline (40.87%), ampicillin + sulbactam (36.09%), doxycycline hydrochloride (10.43%), and cotrimoxazole (9.57%).

Conclusion: *Acinetobacter* species is an emergent and global hospital-acquired pathogen. Drug resistance pattern of *A. baumannii* is quite alarming in our health care settings, so effective infection control practices and judicious use of antibiotics is mandatory.

Keywords: Endotracheal, Intensive care units and wards, Multidrug-resistant *Acinetobacter baumannii*, Nosocomial infection.

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INTRODUCTION

The genus *Acinetobacter* species is nonfermentative and nonmotile, Gram-negative coccobacilli, which comprises 27 known and several unnamed provisional species. Clinically, *A. baumannii* is most often identified as the cause of infection.[1] *Acinetobacter baumannii*, named after Paul Baumann,[2] is ubiquitous in soil and water. Previously, *A. baumannii* was regarded as a low-virulence commensal bacterium. However, it has become a successful pathogen[3] and has emerged as a major cause of healthcare-associated infections, most of which have occurred in critically ill patients in the ICU setting.[4] In recent decades, infections caused by *A. baumannii* have also occurred outside the ICU or in trauma patients after natural disasters and they have even affected patients with comorbidities in the community.[5] Reports of community-acquired *Acinetobacter* infections have increased over the past decade.[6] Several different types of infections, including pneumonia, urinary tract infections, bacteremia, wound infections, and even meningitis, are caused by this organism.[7] These infections often occur in older patients, many of whom have chronic underlying diseases and have previously received antimicrobial treatment.[8,9] The mortality of patients with *A. baumannii* infections in hospitals and in the ICU has ranged from 7.8 to 23% and from 10 to 43% respectively.[10]

The risk factors usually constitute underlying diseases, intravascular lines, mechanical ventilation, old

age, prior treatment with broad-spectrum antibiotics or steroids (including immune-compromised status), prolonged hospitalization, and stay in the ICU. *Acinetobacter baumannii* has an intrinsic propensity to acquire MDR genes, and whole-genome sequencing of resistant strain has proven this.[11] In addition, liberal use of carbapenems and third-generation cephalosporin appears to be related to the development of MDR strains, which helps in turning them into MDR-Ab or pan-resistant.

Acinetobacter baumannii is an opportunistic pathogen of emerging importance in the clinical settings and responsible for up to 20% of infections in ICUs around the globe.[12] The majority of reported clinical cases involved ventilator-associated pneumonia/pulmonary infections, bloodstream infections, skin and soft tissue infections, including burn and surgical wound infections, endocarditis, meningitis, and urinary tract infections. Furthermore, infections caused by *Acinetobacter* are not limited to the hospital settings and reports have emerged unfolding cases involving otherwise healthy individuals of all age groups, occurring in community settings, following natural disasters and during wars.[1,13]

Treatment of infections due to this pathogen is becoming a serious clinical concern, since *A. baumannii* shows extensive resistance to many of the currently used antibiotics, including cephalosporins, aminoglycosides, quinolones, and carbapenems. *Acinetobacter baumannii* is of particular concern due to its predilection to acquire antibiotic resistance determinants.[12]

Acinetobacter baumannii has the capacity to develop antimicrobial resistance by various mechanisms, which is mostly related to mobile genetic elements, such as insertion sequences, plasmids, and antibiotic-resistant islands.[3]

Nosocomial MDR-Ab infection most commonly occurs in ICUs.[14] Outbreaks in ICUs due to MDR-Ab have been reported to be associated with various types of indwelling medical devices and medical procedures used in patient management, especially for respiratory system.[12,13] Moreover, the resistance of *A. baumannii* to common disinfectants and ability to survive for long periods on dry surfaces make it difficult to eradicate from the hospital environment. Current multidrug resistance of this organism ranges from 48 to 85% of clinical isolates, with the greatest burden in Asia, Eastern Europe, and Latin America. Pan resistance of this organism is on the increase, signifying the fact that soon clinicians will face more such infections for which no effective antimicrobial therapeutic option shall be available. So, it is imperative to build up new antimicrobial approaches to fight this emerging threat.[12,15,16]

