Journal of Research in Applied and Basic Medical Sciences 2022; 8(3): 110-117

Original Article

Urmia University of Medical Sciences

RABMS Journal of Research in Applied and Basic Medical Sciences

Comparison of Various Phenotypic Methods in Detection of Carbapenemases and Metallo-Beta-Lactamases (MBL) in Carbapenem Resistant Clinlical Isolates of Acinetobacter Species at A Tertiary Care Centre

Mariya Rouf[1*], Asifa Nazir[2], Ovais Karnain Wadoo[3], Shahida Akhter[4]

[1] *Senior Resident, Department of Microbiology, Government Medical college, Srinagar, India*

[2] *Associate Professor, Department of Microbiology, Government Medical college, Srinagar, India*

[3] *Senior Resident, Department of Physiology, Government Medical college, Srinagar, India*

[4] *Resident, Department of Microbiology, Government Medical college, Srinagar, India*

***Corresponding authors:** Dr. Mariya Rouf, **Address**: Senior Resident, Department of Microbiology, Government Medical college, Srinagar, India, **Email**: drmariyaovais@gmail.com, **Tel**: +917006345867

Abstract

***Background & Aims*:** carbapenem-resistant strains of Acinetobacter baumannii (A. baumannii) have been reported worldwide over the last decade. Detection of the carbapenemases is crucial to determine the severity of the problem. The aim of our study was to detect Carbapenemase and MBL producing strains among Multidrug Resistant (MDR) Acinetobacter species isolated from clinical specimens in this geographical area by Modified Hodge test (MHT) and Imipenem-EDTA double disc synergy test and their evaluation.

***Materials & Methods*:** In this descriptive-prospective study, consecutive, non-duplicate, and resistant-to-carbapenems clinical strains of Acinetobacter species isolated from various clinical samples were included. Antimicrobial sensitivity of Acinetobacter isolates was performed on Mueller Hinton agar plates by Kirby-Bauer disk diffusion method. Carbapenemase production was confirmed by MHT. Confirmation of MBL production was done by subjecting all isolates with positive screen test to combined disc test using imipenem, meropenem, and EDTA. Data analysis was done using Epi Info 7.0. Categorical variables were summarized as frequency and percentage and continuous variables as Mean and SD.

***Results*:** A total of 312 non-duplicate strains of A. baumannii were isolated, out of which 224 (71.79%) strains were resistant and 88 (28.21%) were sensitive to carbapenem. There was 100% sensitivity to Colistin followed by Tigecycline (79%) whereas high degree of resistance was seen against 2nd and 3rd generation cephalosporins and quinolones (>90%). 82.6% were identified as carbapenemase producers on MHT and on Imipenem-EDTA combined disc test (CDT), 21.4% were found to be positive.

***Conclusion*:** Our study showed that tests like MHT are equally efficient to detect carbapenemase production, followed by Imipenem-EDTA combined disc test. These tests are cost-effective and easy to perform and may be used routinely to assess whether carbapenemase producers are present or not.

***Keywords*: Acinetobacter, carbapenems, Modified Hodge Test, Imipenem-EDTA Combined Disc Test**

Received 22 May 2022; accepted for publication 09 July 2022

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

Introduction

Acinetobacter species are aerobic, non-lactose fermenting, oxidase-negative, Gram-negative coccobacilli that are most commonly found in the environments associated with healthcare. Although initially considered of low pathogenic potential in healthy individuals, Acinetobacter species are now considered as important pathogens implicated in nosocomial infections (1).

Originating from the family Moraxellaceae, the genus Acinetobacter contains at least 21 named species, of which Acinetobacter baumannii (A. baumannii) is the most important species in human infections. A. baumannii has been found to be associated with greater resistance to antibiotics and higher mortality among bacteremic patients compared with other genomic species (2). These factors, together with both intrinsic and acquired antibiotic resistance, account for the success of Acinetobacter species as a significant cause of outbreaks and causes its endemic spread of resistant clones throughout the world. Acinetobacter species cause a wide variety of illness in debilitated and hospitalized patients, especially in intensive care units (ICUs). It is an occasional cause of Urinary tract infections (UTIs), being responsible for 1.6% of ICU-acquired UTIs in one study (3). In the localized study, A. baumannii has been reported to be a more common cause of ICU-acquired bloodstream infection than non-ICU-ward infection (1.6% versus 0.9% of bloodstream infections, respectively). Crude mortality overall from A. baumannii bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU (3).

