Phenotypic and Genotypic Detection of Carbapenem Resistant Organism Causing Nosocomial Infection in Patients Attending Tertiary Care Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: Nosocomial infections are the leading cause of mortality. ESKAPE organisms are the primary causes of nosocomial infection as these organisms are more or less carbapenem resistant. This study aimed to isolate and identify the etiological agents responsible for causing nosocomial infection and determine the carbapenemase producing organism by phenotypic and genotypic detection.
Study Design: The study design is cross-sectional study.

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Place and Duration of Study: The study was conducted in the Department of Microbiology, at Index Medical College, Indore, between January 2020 and January 2022.

Methodology: Total of 246 samples was collected from the patients who develop symptoms after 48- 72 hrs of hospitalization. Samples were processed for identification of etiological agents. Gram negative organisms were selected and further identified for carbapenemase enzyme. Screening was done by Kirby Bauer disc diffusion test and further confirmed by Modified Hodge test, Combined Disc test, Double Disc Synergy Diffusion test and Carbapenem Inactivation method. Genotypic detection was done by using multiplex polymerase chain reaction for KPC, NDM and OXA-48 gene.

Results: Out of 107-gram-negative organisms, 19 (17.75%) were carbapenem resistant. Among 19 carbapenem resistant GNR, 13% MHT, 15% CCDT, 17% DDST and 17% mCIM were positive. The sensitivity and specificity of MHT, CCDT, DDST, mCIM were 74%/100, 84%/100,95%/100 and 95%/100respectively. The genotypic detection shows highest percentage of bla_{NDM} 74% which is followed by bla $_{OXA-48}$ 31% and bla_{KPC} 26%.

Conclusion: Hospitals have become the hotspot for various microorganism causing nosocomial infections and are getting carbapenem resistance due to irrational use of antibiotics. Antimicrobial stewardship is one of the effective measures that minimize the resistance. Proper universal precaution can also minimize, spread of resistance in organism. If the last resort drug gets resistant, then it could be challenging for the clinician to treat their patients. Hospitals should have regular HAI meeting and release of antibiogram to know the pattern of these notorious organisms invading infection.

1. INTRODUCTION

Nosocomial infections are defined as the infection that develops in 48 to 72hours after admission of patient; the infection which was not present or not incubating when the patient is hospitalized [1]. Urinary tract infection is the most common nosocomial infection, followed by skin and soft tissue infection, blood stream infection and ventilator associated pneumonia [2]. The organisms responsible in causing nosocomial infection are defined as ESKAPE. It includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species* other includes *Escherichia coli, Proteus, Citrobacter* and members of *Enterobacterals* [3,4]. These bacteria are most common in individual critically ill, immunocompromised and who are multi drug resistance, which lead to life-threatening nosocomial infections [5]. The listed organisms are associated with high mortality and morbidity [6]. According to World Health Organization in 2017 carbapenem resistant *Enterobacterales*, carbapenem resistant *Pseudomonas aeruginosa* and carbapenem resistant *Acinetobacter baumannii* were ranked as critical high priority [7]. Every year antimicrobial resistance is increasing, it is expected that there will be increment of ten times of antimicrobial resistance

by 2050 with the projected scenario of mortality among different continents [8]. Carbapenem have been considered as a potent group of antibiotics to treat extended spectrum beta lactamase producing bacteria in past decade. It is widely prescribed for regimen for multidrug resistant gram-negative bacteria, which are capable in causing systemic infection [9-12]. Carbapenem resistance is associated with the production of enzyme carbapenemase. It is encoded by carbapenemase encoding genes, which were classified into four groups of classes [13].

Class A carbapenemase which include *Klebseilla pneumoniae* carbapenemase (bla_{KPC}), imipenem hydrolyzing beta lactamase (bla_{IMI}) and *Serratia marcescens* enzyme (bla_{SME}).Carbapenemase B are Metallo beta-lactamase which include New Delhi metallobeta lactamase (bla_{NDM}), Verona Integron Metallo beta lactamase (bla_{VIM}), Imipenemase (bla_{IMP}),German Imipenemase (bla_{GIM}) , and Sao Paulo Metallo beta-lactamase (bla SPM). Class D carbapenemase include Oxacillinase consist of bla_{OXA-48}, $bla_{OXA-181}$, bla_{OXA-23}, $bla_{OXA-204}$,$bla_{OXA-162}$, and bla_{OXA-24} [13,14]. Carbapenem resistance can also occur by other modes like porin mutation which leads to reduction of outer membrane permeability, over expression of efflux pump, binding sites (penicillin binding protein), and plasmid encoding