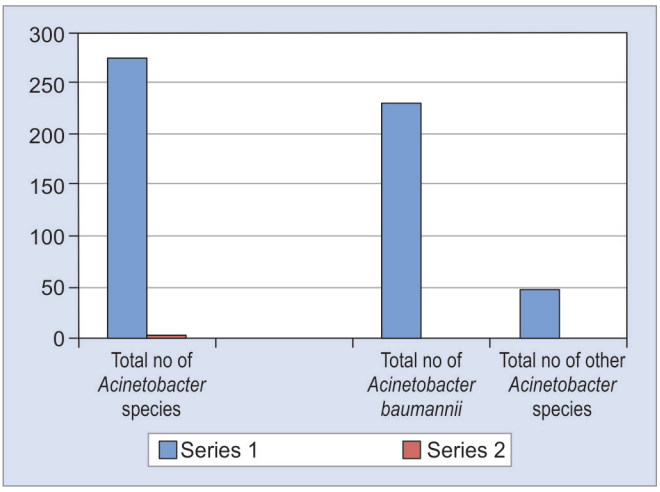
MATERIALS AND METHODS

This study was carried out at the Department of Microbiology, MGH, Sitapura, Jaipur, Rajasthan, India, between March 2016 and February 2017. All clinical samples from patients who were admitted (IPDs) in various wards and ICUs were included for *A. baumannii* infection using standard protocol. A total of 9,540 nonrepetitive samples received in the lab from various wards and ICUs were processed. Samples were collected with universal precautions by prescribed sterile techniques and transported to the laboratory as soon as possible maintaining optimum transportation conditions. Variable samples were collected, which included blood, urine, sputum, endotracheal tip secretions, suction tip secretions, pus, swabs, stents/valves, body fluids, etc. Routine microscopy of samples was done before processing with the help of Gram's staining and other staining, etc. All culture media were obtained from Hi-Media Laboratories, Mumbai, India. Primary inoculation was done on blood agar and MacConkey agar culture media as per standard protocols and incubated for 18 to 24 hours at 37°C aerobically. Cultures were then identified by standard techniques based on colony morphology, Gram staining, hanging drop preparation for motility, and various biochemical tests for indole test, citrate utilization test, urease test, triple sugar iron agar test, oxidase test, phenylalanine deaminase test, and specific tests for species level as oxidative/fermentation glucose test, gelatin hydrolysis test, and growth at 42°C.[17] Antimicrobial susceptibility of the isolate was carried out on Mueller-Hinton agar (Hi-Media Laboratories, Mumbai, India) modified by Kirby Bauer disk diffusion technique by inoculating with the test organism (0.5 McFarland standards) to get a semi-confluent growth as per recommendations of Clinical and Laboratory Standards Institute.[17,18]

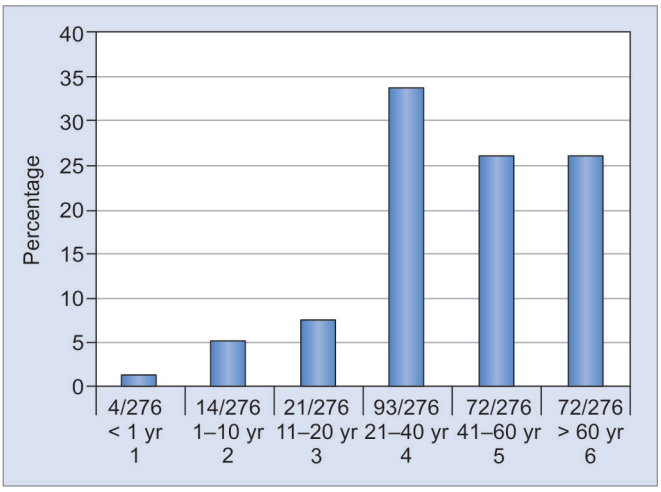
RESULTS

A total of 9,540 nonrepetitive samples from various wards and ICUs [medical ICU (MICU), surgical ICU, coronary care unit, pediatric ICU, neonatal ICU, etc.) of IPD were submitted in the Department of Microbiology, Mahatma Gandhi Medical College, Jaipur, from March 2016 to February 2017 for culture and drug susceptibility testing. Among these 9,540 samples, 4,293 (45%) from various wards and ICUs were culture positive and showed growth of different microorganisms. Out of these positive isolates, 276 (6.42%) were identified as *Acinetobacter* species. Among *Acinetobacter* spp., 230 (83.33%) strains were confirmed as *A. baumannii* strains and remaining 46 (16.67%) strains as other *Acinetobacter* species (Graph 1).