Despite the increased frequency of Multidrug Resistant (MDR) A. baumannii, there is limited information regarding resistance in A. baumannii from the Indian subcontinent; national resistance data based on the few published reports shows the prevalence of resistance to carbapenems ranges from 6.4% to 66%. In the last decade, A. baumannii has emerged as an important nosocomial pathogen in hospitals of India. (4, 5, 6)

Over the last decade A. baumannii has become a serious emerging community and nosocomial pathogen worldwide, known to be responsible for life-threatening infections. Although Carbapenems are the most commonly used antibiotics for treating infections caused by A. baumannii, but an increase in carbapenem-resistant strains of A. baumannii has been reported worldwide over the last decade, mainly through the production of carbapenemases and metallo-β-lactamases (MBLs). Moreover, carbapenem resistance among A. baumannii isolates restricts therapeutic options for treatment of such infections, which might lead to higher morbidity and mortality rates. Detection of the carbapenemases is crucial to determine the severity of the problem and to direct the application of antimicrobial stewardship guidelines to limit further evolution of carbapenem-resistant variants among A. baumannii isolates.

The Modified Hodge test (MHT) is the first growth-based carbapenemase detection test recommended by Clinical & Laboratory Standards Institute (CLSI) in 2009 with high level of sensitivity and specificity in detecting carbapenemases (7); it is one of the most recognized assays for carbapenemases detection. To identify metallo-β-lactamases, Imipenem-EDTA combined disc test (CDT) has been described by Yong et al. (8).

There is no extensive study conducted in the Kashmir valley regarding to detection of Carbapenemase and Metallo-β-lactamase (MBL) producing Acinetobacter species. Hence the aim of our study was to detect Carbapenemase and MBL producing strains among MDR Acinetobacter species isolated from clinical specimens in this geographical area by MHT and Imipenem -EDTA double disc synergy test and their evaluation.

Materials & Methods

This prospective study was carried out in the Department of Microbiology, Government Medical College of Srinagar for a period of 18 months. Isolates of Acinetobacter species obtained from all age and sex groups, admitted at or attending the hospital Outpatients (OPD) and International Patients (IPD) Departments of the associated hospitals were included in this study.

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

All consecutive, non-duplicate clinical strains of Acinetobacter species, isolated from various clinical samples such as blood, pus, urine, respiratory secretions, and other body fluids such as ascitic fluid, etc. resistant to carbapenems were included in the study. Duplicate clinical strains of carbapenem resistant strains of Acinetobacter species isolated from the same patient and susceptible strains of Acinetobacter species were not included in the study. Antimicrobial sensitivity of Acinetobacter isolates was performed on Mueller Hinton agar plates by Kirby-Bauer disk diffusion method according to CLSI guidelines. (9)

The following antibiotic discs were used; amikacin-30µg, gentamicin-10µg, ciprofloxacin-5µg, ceftazidime-30µg, cefipime-30µg, piperacillin-100µg and tazobactum-10µg, imipenem-10µg, tigecycline-15µg and polymyxin B-300 units. In addition, nitrofurantoin-300µg discs were used for isolates recovered from urine. All the discs were procured from Hi media, Mumbai. The sizes of the zones of inhibition were interpreted as per CLSI guidelines.

Carbapenemase production was confirmed by Modified Hodge test. The test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (E. coli ATCC 25922) towards a carbapenem disk. Positive Control was named carbapenemase producing Acinetobacter strain and Negative Control was named carbapenemase negative Acinetobacter strain. Confirmation of MBL production was done by subjecting all screen test positive isolates to combined disc test (CDT) using imipenem, meropenem, and EDTA.

