Molecular Surveillance of Carbapenem Resistant Acinetobacter baumannii (CRAB) from clinically significant Non fermenting GramNegative bacilli in a tertiary care hospital

Mathavi Sureshkumar[1]*, Sarath Chandar[2], Nalina C[3]

[1] Principal Investigator – Professor & Head, Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Salem, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India. drmathavimicro@gmail.com

[2] Postgraduate, Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Salem, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India. drsarath6009@gmail.com

[3] Postgraduate, Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Salem, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India. nalinachitrarasu@gmail.com

Abstract

Background: *Acinetobacter baumannii* has emerged as a significant nosocomial pathogen, particularly in intensive care settings due to its remarkable ability to acquire resistance to multiple antimicrobial agents. Carbapenem-resistant Acinetobacter baumannii (CRAB) significantly limits therapeutic options and poses a serious clinical challenge. Molecular surveillance is essential to understand the distribution of resistance determinants and to guide infection control strategies.

Objectives: To perform molecular surveillance of carbapenem-resistant *A. baumannii* isolated from clinically significant non-fermenting gram-negative bacilli (NFGNB) in a tertiary care hospital and to characterize the major carbapenem resistance genes.

Methodology: A total of 196 clinically significant NFGNB isolates were collected from various clinical specimens over a defined study period. Acinetobacter baumannii were identified using conventional biochemical methods and confirmed by VITEK 2 automated system. Antimicrobial susceptibility testing was performed according to CLSI guidelines. Carbapenem resistance was defined based on resistance to imipenem and/or meropenem. Molecular detection of carbapenemase-encoding genes, including blaOXA-23, blaOXA-51, blaOXA-58, blaNDM and blaVIM was carried out using polymerase chain reaction (PCR).

Results: Out of 196 NFGNB isolates, 39 isolates were *A. baumannii*, of which 31 (79.5%) were carbapenem resistant. Molecular analysis revealed the presence of intrinsic blaOXA-51 like gene in all carbapenem resistant *A.baumannii* isolates, with blaOXA-23 (74.2%) being the predominant acquired carbapenemase gene followed by blaNDM(19.3%) and a combination of blaOXA-58, blaNDM and blaVIM (6.5%).

Conclusion: The study highlights the alarming burden of carbapenem resistance among A. baumannii isolates in the hospital setting. The predominance of blaOXA-23 underscores its major role in mediating resistance. Continuous molecular surveillance, strict infection control measures and robust antimicrobial stewardship programs are essential to curb the spread of CRAB and optimize patient management.

Keywords: *Acinetobacter baumannii*, Carbapenem resistance, OXA-type β-lactamases

Introduction

Acinetobacter baumannii has emerged as a significant nosocomial pathogen in tertiary care hospitals due to its ability to survive in hospital environments and cause outbreaks, particularly in intensive care units (ICUs) [1]. It is frequently associated with ventilator-associated pneumonia, bloodstream infections, wound infections and urinary tract infections, predominantly affecting critically ill and immunocompromised patients [2].

A major concern with *A. baumannii* is its remarkable ability to acquire multidrug resistance, especially resistance to carbapenems, which are often considered last-line agents for severe gram-negative infections [3]. The increasing emergence of carbapenem-resistant A. baumannii (CRAB) has significantly limited therapeutic options and is associated with increased morbidity, mortality, prolonged hospital stay and higher healthcare costs [4].

Carbapenem resistance in *A. baumannii* is primarily mediated by the production of carbapenem-hydrolyzing β-lactamases, particularly OXA-type enzymes [5]. Among these, blaOXA-23 is the most widely distributed and clinically significant resistance determinant, while the intrinsic blaOXA-51 gene may contribute to resistance when overexpressed [6]. In addition, the acquisition of metallo-β-lactamases such as blaNDM further complicates the resistance profile and poses a serious threat due to its potential for horizontal gene transfer [7].

Non-fermenting gram-negative bacilli constitute an important group of hospital pathogens, with *A. baumannii* being one of the most clinically significant members because of its high resistance potential [8]. Molecular surveillance of carbapenem resistance genes among CRAB isolates is therefore essential for understanding local epidemiology, correlating phenotypic and genotypic resistance and guiding infection control and antimicrobial stewardship strategies [9].