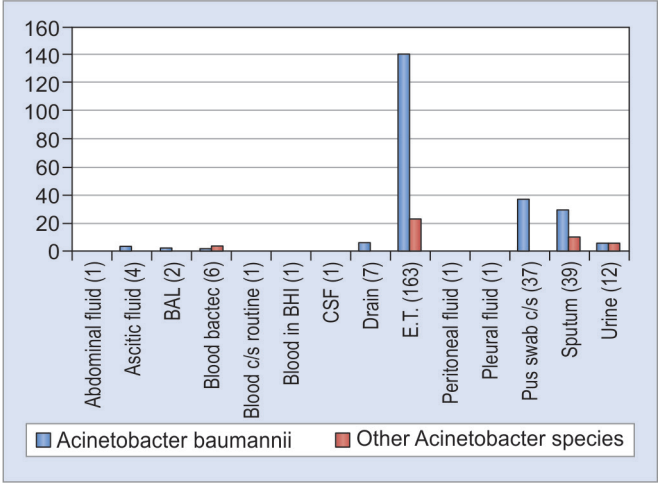
Among the clinical specimens yielding growth of *Acinetobacter* spp., 75.36% (208/276) positive culture was seen in male patients followed by female patients of 24.28% (67/276), and 1 patient (1/276) was 6-year-old



Graph 1: Frequency of *Acinetobacter* species in IPD patients from various wards and ICUs



Graph 2: Distribution of *Acinetobacter* isolates according to age group



Graph 3: Distribution of isolated *Acinetobacter* species organisms in various specimens in IPD patients

transgender admitted in pediatric ward. Maximum numbers of *Acinetobacter* isolates were recovered from 21 to 40 years age group (Graph 2).

Maximum number of isolates was recovered from endotracheal (ET) secretions (163), sputum (39), pus swab c/s (37), and urine (12) (Graph 3).

Maximum number of *A. baumannii* strains was recovered from ICU (80), MICU (19), Neuro Surgical Ward (14), General Air Conditioner Ward (12), Intensive Cardiac Care Unit (11), Male Cardiac Ward (11), high dependency unit (11) and Hepatobiliary Critical ICU (10). Antibiotic susceptibility testing of 23 different antibiotics against *Acinetobacter* spp. revealed that colistin was the most sensitive antibiotic to which 99.13% of the tested isolates were sensitive and only two isolates (0.87%) were resistant to colistin drug. Other sensitive drugs were tigecycline (67.83%), cefoperazone/sulbactam (44.78%), minocycline (40.87%), ampicillin + sulbactam (36.09%), doxycycline hydrochloride (10.43%), cotrimoxazole (9.57%), moxifloxacin (9.13%), meropenem (6.96%), and amikacin (4.78%). Intermediate sensitivity was also seen in cefoperazone + sulbactam (30.00%), minocycline (22.18%), and tigecycline (16.52%). Sensitivity to all other antimicrobials was 15% or less. Sensitivity of *A. baumannii* isolates to different drugs is shown in Table 1.

DISCUSSION

Multidrug-resistant Gram-negative pathogens are associated with high morbidity and mortality. Multidrug resistant *Acinetobacter* spp. has been reported worldwide and has now emerged as one of the hardiest healthcare-associated infections to control and treat. Patients admitted in burn unit, ICU, and those wards with central intravenous catheters and respiratory devices are the main targets of this organism.[13,19] Delay in receiving adequate empirical antimicrobial therapy has an adverse effect on clinical outcomes in hospital-acquired infections caused by *A. baumannii*.[20] *Acinetobacter*-associated nosocomial infections in critically ill patients are on the rise.[21,22] Its MDR phenotype is capable of acquiring new mechanisms of resistance and nosocomial outbreaks.[5] Resistance to antibiotic poses a serious and growing problem, because such resistant infectious diseases are becoming more difficult to treat. Resistant bacteria do not respond to the antibiotics and continue to cause infection.[23] In the last few decades, there has been a general trend of increasing incidence of infection due to this pathogen around the globe.[24]

In the present study, number of positive cultures is 45%, which is similar to the study by Mohammadi-Mehr et al.[25] In the study of Sabir et al,[26] the percentage of positive culture is 87.17%, which is much higher than the present study. In the present study, the percentage of *Acinetobacter* isolates (6.42%) is found to be similar in both the studies by Fayyaz et al[27] (10.9%) and Goossens[28] (4.9%).