Data were entered in a Microsoft Excel sheet. Categorical variables were summarized as frequency and percentage and continuous variables were summarized as Mean and SD. Data analysis was done using Epi Info 7.0.

Results

A total of 312 non-duplicate strains of Acinetobacter species were isolated from patients; out of which, 224 (71.79%) were carbapenem resistant and 88 (28.21%) were carbapenem sensitive. Two species were isolated; maximum number of isolates were of A. baumannii 200 (89.3%) and 24 (10.7%) were Acinetobacter lowffii. Males (146; 65.2%) were affected predominantly more than females (78; 34.8%) in our study. Maximum isolates were found in the age group of 60 years and over (25.4%) followed by an age of less than 10 years (17.4%). Sample wise distribution of Acinetobacter isolates is shown in Figure 1. Distribution of carbapenem resistant Acinetobacter based on its diagnosis is shown in Figure 2.

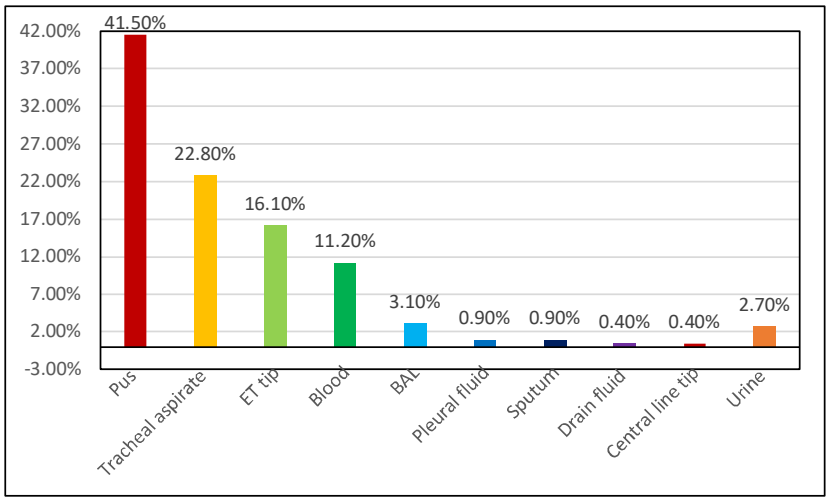

Fig 1. Sample wise distribution of carbapenem resistant Acinetobacter isolates

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

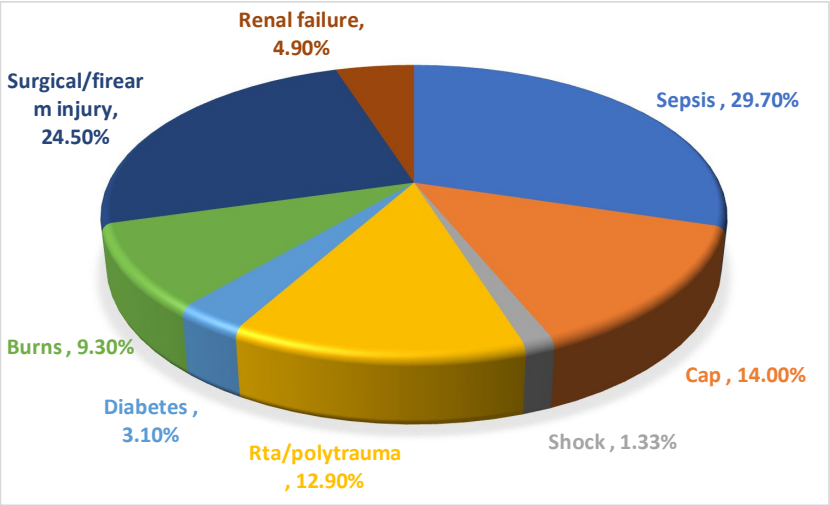

Fig 2. Diagnosis Wise Distribution of Carbapenem Resistant Acinetobacter Isolates.

The antimicrobial sensitivity patterns of the various isolates are depicted in Figure 3.