Aim

1. To evaluate the burden of carbapenem-resistant Acinetobacter baumannii among clinically significant non-fermenting gram-negative bacilli.
2. To determine the antimicrobial susceptibility profile of A. baumannii isolates using conventional and VITEK-2 methods.
3. To detect major carbapenem resistance genes (blaOXA-23, blaOXA-51, blaOXA-58 and blaNDM) in carbapenem-resistant isolates.

Methodology

This prospective cross-sectional study was conducted in the Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College and Hospitals, Salem, over a period of 2 years from October 2023 to October 2025. The study included patients of all age groups presenting with clinically suspected infections.

Sample Processing and Isolation

Clinical specimens including blood, sputum, endotracheal aspirates, urine, pus, wound swabs and body fluids were processed following standard microbiological procedures. Samples were subjected to Gram staining and those showing gram-negative bacilli were inoculated onto MacConkey agar and blood agar.
After overnight incubation, isolates producing non-lactose-fermenting colonies on MacConkey agar were selected for further identification.

Identification of Non-Fermenting Gram-Negative Bacilli (NFGNB)

Presumptive non-fermenting gram-negative bacilli were identified using standard biochemical tests including oxidase test, catalase test, oxidative–fermentative (OF) test, citrate utilization, urease test, nitrate reduction test and motility test. Species-level identification of *Acinetobacter baumannii* were further confirmed using the VITEK-2 automated identification system.

Antimicrobial Susceptibility Testing

All confirmed *Acinetobacter baumannii* isolates were subjected to antimicrobial susceptibility testing (AST) by both conventional method (Kirby–Bauer disc diffusion) and the VITEK-2 automated system and the results were interpreted according to CLSI guidelines. Following antibiotic discs (HiMedia) were used : Amikacin (30µg), Ampicillin-sulbactam (10µg+10µg), Ceftazidime (30µg), Cefepime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Co-trimoxazole (25µg), Gentamicin (10µg), Imipenem (10µg), Levofloxacin (5µg), Meropenem (10µg), Minocycline (30µg) and Piperacillin-Tazobactam (100µg + 10µg).In addition to that, for urine isolates following discs were used: Nalidixic acid (30µg), Norfloxacin (10µg), Nitrofurantoin (300µg) and Netilmicin sulphate (30µg).
Carbapenem resistance was defined based on resistance to imipenem and/or meropenem.

Selection of Carbapenem Resistant Isolates

Only isolates identified as carbapenem-resistant Acinetobacter baumannii (CRAB) were subjected for molecular analysis.

DNA Extraction

Genomic DNA was extracted from CRAB isolates using the HiMedia bacterial DNA extraction kit, following the manufacturer's instructions.

Molecular Detection of Carbapenem Resistance Gene:

Polymerase chain reaction (PCR) was performed to detect major carbapenemase-encoding genes, including blaOXA-23, blaOXA-51, blaOXA-58, blaVIM and blaNDM. PCR amplification was carried out using gene-specific primers under standardized cycling conditions.

Results

Out of 196 NFGNB isolates, *Pseudomonas aeruginosa* were predominant with 121 isolates and 39 *Acinetobacter baumannii* identified by conventional methods and remaining 36 isolates were reported as NFGNB and was identified as *Stenotrophomonas maltophilia* by automated VITEK 2 method. This distribution highlights the clinical relevance in routine microbiology laboratories.

Figure 1: Distribution of organisms among the isolated NFGNB (N = 196)

