A Microbiological Study of *Acinetobacter calcoaceticus baumannii* with Special Reference to Multidrug Resistance

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Abstract

Introduction The outbreak of *Acinetobacter calcoaceticus baumannii* (*ACB*) is mainly reported to be a notorious pathogens at health-care settings. It is the major problem on the health-care system with high morbidity and mortality rates because of the broad range of antibiotic resistance and lack of understanding the mechanism of developing new antibiotic resistance rapidly. It emphasizes the importance of local surveillance in describing or understanding and predicting microbial resistance patterns so that there will be limited use of antibiotics by developing strategies to control the extensive use of antimicrobial chemotherapy in clinical environment, which is still considered as one of the factors in the emergence of multidrug resistance microorganisms.

Objectives The study aims to detect the occurrence rate of *ACB* infections from various clinical samples, identify the resistance levels to different groups of antimicrobial agents, and the occurrence rate of multidrug resistant (MDR) *ACB* clinical isolates from a tertiary hospital in Durgapur, West Bengal, India.

Material and Methods The study was performed in the Department of Microbiology of the IQ City Medical College and Hospital, Durgapur, West Bengal, India, for the 24 months duration, that is, from January 1, 2018 to December 31, 2019. Altogether 15,800 clinical samples consisting of endotracheal tube aspirates, sputum, pus, blood, catheter tips, urine, tissue, and other body fluids were studied. *ACB* from clinical samples were identified by its characteristic colonies (nonlactose fermenting, glistening, small mucoid colonies), Gram-staining pattern (Gram-negative coccobacillus), and standard biochemical reactions. It was further confirmed in the Department of Microbiology of the Healthworld Hospital, Durgapur, West Bengal, India, by Vitek2 compact system (bioMerieux, Inc., Durham, North Carolina, United States). Antibiotic susceptibility testing was performed using automated broth microdilutions by Vitek2 compact system (bioMerieux, Inc.) and Kirby-Bauer disk diffusion test on Mueller-Hinton Agar (HiMedia).

Keywords

- *Acinetobacter calcoaceticus baumannii*
- antibiotic susceptibility testing
- MDR strains
- Vitek2 compact system

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Results Nonrepetitive 289 *ACB* were isolated from various clinical samples. A total of 277 (96%) isolates of *ACB* were MDR strains.

Conclusion *ACB* was mostly isolated from the intensive care unit department and was found to be the most MDR type in the tertiary care hospital by this study.

Introduction

Acinetobacter calcoaceticus baumannii (*ACB*) are mainly reported to be the most important nosocomial pathogens at the intensive care units (ICUs) and health care settings housing very ill patients.[1] Recent studies have reported its colonization at body sites such as the oropharyngeal wall, skin, and intestinal tract.[2] About 8.3 to 41% of ICU patients are reported with colonization of intestinal tract.[3] Various geographical areas have been reported for the outbreak of *ACB*, mainly *Acinetobacter baumannii*[4] in which India has also been the forefront of such studies. Pan drug resistant *A. baumannii* isolates have been reported from Asia and the Middle East.[5]

The major alarm is the treatment of infections which are rapidly acquiring resistant to antibiotics. This includes extended spectrum β-lactamases to β-lactam and β-lactamase inhibitor, cephalosporins, and carbapenems. Furthermore, it has been linked to the loss or reduced expression of porins or overexpression of multidrug efflux pumps and mutations that change targets or cellular functions.[6,7] Due to the emergence of carbapenem resistance in the strains of *A. baumannii*, largely through a clonal spread, the therapeutic options are decreasing.[6,8,9] *A. baumannii* comes out to be an important agent of nosocomial infection in ICU patients associated with ventilator-acquired pneumonia, urinary tract infection, and other infections.[10] Moreover, such type of infections are becoming more problematic due to the emergence of the multidrug resistant (MDR) strain.[11–13] In a recent surveillance study in Greece, 3% of *A. baumannii* strains derived from ICU patients were colistin resistant.[14] The mortality rates varies from 7.8 to 75%.[2] The MDR strain rarely occur outside of health care settings; however, community-acquired *A. baumannii* has been reported and progresses more rapidly with a higher mortality rate compared with hospital setting.[11] Thus, it accentuates the importance of local surveillance in describing or understanding and predicting microbial resistance patterns so that there will be limit use of antibiotics by developing strategies to control on extensive use of antimicrobial chemotherapy in clinical environment which is still considered as one of the factors in the emergence of multidrug resistance microorganisms. The purpose of this study is to find out the occurrence rate of ACB infections from various clinical samples, identify the resistance levels to different antimicrobial agents and occurrence rate of MDR *ACB* clinical isolates from a tertiary hospital in Durgapur, West Bengal, India.