Table 1: Antimicrobial susceptibility pattern of *A. baumannii* isolates

	Resistance		*Intermediate*		*Sensitive*	
Name of antibiotics	*No.*	*%*	*No.*	*%*	*No.*	*%*
Amikacin (AK)	217	94.35	2	0.87	11	4.78
Ampicillin + Sulbactam (A/S)	147	63.91	–	–	83	36.09
Azithromycin (AZM)	222	96.52	–	–	8	3.48
Cefepime (CPM)	227	98.69	1	0.44	2	0.87
Cefoperazone + Sulbactam (CFS)	58	25.22	69	30.00	103	44.78
Cefotaxime (CTX)	226	98.26	2	0.87	2	0.87
Ceftazidime (CAZ)	228	99.13	–	–	2	0.87
Ceftriaxone (CTR)	228	99.13	–	–	2	0.87
Ciprofloxacin (CIP)	221	96.09	–	–	9	3.91
Colistin (CL)	2	0.87	–	–	228	99.13
Cotrimoxazole (COT) (trimethoprim/sulfamethoxazole)	208	90.43	–	–	22	9.57
Doripenem (DOR)	207	90.00	8	3.48	15	6.52
Doxycycline hydrochloride (DO)	206	89.57	–	–	24	10.43
Gentamicin (GEN)	223	96.96	–	–	7	3.04
Imipenem (IPM)	225	97.83	–	–	5	2.17
Levofloxacin (LE)	218	94.78	6	2.61	6	2.61
Meropenem (MRP)	214	93.04	–	–	16	6.96
Minocycline (MI)	85	36.95	51	22.18	94	40.87
Moxifloxacin (MO)	208	90.43	1	0.43	21	9.13
Netilmicin sulfate (NET)	227	98.70	–	–	3	1.30
Piperacillin (PI)	228	99.13	–	–	2	087.
Piperacillin/tazobactam (PIT)	222	96.52	–	–	8	3.48
Tigecycline (TGC)	36	15.65	38	16.52	156	67.83
Total number of isolates: 230						

In our study, the percentage of *A. baumannii* (83.33%) and other *Acinetobacter* species (16.67%) is found to be approximately similar in the studies by Fayyaz et al[27] and Gupta et al.[29] In the present study, the percentage of *Acinetobacter* species in male (75.36%) and female (24.28%) is found to be approximately similar in the studies by Fayyaz et al[27] and higher than the studies by Tahseen and Talib[30] and Saleem et al.[31] In our study, 1 patient was transgender admitted in pediatric ward. In the present study, the maximum frequency of *Acinetobacter* isolates from respiratory tract intubated patients (ET samples: 59.5%) followed by sputum (14.13%), pus swab c/s (13.40%), urine (4.35%), and blood (2.54%), which is higher than in the studies by Chim et al,[32] Markogiannakis et al,[22] and Alvarez-Lerma et al.[33] In the present study, the maximum frequency of *A. baumannii* isolates was recovered from ICUs (63.04%) compared with wards, which is found to be similar in the studies by Xia et al[13] and Tahseen and Talib.[30]