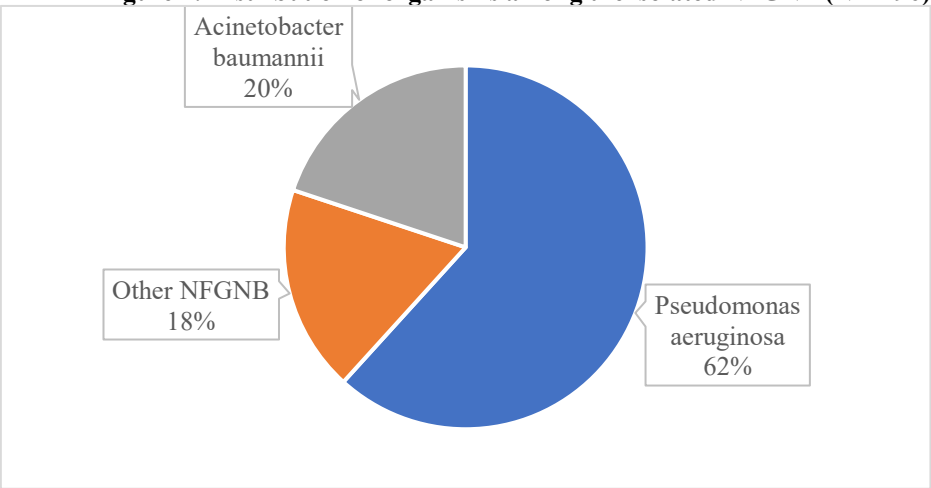
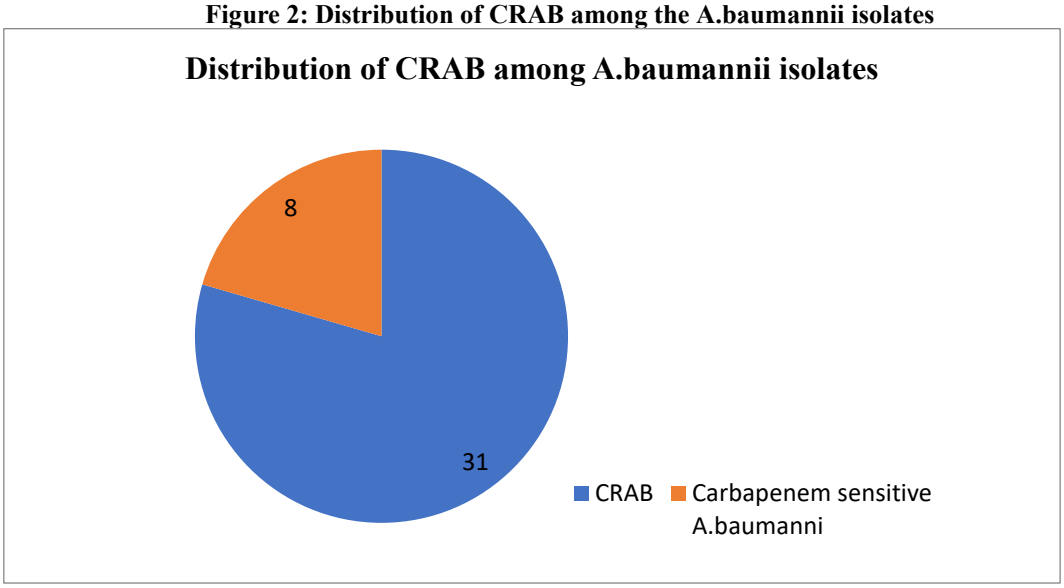


Table 1: Specimen wise distribution of Acinetobacter baumannii Isolates (N=39)

S.No	Type of sample	NFGNB (N=196)	A.baumannii isolates (N=39)	CRAB (N=31)	Percentage
1	Urine	27	5	4	18.5 %
2	Sputum	34	10	6	29.4 %
3	Pus	78	7	5	9.0 %
4	ET aspirate	28	14	14	50.0 %
5	Blood	18	2	1	11.1 %
6	Central line tip	11	1	1	9.1 %

Acinetobacter baumannii were predominantly isolated from 14 (50%) endotracheal aspirates followed by sputum (29.4%) and pus (9.0%), highlighting its frequent recovery from respiratory samples.

Figure 2: Distribution of CRAB among the A.baumannii isolates



Among 39 A.baumannii isolates, 31(79.5%) were carbapenam-resistant and 8(20.5%) were carbapenem-sensitive.

Table 2: Department wise distribution of CRAB isolates

Departments	No. of A. baumannii isolates (%)	No. of CRAB isolates (%)
General Medicine	2 (5.1%)	1(2.5%)
Emergency Medicine	3 (7.6%)	1(2.5%)

General Surgery	4 (10.2%)	3(7.6%)
Pediatrics	1 (2.5%)	0 (0%)
Orthopedics	6 (15.3%)	4(12.9%)
ICU	14 (35.8%)	14(45.1%)
Chest & TB	9 (23%)	8(25.8%)
Total	39	31

ICU accounted for the highest number of CRAB isolates 14 (45.1%) indicating a substantial burden of carbapenem resistance. Pulmonary medicine ward also reported a considerable number of CRAB isolates. Overall, a high proportion of A. baumannii isolates were carbapenem resistant, underscoring the widespread occurrence of resistance.