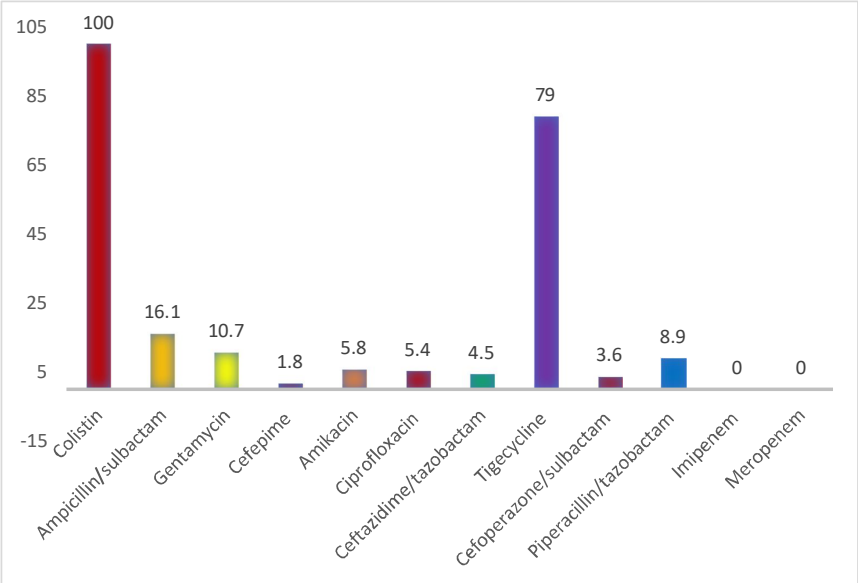

Fig 3. Antimicrobial susceptibility pattern of acinetobacter isolates

100% of the isolates were sensitive to colistin and there was a variable sensitivity to other antimicrobials tested.

In our study, among carbapenem resistant Acinetobacter isolates, 94.4% were carbapenemase producers i.e., had positive MHT results and 70.5% were Metallo-β-lactamase producers (CDT positive). MHT and CDT positive isolates predominated among males (62.09%) and (46.4%) respectively as compared to females (32.5%) and (24.1%) respectively.

The antibiogram of carbapenem resistant isolates is shown in Table 1. It was observed that maximum

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

resistance among the carbapenemase producing isolates was seen for cephalosporins cefepime (98.2%) whereas least resistance was against tigecycline (21.0%), and no resistance was seen against polymyxin b and it was 100% sensitive.

Table 1. Antimicrobial susceptibility profile of MHT positive and CDT positive isolates

Antibiotic	MHT positive	CDT positive
	Sensitive	Sensitive
Colistin	(100.0%)	(67.4%)
Amikacin	(5.8%)	(4.0%)
Gentamycin	(10.7%)	(7.1%)
Ciprofloxacin	(5.4%)	(4.0%)
Ceftazidime/tazobactam	(4.5%)	(3.6%)
Tigecycline	(75.0%)	(55.4%)
Cefoperazone /sulbactam	(3.1%)	(2.2%)
Piperacillin/tazobactam	(7.6%)	(5.4%)
Imipenem	(00.0%)	(00.0%)
Meropenem	(00.0%)	(00.0%)

MHT positive isolates show the maximum sensitivity to colistin and tigecycline 100% and 75%, respectively. CDT positivity was most sensitive to colistin 64% and tigecycline 55.4%. However, the maximum resistance was seen against Meropenem, Imipenem, cefoperazone/ sulbactam (94.6%, 94.6%, and 91.5%, respectively). For CDT positivity, the maximum resistance was seen with Meropenem, Imipenem, cefoperazone/ sulbactam (68.3%, 70.5% and 70.5%, respectively).