Materials and Methods

The study was performed in the Department of Microbiology of the IQ City Medical College and Hospital, Durgapur, West Bengal, India, for the 24 months duration, that is, from January 1, 2018 to December 31, 2019. A total of 289 *ACB* nonrepetitive isolates from 15,800 clinical samples consisting of endotracheal tube aspirates, sputum, pus, blood, catheter tips, urine, tissue, and other body fluids were involved in the study. *ACB* from clinical samples were identified by its characteristic colonies (nonlactose fermenting, glistening, small mucoid colonies), Gram-staining pattern (Gram-negative coccobacillus), and standard biochemical reactions. It was further confirmed in the Department of Microbiology of the Health World Hospital, Durgapur, West Bengal, India, by Vitek2 compact system (bioMerieux, Inc., Durham, North Carolina, United States). Antibiotic susceptibility testing was performed using automated broth microdilutions by Vitek2 compact system (bioMerieux, Inc.) and antibiotic disc diffusion test (Kirby–Bauer) according to the Central Laboratory Standards Institute (CLSI) interpretive criteria.[15]

For disc diffusion test, the antibiotic drugs with their concentration were piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), ticarcillin-clavulanate (75/10 µg), cefepime (30 µg), ceftazidime (30µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), doxycycline (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim-sulfamethoxazole or co-trimoxazole (1.25/23.75 µg), meropenem (10 µg), imipenem (10 µg), and doripenem (10 µg). These antibiotic discs were obtained commercially from HiMedia and BioRad distributors. Disk diffusion in colistin was not performed as broth microdilution is the only approved minimum inhibitory concentration test.

The disc diffusion test was performed on Mueller–Hinton Agar (HiMedia) as per the Kirby–Bauer method. Each zone size is interpreted according to the CLSI guideline and grouped as "susceptible (S), intermediate (I), and resistance (R)."

The resistance levels to different groups of antimicrobial agents were determined and the isolates were categorized as MDR, that is, resistance to one or more agents in at least three antimicrobial categories.[11]

Results

In our study, 289 nonrepetitive *ACB* were isolated, which contributes 1.83% out of all the different 15,800 clinical samples. *ACB* was most isolated from ICU, that is, 145 (50.17%); followed by medicine ward, that is, 97 (33.56%); surgical ward, that is, 32 (11.07%), pediatric ward 13 (4.50%); and cardiology 2 (0.70%) as shown in ►**Table 1**.

A total of 115 (39.79%) of the *ACB* isolates were from endotracheal tubes which were followed by sputum 63 (21.80%), pus 62 (21.45%), blood 34 (11.77%), fluid 7 (2.42%), urine 4 (1.38%), other specimens like catheterized tips or tissue 3 (1%), and throat swab 1 (0.35%) as shown in ►**Table 2**.

Table 1 Distribution of isolation of *Acinetobacter calcoaceticus baumannii* ($N = 289$)

Wards or Units	Isolation	Percentage
Intensive care unit (ICU)	145	50.17
Medicine	97	33.56
Surgery	32	11.07
Pediatric	13	4.50
Cardiology	2	0.70
Total	289	100

Table 2 Clinical specimens showing isolation rates from different clinical samples

Types of samples	Isolates of *Acinetobacter calcoaceticus baumannii*	Percentage
Endotracheal secretions	115	39.79
Sputum	63	21.80
Pus	62	21.45
Blood	34	11.77
Fluid	7	2.42
Urine	4	1.38
Other specimens	3	1
Throat swab	1	0.35
Total	289	100

Among all the *ACB* isolates, 88% were resistant to amikacin, 2% to tigecycline, and 96% to trimethoprim-sulfamethoxazole (co-trimoxazole). The other antibiotics like piperacillin-tazobactam, ticarcillin-clavulanate, cefepime, ceftazidime, gentamicin, levofloxacin, ciprofloxacin, meropenem, imipenem, and doripenem were 100% resistant as shown in ►**Table 3**. All the isolates were sensitive to colistin and doxycycline antibiotics. Note that 277 (96%) isolates of *ACB* were MDR strains.

The data generated in the antibiotic susceptibility test given by the Kirby–Bauer disc diffusion test and automated microbroth dilution method as shown in ►**Table 3** was not so much variable, but they are qualitatively comparable to each other. The susceptibility pattern of piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidime, meropenem, imipenem, and doripenem showed similar pattern both in these two methods.