carbapenem which can be through horizontal plasmid mediated transmission [9,15]. There are various methods for the detection of carbapenem [16,17]. The detection of carbapenemase is essential for antimicrobial stewardship to get updated about resistance pattern of antibiotics. There are various range of metholies for detection of carbapenem resistance from conventional Kirby Bauer disc diffusion method to phenotypic methods like modified hodge test, combined disc diffusion test, double disc synergy test and Modified carbapenem inactivation method. Genotypic detection is be performed by polymerase chain reaction. Antimicrobial stewardship is crucial to minimize the rate of nosocomial infection, the isolates and identification of etiological agent and their resistance pattern are essential perquisite, to prevent further dissemination of infection and reduce hospital burden. As carbapenem resistance have increased globally, in this regard this study aimed to screen carbapenem producing Gram negative organism causing Nosocomial infection using both phenotypic and genotypic method.

2. MATERIALS AND METHODS

2.1 Study Design

A cross-sectional study was out carried on 246 clinical samples of patients admitted and who develop symptoms after 48 hours. The study was conducted at Index Medical College, Hospital and Research Centre, Indore Madhya Pradesh. The study was carried out from January 2020 to January 2022. All of the methods were under standard guidelines set out by the Clinical and Laboratory Standards Institute (CLSI).

3. BACTERIAL ISOLATION AND IDENTIFICATION [18]

Gram-negative bacteria were isolated and identified by standard manual conventional method from the culture of the routine clinical samples which include Urine, Blood, Pus, Sputum, Endotracheal Aspirate, HAI's sample (Pleural fluid, Ascitic fluid, Peritoneal fluid, Rectal swab). The samples were inoculated on blood agar (Himedia), MacConkey agar (Himedia). Blood and respiratory samples were inoculated also include on chocolate agar (Himedia). Urine sample was inoculated on CLED agar (Himedia). The samples were incubation at 37°C ± °C, for 24 hours. Colony morphology of growth plates

was performed and Gram staining (Himedia) was performed from the samples which show growth. Biochemical identification was performed on the isolates which were gram-negative bacilli. The biochemical tests involve Catalase test, Oxidase test, Indole test, Methyl red, Voges-Proskauer test, Citrate utilization test, Urea hydrolysis test, Motility test, Triple sugar iron test, Hugh and Leifson test and nitrate reduction test were performed for identification of gram-negative bacilli.

4. CARBAPENEM-RESISTANCE SCREENING

All the gram-negative bacilli were screened for Carbapenem-resistance. It was done by using Meropenem (10ug) disc, Imipenem (10ug) disc and Ertapenem (10ug) disc. Interpretation of result was done under the guidelines of Clinical and Laboratory Standards Institute.

4.1 Phenotypic Detection

4.1.1 Detection of carbapenemase production

4.1.1.1 Modified hodge test [19]

According to Clinical and Laboratory Standards Institute (CLSI)(2018) guidelines, all isolates were subjected to the modified Hodge test. A lawn culture of the 1:10 dilution of 0.5 McFarland suspension of *Escherichia coli* ATCC 25922 was carried out on Mueller Hinton agar plate. Meropenem (10ug) disc was placed in the center of the charged with *Escherichia coli* ATCC 25922 on Mueller Hinton agar plate. A straight line was drawn from the edge of the disk to periphery of the plate with the test organism. A control strains (positive and negative) was also tested on the same plate drawn from the edge of the disk to periphery of the plate. The plates were incubated at 35 °C ± 2 °C for 24 hours. Interpretation of Positive modified Hodge observe by clover leaf-like indentation of the *Escherichia coli* 25922 strain growing along with the test organism growth streak within the disk diffusion zone indicating the production of enzyme carbapenemase and a negative test showed no growth of the *Escherichia coli* ATCC 25922 along the test organism growth streak within the disk diffusion zone. *Klebsiella pneumoniae* (ATCC BAA-1705) and *Klebsiella pneumoniae* (ATCC BAA-1706) were used as positive and negative controls respectively.