Discussion

In the present study, a total of 312 non-duplicate strains of Acinetobacter species were isolated over the study period; out of which, 224 (71.79%) strains were resistant to and 88 (28.21%) were sensitive to carbapenem. These findings are in agreement with the study of Padmalaxmi et al. (10), who reported 70% of carbapenem resistance in the isolates of their study. Similar high proportion of resistance (71%) has been reported in another Indian study done by Kumar et al. (11). Studies from Iran and Taiwan reported 62% and 91.7% resistance to carbapenem among Acinetobacter species (12, 13). The prevalence rate was higher in our study maybe because the most of our isolates were obtained from patients with prolonged hospital stays. Most of these patients were from the intensive care unit. Isolation rate was increased because intensive care units are associated with many risk factors like mechanical ventilation and indwelling catheters. 70% of these patients were treated with more than one broad spectrum antibiotics.

Of 224 samples sent to the microbiology laboratory during the study period, maximum number of Acinetobacter strains were identified from the respiratory tract specimens 98 (43.75%). Similar results were obtained by Noori et al. (2014) (12), who found the highest percentage of A. baumannii in respiratory tract specimens (52.8%) followed by urine (26.9%) and blood (7.4%). Another study conducted by de Carvalho et al. (2013) (14) described that the most frequent site of isolation was tracheal secretion (56.3%), followed by the catheter tip (16.9%), blood (7%), and urine (7%).

In the present prospective study, the risk factors for acquiring Acinetobacter infections were analyzed. It was found out that from 224 patients, 74 (20.9%) had a history of previous antibiotic intake, 43 (19.1%) had history of hospital stay, 13 (5.8%) were immunocompromised, 11 (4.91%) had recurrent infections, and 4 had history of hemodialysis.

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

Patients who stay for a long period in the hospital, acquire infection from other persons who are colonized with Acinetobacter species. Longer duration of stay in the intensive care unit further increases risk of infection due to contamination caused by health care workers and also from equipment such as ventilators, catheters, i.v lines. Other risk factors include immunosuppression, unscheduled admissions, use of broad-spectrum antimicrobials, sepsis in the ICU, invasive procedures like urinary and intravenous catheterizations, and patients on ventilators due to respiratory failure. A similar observation is found in the study by Garmendia et al. (15) who found that male preponderance, mechanical ventilation, and previous infection increased length of stay in the ICU and were associated with Acinetobacter infections. Most of our patients were exposed to invasive procedures like mechanical ventilation, intravenous catheters, urinary catheters, central venous lines, endotracheal intubation and tracheostomy. Various studies done by Mindolli et al. (16), Neetu Gupta et al. (17), Garmendia et al. (15), and Lone et al. (18) have identified all these co-morbid conditions as relative risk for acquiring MDR Acinetobacter isolates.

The present study highlighted the most alarming situation of highly diverse antibiotics resistance rates against cephalosporin's (first, second, and third) ranging from 95.5 % to 96.4%; cefepime (98.2%), cefoperazone/sulbactam (96.4%), ceftazidime/tazobactam (95.5%), and piperacillin/tazobactam (91.5%). Also, 94.6% of the isolates showed resistance against ciprofloxacin, 94.2% against amikacin, 89.3% against gentamicin, and 83.9% against ampicillin plus sulbactam. 71.79% of the isolates were carbapenem resistant and 28.21% were carbapenem sensitive. Colistin and tigecycline sensitivity were 100 % and 79%, respectively, gained by disk diffusion method. In the study of Ahmed Nahid (19), the resistance level to antibiotics was 100% for each of meropenem, ceftazidime, cefepime, ciprofloxacin, and piperacillin/tazobactam, and was 80% or more to amikacin, aztreonam, gentamicin, and tobramycin, which was higher than the results of our study. Resistance pattern to ampicillin, amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, and piperacillin/tazobactam was 100%, 50%, 66.7%, 83.3%, 40%, 73.3%, and 70%, respectively in a study done by Nermin H. Ibrahim. (20). Results of our study matched with the results of Fouad et al. (21) in Cairo who found high resistance rate of Acinetobacter species to antibiotics: they found 100% resistance for each of ceftazidime, cefepime, ciprofloxacin, and piperacillin/tazobactam and above 80% for amikacin, aztreonam, gentamicin, and tobramycin (21).