In the present study, a discordant result among the seven studied antibiotics was observed. The number of susceptible (S) isolates toward amikacin was more in the automated broth microdilution method, that is, 5% susceptible in disk diffusion and 12% susceptible in the automated broth microdilution method. However, the numbers of susceptible isolates were found to be decreased in the automated broth microdilution method, that is, cefepime, gentamicin, and levofloxacin showed 1, 3, and 4% higher susceptible in disk diffusion, respectively, as compared with the automated

Table 3 Comparison of antibiotic susceptibility pattern by Kirby–Bauer disk diffusion test and automated broth microdilution method

Name of antibiotics	% of Disk diffusion	% of MIC
Piperacillin/ Tazobactam	S = 0, I = 0, R = 100	S = 0, I = 0, R = 100
Ticarcillin/ Clavulanate	S = 0, I = 0, R = 100	S = 0, I = 0, R = 100
Ceftazidime	S = 0, I = 0, R = 100	S = 0, I = 0, R = 100
Cefepime	S = 1, I = 1, R = 98	S = 0, I = 0, R = 100
Amikacin	S = 5, I = 7, R = 88	S = 12, I = 0, R= 88
Gentamicin	S = 3, I = 3, R = 94	S = 0, I = 0, R = 100
Levofloxacin	S = 4, I = 0, R = 96	S = 0, I = 0, R = 100
Ciprofloxacin	S = 4, I = 0, R = 96	S = 0, I = 0, R = 100
Co-trimoxazole	S = 4, I = 3, R = 93	S = 4, I = 0, R = 96
Tigecycline	S = 98, I = 0, R = 2	S = 97, I = 1, R = 2
Doxycycline	S = 100, I = 0, R = 0	Not applicable[a]
Meropenem	S = 0, I = 0, R = 100	S = 0, I = 0, R = 100
Imipenem	S = 0, I = 0, R = 100	S = 0, I = 0, R = 100
Doripenem	S = 0, I = 0, R = 100	S = 0, I = 0, R = 100
Colistin	Not applicable[b]	S = 100, I = 0, R = 0

Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, sensitive.
[a]Doxycycline antibiotic is not available in the testing set of automated broth microdilution method.
[b]Colistin antibiotic is not recommended for disk diffusion test according to Central Laboratory Standards Institute (CLSI).

broth microdilution method. The numbers of resistance (R) isolates were mostly found in the automated broth microdilution method whereas there was no finding of intermediate (I) isolates. Both the data of antibiotic susceptibility has its associated advantages and disadvantages. The disc diffusion test is relatively easy to setup and inexpensive, but it does not provide quantitative data.

►**Table 4** shows the percentage of resistant isolates particularly amikacin, co-trimoxazole, and tigecycline in the

Table 4 Distribution of resistant *Acinetobacter calcoaceticus baumannii* isolates particularly in amikacin, co-trimoxazole, and tigecycline in the different units of the hospital ($N = 289$)

Wards or Units	Amikacin No. of resistant isolates (%)	Co-trimoxazole No. of resistant isolates (%)	Tigecycline No. of resistant isolates (%)
Intensive care unit (ICU)	145 (100)	145 (100)	6 (4.13)
Medicine	73 (75.25)	95 (97.93)	0 (0)
Surgery	32 (100)	30 (93.75)	0 (0)
Pediatric	2 (15.38)	5 (38.46)	0 (0)
Cardiology	2 (100)	2 (100)	0 (0)

Table 5 Distribution of resistant *Acinetobacter calcoaceticus baumannii* isolates in the various samples ($N = 289$)

Name of antibiotics	Sputum No. of isolates (%)	Urine No. of isolates (%)	Blood No. of isolates (%)	Endotracheal tube (ET) No. of isolates (%)	Pus No. of isolates (%)	Fluid No. of isolates (%)	Other specimens like catheterized tips or tissue No. of isolates (%)	Throat swab No. of isolates (%)
β-lactam combination agent								
Piperacillin/Tazobactam	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Ticarcillin/Clavulanate	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Cephems (parenteral)								
Ceftazidime	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Cefepime	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Carbapenems								
Meropenem	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Imipenem	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Doripenem	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Aminoglycosides								
Amikacin	49 (77.8)	4 (100)	25 (73.52)	113 (98.26)	52 (83.87)	7 (100)	3 (100)	1 (100)
Gentamicin	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Tetracyclines								
Tigecycline	2 (3.17)	0 (0)	4 (11.76)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Minocycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fluoroquinolones								
Ciprofloxacin	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Levofloxacin	63 (100)	4 (100)	34 (100)	115 (100)	62 (100)	7 (100)	3 (100)	1 (100)
Folate pathway antagonists								
Trimethoprim-sulfamethoxazole (co-trimoxazole)	56 (88.88)	4 (100)	34 (100)	115 (100)	57 (91.93)	7 (100)	3 (100)	1 (100)

different units of the hospital in which the ICU has got the highest resistant isolates, that is, 145 out of which 4.13% were resistant to tigecycline.