Carbapenems were previously known to be effective against MDR-Ab, but since the emergence of pan-resistant *Acinetobacter* spp., it is even more difficult to treat this pathogen.[34,35] The Centers for Disease Control and Prevention has reported an increasing rate of carbapenems resistant among *A. baumannii* from 9% in 1995 to 40% in 2004.[19] The majority of *A. baumannii* isolated from our patients showed resistance to more than one group of antibiotics. A study from India revealed 87% isolates were MDR and 20% carbapenem resistant.[36] Another study from India showed 33% carbapenem resistance,[37] a study from Korea reported 55.8% carbapenem resistance,[20] and similar carbapenem resistance among isolates of *Acinetobacter* spp. was reported from the Aga Khan University Hospital at Karachi.[38] A study from Norway revealed that about 9% isolates were *A. baumannii* and 95.6% of these isolates of *A. baumannii* were resistant to ciprofloxacin, nalidixic acid, trimethoprim/sulfamethoxazole and gentamicin, and intermediately susceptible to amikacin[39]; almost similar results were depicted in the present study. A study from Mayo Hospital, Lahore also reported about 11.8% isolates of *A. baumannii*.[40] A study from Saudi Arabia revealed that *A. baumannii* isolates showed high resistance to piperacillin (93.1%), aztreonam (80.5%), ticarcillin, ampicillin, and tetracycline (76.4%, each), and cefotaxime (75%). Only amikacin showed low rate of resistance compared with other antibiotics (40.3%).[41] In the present results, amikacin was resistant in 94.35% of isolates. Another study from India showed that 87% *Acinetobacter* spp. were resistant to third-generation cephalosporins, aminoglycosides, and quinolones, indicating high prevalence of MDR.[37] Alarming situation is reported from a study in USA that colistin resistance is reported in 18% isolates of *A. baumannii* recovered from solid organ transplant patients.[42] In the

present study, *A. baumannii* isolates showed high rate of resistance to ceftriaxone (99.13%), ceftazidime (99.13%), piperacillin (99.13%), cefepime (98.69%), cefotaxime (98.26%), netilmicin sulfate (98.70%), imipenem (97.83%), gentamicin (96.96%), azithromycin (96.52%), piperacillin/tazobactam (96.52%), and ciprofloxacin (96.09%), which is similar to a study done by Fayyaz et al[27] and found higher than the studies done by Prashanth and Badrinath[43] and Armin et al.[44]

In our study, colistin showed 99.13% sensitivity against *A. baumannii* isolates. Only two isolates of *A. baumannii* (0.87%) were resistant to colistin drug in the present study. Other sensitive drugs were tigecycline (67.83%), cefoperazone/sulbactam (44.78%), minocycline (40.87%), ampicillin + sulbactam (36.09%), doxycycline hydrochloride (10.43%), cotrimoxazole (9.57%), moxifloxacin (9.13%), meropenem (6.96%), and amikacin (4.78%), which has similar sensitivity rate in the study by Qureshi et al.[45] Intermediate sensitivity was also seen in cefoperazone + sulbactam (30.00%), minocycline (22.18%), and tigecycline (16.52%). These results were found similar to study done by Tahseen and Talib.[30] No pan-resistant *A. baumannii* was seen in our study. We have observed good susceptibility to tigecycline (67.83%), cefoperazone/sulbactam (44.78%), minocycline (40.87%), ampicillin + sulbactam (36.09%), and doxycycline hydrochloride (10.43%) in our study.

The overall frequency of *Acinetobacter* isolates infection in our setup was about 6.42% (*A. baumannii* 5.36%, other *Acinetobacter* species 1.07%) in total positive samples. Antimicrobial susceptibility of this pathogen varies significantly among regions/centers. Therefore, local surveillance studies are required to look for the most suitable empirical therapy. Since there are various mechanisms of resistance in *A. baumannii* for the development of valuable strategies, it is important to comprehend the interplay of various resistant mechanisms.

CONCLUSION

Acinetobacter species is an emergent and global nosocomial pathogen. About 6.42% of infections in IPD patients (wards and ICUs) are caused by *Acinetobacter* species in positive cultures in our setup. *Acinetobacter baumannii* is the most prevalent (83.33%) among *Acinetobacter* spp. Maximum frequency of *A. baumannii* isolates was recovered from ICUs (63.04%) compared with wards. Colistin (polymyxin-E) was the most sensitive antibiotic to which 99.13% of the tested isolates were sensitive. Other drugs of choices are tigecycline (67.83%), cefoperazone/sulbactam (44.78%), minocycline (40.87%), ampicillin + sulbactam (36.09%), doxycycline hydrochloride (10.43%), and cotrimoxazole (9.57%). Sensitivity to all other antimicrobials was 8% or less. Resistance pattern of *A. baumannii* is quite alarming in our health care settings, so effective infection control practices and judicious use of antibiotics is mandatory, as well as clinical guidance regarding the potential risks for therapeutic failure is imperative.

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