Carbapenem-resistant Acinetobacter baumannii was screened for the presence of carbapenemases by MHT. Out of 224 samples, 212 (94.6%) were positive with MHT in our study. These results are supported by several studies which have found that the MHT is a useful screening test for carbapenemase production (22, 23). The carbapenem resistant isolates were further screened for Metallo-β-lactamase production by performing the Imipenem-EDTA combined disc test (CDT test). 70.5% were positive by CDT in our study. In our study, 94.6% were positive with MHT, but only and 70.5% were positive with CDT. This may be because of the production of carbapenemase other than Metallo-β-lactamase, which is dependent on serine instead of zinc for its function.

In this study, MHT showed the highest sensitivity (94.6%) for detection of carbapenemase producers compared to CDT (70.5%). Similar findings were observed in the study conducted by Lee et al. (24). MHT, which precludes the use of EDTA, detects only carbapenemase activity. Moreover, isolates of Acinetobacter species that were carbapenem resistant and had positive carbapenemase by MHT may be negative for MBL due to non-MBL carbapenemase, which is not dependent on the zinc ion for its action. (25). The modified Hodge test has been extensively used as a phenotypic technique for detecting carbapenemase activity, since it is routinely available in the clinical laboratory and recommended by the CLSI. Though MHT is a feasible test and is easy and economical to check possible production of MBL, the subjective interpretation of its results and possible lack of an

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

appreciable distorted zone of inhibition are the major drawbacks. Also, on the basis of our results and the related discussion, we recommend Imipenem-EDTA combined disc test for routine detection of MBL as it has good sensitivity (70.5%), is easy to perform, has objective interpretation of result, and is economical.

Conclusion

To conclude, this study highlighted the high prevalence of multidrug-resistant A. baumannii isolates, which are emerging as a predominant pathogen in hospitals. Initial screening of the putative carbapenemase producers will help to manage infection-control policy and early directed therapy. Easy detection methods are required for routine clinical labs. Our study showed that tests like MHT are equally efficient to detect carbapenemase production, followed by Imipenem-EDTA combined disc test (CDT). These tests are cost-effective and easy to perform and may be used routinely to assess whether carbapenemase producers are present or not.

Acknowledgments: None

Conflict of interest

The authors have no conflict of interest in this study.

Financial support: Nil

Ethical Statement

Ethical approval for this study was obtained from the Institutional Ethics Committee of Government Medical College of Srinagar.

References

1. Kempf M, Rolain JM. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents 2012;39(2):105–14.
2. Sanjeev H, Swathi N, Asha P, Rekha R, Vimal K, Ganesh HR. Systematic review of Urinary tract infection caused by Acinetobacter species among hospitalised patients. J health Allied Sci 2013;3(04):007-9.
3. Gaur A, Garg A, Prakash P, Anupurba S, Mohapatra TM. Observations on carbapenem resistance by minimum inhibitory concentration in nosocomial isolates of Acinetobacter species: An experience at a tertiary care hospital in north India. J Health Popul Nutr 2008;26(2):183–8.
4. Prashanth K, Badrinath S. Nosocomial infections due to Acinetobacter species: Clinical findings, risk and prognostic factors. 2006;24(1):39-44.
5. RU, Rao RS, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, Prashanth K. Phenotypic and genotypic assays for detecting the prevalence of metallo-β-lactamases in clinical isolates of Acinetobacter baumannii from a South Indian tertiary care hospital. J Med Microbiol 2009;58(4):430-5.
6. Poirel L, Mansour W, Bouallegue O, Nordmann P. Carbapenem-resistant Acinetobacter baumannii isolates from Tunisia producing the OXA-58-like carbapenem-hydrolyzing oxacillinase OXA-97. Antimicrob Agents Chemother 2008;52(5):1613-7
7. Clinical & Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 19th ed; 2009. CLSI supplement M100-S28.
8. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disc method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002;40:3798-801.
9. Clinical & Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 19th ed; 2009. CLSI supplement M100-S28.
10. Padmalakshmi Y, Shanthi M, Sekar U, Arunagiri K. Phenotypic and Molecular Characterisation of Carbapenemases in Acinetobacter Species in a Tertiary Care Centre in Tamil Nadu, India. Nat J Lab Med 2015;4(3):55-60.
11. Kumar SH, De Anuradha S, Baveja SM, Gore MA. Prevalence and risk factors of metallo β-lactamase producing Pseudomonas aeruginosa and Acinetobacter species in burns and surgical wards in a tertiary care hospital. J Lab Physician 2012;4(1):039-42.
12. Noori M, Karimi A, Fallah F, Hashemi A, Alimehr S, Goudarzi H, Aghamohammad S. High Prevalence of Metallo-beta-lactamase Producing Acinetobacter. Arch Pediatr 2014 ;2(3):e15439.