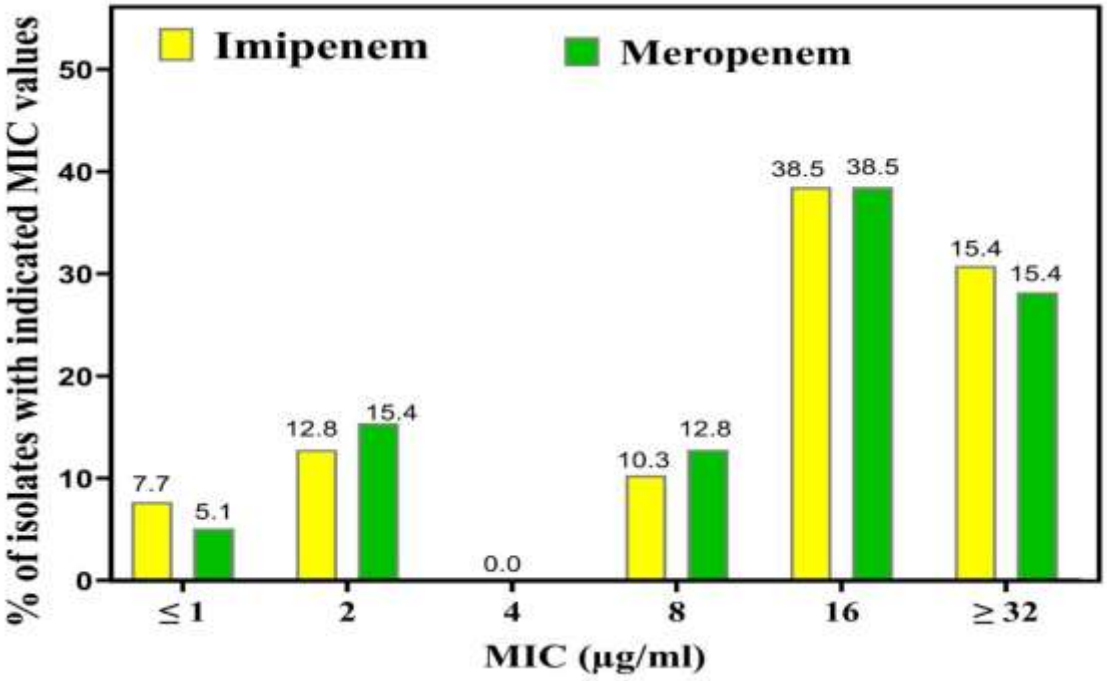
Table 3: Antimicrobial susceptibility pattern of Acinetobacter baumannii (N=39) Conventional and automated method

Drugs	**Conventional AST(N=39)**			**VITEK-2 AST(N=39)**		
	Sensitive n (%)	**Moderately sensitive n(%)**	**Resistant n (%)**	**Sensitive n (%)**	**Moderately sensitive n(%)**	**Resistant n (%)**
Ampicillin-Sulbactam (10µg+10µg)	12 (30.8)	5(12.8)	22(56.4)	13 (33.3)	5(12.8)	21(53.9)
Amikacin (30µg)	8(20.5)	4 (10.3)	27(69.2)	8(20.5)	4 (10.3)	27(69.2)
Gentamicin (10µg)	6(15.4)	3(7.7)	30(76.9)	6(15.4)	3(7.7)	30(76.9)
Cefepime (30µg)	4(10.3)	2(5.1)	33(84.6)	4(10.3)	2(5.1)	33(84.6)
Ceftazidime (30µg)	3(7.7)	2(5.1)	34(87.2)	3(7.7)	2(5.1)	34(87.2)
Cefotaxime (30µg)	2(5.1)	1(2.6)	36(92.3)	2(5.1)	1(2.6)	36(92.3)
Ceftriaxone (30µg)	2(5.1)	1(2.6)	36(92.3)	2(5.1)	1(2.6)	36(92.3)
Ciprofloxacin (5µg)	7(17.9)	3(7.7)	29(74.4)	7(17.9)	3(7.7)	29(74.4)
Levofloxacin (5µg)	7(17.9)	3(7.7)	29(74.4)	7(17.9)	3(7.7)	29(74.4)
Cotrimoxazole (25µg)	10(25.6)	4(10.3)	25(64.1)	10(25.6)	4(10.3)	25(64.1)
Minocycline (30µg)	14(35.9)	5 (12.8)	20(51.3)	15(38.5)	5 (12.8)	19(48.7)
Imipenem (10µg)	8(20.5)	0	31(79.5)	8(20.5)	0	31(79.5)
Meropenem (10µg)	8(20.5)	0	31(79.5)	8(20.5)	0	31(79.5)
Piperacillin-Tazobactam (100 + 10µg)	9(23.1)	4(10.3)	26(66.6)	9(23.1)	4(10.3)	26(66.6)
Colistin	-			-	35(89.7)	4(10.3)

Colistin susceptibility testing was performed by the VITEK 2 automated system. CLSI-recommended colistin broth disc elution method or reference broth microdilution methods were not performed for confirmation of colistin susceptibility results.