►**Table 5** shows the distribution of resistant *ACB* isolates in the various samples in which we found out that the resistant to amikacin were highly isolated from endotracheal specimen, that is, 113 (98.26%), followed by 52 (83.87%) isolates from pus, 49 (77.77%) from sputum, and 25 (73.52%) from blood specimen. Furthermore, the resistant to co-trimoxazole were highly isolated from pus and sputum, that is, 57 (91.93%) and 56 (88.88%), respectively. The tigecycline resistant isolates were isolated from blood specimen and sputum, that is, 4 (11.76%) and 2 (3.17%), respectively.

Discussion

Our study reveals that *ACB* are commonly isolated from ICU (50.17%). In the study done by Talukdar et al,[16] the highest isolates (51.41%) were from ICU, followed by the medicine ward (13%) and surgical ward (11%); pointing toward the fact

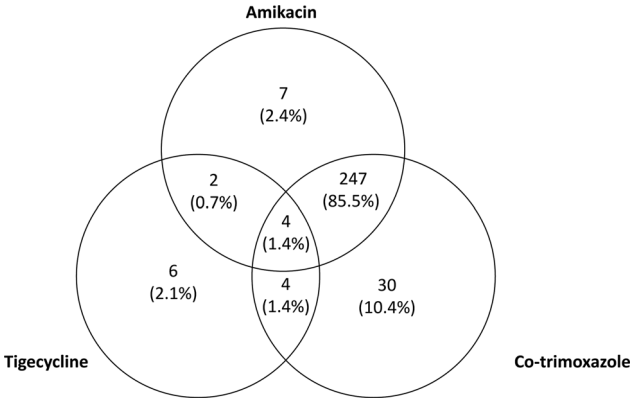

Fig. 1 Venn diagram showing association between three classes of antibiotics namely aminoglycosides (amikacin), folate pathway antagonists (co-trimoxazole), and tetracycline (tigecycline). Within the Venn diagram circles are 284 isolates representing varying degrees of antibiotic resistance (moderate resistance or fully resistant), while there are 5 isolates outside the Venn diagram, representing the susceptible isolates. Together, these constitute the entire population of *Acinetobacter calcoaceticus baumannii* complex isolates examined in this study (i.e., $N = 284 + 5 = 289$ isolates).

that *ACB* is a predominantly ICU bug. Our result substantiates the point that various risk factors are linked to ACB infection in ICU like chances of colonization among the sick and immune compromised patients. Those patients having multiple wounds, indwelling devices, and high antibiotic selective pressures are inclined for cross-transmission.

In our study, 277 (96%) isolates of *ACB* were reported as MDR strains with 100% resistance to most of the applied antibiotic except colistin and doxycycline. Banerjee et al showed reports of 88% MDR isolates with 100% resistance to most of the applied antibiotics except colistin and tigecycline.[17,18] The promising available treatments for MDR strains are tigecycline or colistin. Unfortunately, our findings reveal 2% resistance among all isolates (289 in numbers) to tigecycline. In a recent surveillance study from Germany, tigecycline resistance among 215 *A. baumannii* was 6%.[19] MDR *ACB* has associated with large morbidity and mortality after carbapenem-resistant *Enterobacteriaceae* and extended-spectrum beta-lactamase producing *Enterobacteriaceae*.[20] With high antibiotic selective pressures, the MDR organisms acquire various antibiotic resistance mechanisms as a strategy for survival. In our present study, out of 145 isolates in the ICU ward, we found out 6 isolates, that is, 4.13%, were resistant to tigecycline in the ICU as shown in ►**Table 4**. Therefore, we assumed that the high use of tigecycline in managing the treatment of *ACB* infection in ICU nowadays provide the emergence of tigecycline resistant strain in such environment although the relative contribution of these mechanisms are not yet known. Moreover, we highlighted firm application of antibiotic management program with strict hospital infection control measures to prevent nosocomial infection of MDR mostly in ICU. This study had limitations in aspect of number of isolates, short study period, and a single-center study. Therefore, a generalized finding with multicenter settings and long surveillance data has to be studied more especially molecular-based study.

Conclusion

MDR is being increasingly reported in *ACB* and posing a threat to hospitalized patients due to the limitation of therapeutic options. This may be caused due to highly antibiotic selective pressure that we can easily see in the ICU environment. This is a significant burden on the health care system in excess costs due to an extensive treatment of symptoms and lack of understanding in how they persist.

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None.

Authors' Contributions
L.L.A.: Literature search, review of literature, manuscript preparation, concept and design of the study, data collection, statistically analyzed and interpreted, prepared first draft of manuscript, and critical review of study proposal.
D.C.T.: Scientific advisor, critical review of study proposal, and helped in preparing first draft of manuscript.
D.D.: Scientific advisor, critical review of study proposal, and data collection.

Conflict of Interest
None declared.

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