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]

13. Sung JY, Kwon KC, Park JW, Kim YS, Kim JM, Shin KS, et al. Dissemination of IMP-1 and OXA Type beta-Lactamase in Carbapenem resistant Acinetobacter baumannii. Korean J Lab Med 2008;28:16-23.
14. Carvalho RM, Marques SG, Gonçalves LH, Abreu AG, Monteiro SG, Gonçalves AG. Phenotypic detection of metallo-β-lactamases in Pseudomonas aeruginosa and Acinetobacter baumannii isolated from hospitalized patients in São Luis, State of Maranhão, Brazil. Rev Soc Bras Med Trop 2013;46:506-9.
15. García-Garmendia JL, Ortiz-Leyba C, Garnacho-Montero J, Jiménez-Jiménez FJ, Pérez-Paredes C, Barrero-Almodóvar AE, Miner MG. Risk factors for Acinetobacter baumannii nosocomial bacteremia in critically ill patients: a cohort study. Clin Infec Dis 2001 1;33(7):939-46.
16. Mindoli Pb, Salmani Mp, Vishwanath G, hanumanthapa Ar, identification and speciation of Acinetobacter and their antimicrobial susceptibility testing. Al Amen J Med Sci 2010;3:3459.
17. Gupta N, Gandham N, Jadhav S, Mishra RN. Isolation and identification of Acinetobacter species with special reference to antibiotic resistance. Journal of natural science, biology, and medicine. 2015 Jan;6(1):159-62.
18. Lone R, Shah A, Kadri SM, Lone S, Faisal S. Nosocomial multi-drug-resistant Acinetobacter infections - clinical findings, risk factors and demographic characteristics. Bangladesh J Med Microbiol 2009;3(1):34-8.
19. Ahmed NH, Baba K, Clay C, Lekalakala R, Hoosen AA. In vitro activity of tigecycline against clinical isolates of carbapenem resistant Acinetobacter baumannii complex in Pretoria, South Africa. BMC Res Notes 2012;5(1):1-4.
20. Ibrahim NH, Somily AM, Bassyouni RH, El-Aabedien AZ. Comparative study assessing the effect of tigecycline and moxifloxacin in prevention of Acinetobacter baumannii biofilm. Life Sci J 2012;9(3):1016-24 .
21. Fouad M, Attia AS, Tawakkol WM, Hashem AM. Emergence of carbapenem-resistant Acinetobacter baumannii harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. Int J Infect Dis 2013 1;17(12):e1252-4.
22. AL-Harmoosh A, Jarallah EM. First detection of the blaoxa-23 genes in a clinical isolates of acinetobacter baumannii in Hillah hospitals-IRAQ. J Pharm Biol Sci 2015;10:77–83.
23. Aziz RAR, Al-Jubori SS. Molecular analysis of genetic elements responsible for xdr in highly successful pathogen acinetobacter baumannii isolated from clinical samples of Iraqi patients. J Global Pharm Technol 2017;9:26–39.
24. Lee SO, Kim NJ, Choi SH, Kim TH, Chung JW, Woo JH et al. Risk factors for the acquisition of imipenem resistant Acinetobacter baumannii: a case-control study. Antimicrob Agents Chemother 2004;48:224-8.
25. Queenan A, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev 2007;20:440-58.

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

[Downloaded from ijrabms.umsu.ac.ir on 2024-10-12]

[DOI: 10.52547/rabms.8.3.110]