A.baumannii exhibited high resistance to most β-lactams, aminoglycosides, fluoroquinolones, and carbapenems confirming multidrug resistance. Minocycline showed comparatively better activity, while colistin/polymyxin B susceptibility was detected by VITEK-2.

Table 4: MIC distribution of Imipenem and Meropenem among Acinetobacter baumannii isolates(N=39)



Susceptible (MIC ≤2 µg/ml), Intermediate (MIC =4 µg/ml), Resistant(MIC ≥8 µg/ml)

The majority of carbapenem resistant *A.baumanii* isolates exhibited high level imipenem and meropenem MIC values (≥ 16 µg/ml). Carbapenem resistance was observed in 79.5% of the isolates.

Table 5: Distribution of Carbapenem Resistance genes among CRAB isolates (N=31)

Carbapenem resistance gene profile	No of isolates (n)	Percentage (%)
blaOXA-23	23	74.2
blaNDM	6	19.3
blaOXA-58 + blaNDM + blaVIM	2	6.5
Total	31	100

blaOXA-51 is intrinsic to A. baumannii and was detected in all isolates. Acquired carbapenemase genes detected in the study included blaOXA-23, blaOXA-58, blaNDM and blaVIM.

blaOXA-23 was the predominant carbapenem resistance gene followed by blaNDM. A small proportion of isolates harbored blaOXA-58 with blaNDM, indicating emergence of high level resistance. Overall, OXA type carbapenemases were the principle mechanism of carbapenem resistance.

Discussion

Non-fermenting gram-negative bacilli (NFGNB) have emerged as important nosocomial pathogens due to their intrinsic resistance and ability to acquire multiple resistance mechanisms. In the present study, among 196 NFGNB isolates, Pseudomonas aeruginosa was the predominant organism, followed by Acinetobacter baumannii. Similar distributions have been reported in earlier studies from India and other countries, where P. aeruginosa and A. baumannii consistently constitute the major NFGNB isolated from clinical specimens [9,10]. This highlights their persistent role in hospital-acquired infections.

Specimen-wise analysis showed that A. baumannii was most frequently isolated from endotracheal aspirates (50%), followed by sputum and pus. This finding correlates well with studies by Dash et al. and Kaur et al., who reported respiratory samples as the commonest source of A. baumannii, particularly among ventilated patients [11,12].

Department-wise distribution revealed the highest burden of carbapenem-resistant A. baumannii (CRAB) isolates from ICU (45.1%), followed by Chest & TB wards. Similar observations have been reported by Manchanda et al.

and Pragasam et al., where ICUs were the primary reservoirs of CRAB [13,14]. The heavy antibiotic pressure, prolonged hospital stay and frequent use of invasive devices in ICUs contribute significantly to the selection and persistence of resistant strains.

Antimicrobial susceptibility testing demonstrated high resistance of A. baumannii to β-lactams, aminoglycosides, fluoroquinolones and carbapenems, confirming its multidrug-resistant nature. Comparable high resistance rates have been documented by studies across India and globally [15]. In the present study, only about 20% of isolates remained susceptible to imipenem and meropenem, indicating alarming levels of carbapenem resistance.

Minocycline showed comparatively better activity against A. baumannii, consistent with reports by Vila et al. and Sahu et al., who described minocycline as a useful alternative for MDR A. baumannii infections [16,17]. Colistin / polymyxin-B susceptibility was detected only by VITEK-2, reflecting their role as last-line agents. However, reliance on these drugs is concerning due to nephrotoxicity and the potential emergence of resistance.

Carbapenem resistance was detected in 79.5% (31/39) of A. baumannii isolates. Most resistant isolates demonstrated high imipenem and meropenem MICs (≥16 µg/mL). These findings are comparable to those reported by Manoharan et al. and Khurana et al., who also observed high levels of carbapenem resistance among clinical A. baumannii isolates. The high MIC values suggest the dissemination of carbapenemase-producing strains in the hospital environment [18,19].

The intrinsic blaOXA-51-like gene was detected in all A. baumannii isolates, consistent with previous studies. Among the acquired carbapenemase genes, blaOXA-23 was the most prevalent, indicating that it is the major mechanism of carbapenem resistance in our setting. Similar findings have been reported in studies by Mugnier et al. and Vijayakumar et al., where blaOXA-23 was the predominant carbapenemase gene. The detection of blaNDM, blaOXA-58, and blaVIM further highlights the emergence of diverse resistance mechanisms among CRAB isolates [20,21].

Overall, the findings underscore the growing threat posed by CRAB in tertiary care hospitals and emphasize the need for strict infection control measures, antimicrobial stewardship and continuous surveillance to curb further spread.

Conclusion

Pseudomonas aeruginosa was the most common non-fermenting gram-negative bacillus, while Acinetobacter baumannii predominated in ICU and Pulmonary medicine/TB wards, especially in endotracheal samples, suggesting a link to ventilator-associated infections. *A. baumannii* exhibited extensive resistance to β-lactams, aminoglycosides, fluoroquinolones and carbapenems with only minocycline showing moderate activity and reliable colistin/polymyxin B detection by VITEK-2. Carbapenem resistance was mainly driven by blaOXA-23 and a few isolates harbored blaNDM, indicating rising high-level resistance. These patterns highlight the critical need for ongoing surveillance and strong antimicrobial stewardship.

References

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538-82.
2. Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med. 2008;358(12):1271-81.
3. Doi Y, Murray GL, Peleg AY. Acinetobacter baumannii: evolution of antimicrobial resistance—treatment options. Semin Respir Crit Care Med. 2015;36(1):85-98.
4. Falagas ME, Bliziotis IA, Siempos II. Attributable mortality of Acinetobacter baumannii infections. Crit Care. 2006;10(2):R48.
5. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant Acinetobacter baumannii. J Antimicrob Chemother. 2010;65(2):233-8.
6. Evans BA, Amyes SGB. OXA β-lactamases in Acinetobacter baumannii. Clin Microbiol Rev. 2014;27(2):241-63.
7. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases. Clin Microbiol Infect. 2014;20(9):821-30.
8. Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant Acinetobacter species and Pseudomonas aeruginosa as pathogens in serious infections. Clin Infect Dis. 2001;32(2):S104-13.
9. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: WHO; 2017.
10. Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
11. Dash M, Padhi S, Narasimham MV, Pattnaik S. Antimicrobial resistance in Acinetobacter baumannii from tertiary care hospital in India. J Clin Diagn Res. 2013;7(6):1184-6.
12. Kaur A, Singh S. Prevalence of carbapenem resistant Acinetobacter baumannii in intensive care units. J Clin Diagn Res. 2014;8(4):DC09-11.
13. Manchanda V, Sanchaita S, Singh N. Multidrug resistant Acinetobacter. J Glob Infect Dis. 2010;2(3):291-304.
14. Pragasam AK, Veeraraghavan B, Bakthavatchalam YD, Mathai D. Molecular mechanisms of carbapenem resistance in Acinetobacter baumannii. Indian J Med Microbiol. 2016;34(2):191-6.
15. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008;46(8):1254-63.
16. Vila J, Pachón J. Therapeutic options for Acinetobacter baumannii infections. Expert Opin Pharmacother. 2008;9(4):587-99.

17. Sahu S, Mohanty I, Narasimham MV, Padhi S. In vitro activity of minocycline against multidrug resistant Acinetobacter baumannii. Indian J Med Res. 2018;147(4):417-21.
18. Manoharan A, Chatterjee S, Mathai D, SARI Study Group. Detection and characterization of metallo-beta-lactamases producing Pseudomonas aeruginosa and Acinetobacter baumannii in India. Indian J Med Microbiol. 2010;28(3):241-244.
19. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial gram-negative pathogens from North and South Indian hospitals. J Med Microbiol. 2017;66(7):999-1004.
20. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the blaOXA-23 carbapenemase gene of Acinetobacter baumannii. Emerg Infect Dis. 2010;16(1):35-40.
21. Vijayakumar S, Gopi R, Gunasekaran P, Bharathy M, Walia K, Anandan S, et al. Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii isolates reveals the emergence of blaOXA-23 and blaNDM-1 encoding international clones in India. Infect Genet Evol. 2019; 75:103986.