4.1.1.2 Modified carbapenem Inactivation method (mCIM) [19]

The mCIM method was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines with use of imipenem disc. A loopful of bacterial isolate from an overnight culture plate, was emulsified in 2 mL of Tryptone Soya Broth (TSB). A imipenem disc (10µg) was immersed in Tryptone Soya Broth. Tryptone Soya Broth (TSB) containing imipenem disc was incubated at 35 °C ± 2 °C for two -four hours. 0.5 McFarland suspension of *Escherichia coli* ATCC 25922 in nutrient broth was made with the help of cotton swab lawn culture on a Mueller Hinton agar. *Escherichia coli* ATCC 25922 will act as susceptible strain for potent imipenem disc. Now allow the plates to dry for 3-10 minutes. Imipenem disc from TSB-imipenem disc suspension was picked before applying on the plate, pressed the excess fluid with help of sterile inoculating loop. With the help of loop removed the disc from the tube and then placed on the Mueller Hinton agar plate previously charged with *Escherichia coli* ATCC 25922 imipenem susceptible. Plates were incubated at 35 °C ± 2 °C for 18-24 hours. Interpretation of positive result show zone between 6-15 mm or presence of colonies within a 16-18 mm zone. Presence of enzyme carbapenemase, the imipenem disc will be hydrolysed and there will be no inhibition of the imipenem-susceptible *Escherichia coli* ATCC 25922. Negative result show zone more than 19 mm. Absence of enzyme carbapenemase, the imipenem disc will not be hydrolysed and will inhibit growth of the imipenem-susceptible E. coli ATCC 25922. Indeterminate show zone between 16-18 mm. *Klebsiella pneumoniae* ATCC BAA-1705 and *Escherichia coli* ATCC 25922 were used as positive and negative controls respectively.

5. DETECTION OF METALLO-β-LACTAMASE

5.1 Combined Disc Test (CDT) [20]

The test organisms were inoculated on Mueller Hinton agar plate as per Clinical and Laboratory Standards Institute (CLSI) guidelines. After drying, two 10 µg Meropenem discs were placed on the lawn culture with 20 mm distance from centre to centre of the discs. A 10 µl of 0.5 M EDTA (Himedia) was added to one of the Meropenem discs and incubated overnight. Incubate at 35 °C ± 2 °C for 24 hours. The inhibition zones of Meropenem and Meropenem plus EDTA were compared after incubation. Interpretation of positive result show increase in zone of diameter for Meropenem plus EDTA of ≥5 mm than Meropenem alone.

5.2 Meropenem EDTA Double Disc Synergy Test (DDST) [21]

0.5 McFarland was inoculated on Mueller Hinton agar plate. After drying, a Meropenem disc (10 µg) and a blank filter paper disk was placed 10mm apart from edge to edge, 10 µl of 0.5 M EDTA (Himedia) solution was then applied to the blank filter paper disc, to achieve the concentration of 750 µg . The plates were incubated at 37°C for 24 hours. Interpretation of positive is indicated as enhancement in the zone of diameter of > 5 mm.

6. GENOTYPIC DETECTIONS [22]

DNA was extracted by using Qiagen miniprep kit as per the brochure provided in the kit. The DNA of each isolate were subjected to multiplex PCR of bla_{NDM}, bla_{KPC}, $bla_{OXA\ 48}$ were according to Martha F. Mushi et al. 20 µL PCR reaction mixture includes 10 µL of master mix, 0.5 µL of primer and 4 µL nuclease free water, 2 µL Q buffer, and 200ng of purified DNA template. The PCR amplification was done using Applied Biosynthesis thermal cycler. A total 30 cycles were programmed with the initial denaturation cycle for 10 minutes at 95^0C, followed by 30 seconds denaturation at 94^0C, Annealing for 30 seconds at 55^0C and Extension for 1 minute at 72^0C for bla_{NDM} bla_{KPC}, $bla_{OXA\ 48}$. Additional final extension is required for 7 minutes at 72^0C.

7. RESULTS

A total of 246 clinical samples were collected during the study period from Jan 2020 to Jan 2022. Out of 246, 107 (43.49%) were gram negative bacilli. From 107-gram negative bacteria, the highest percentage were of *Escherichia coli*(32.55%) followed by, *Klebseilla pneumoniae* (17.5%), *Citrobacter species* and Pseudomonas aeruginosa (8.52), *Acientobacter Species* (7.55%), Proteus mirabilis (4.56%) and *Enterobacter species* (3.87%). On performing Kirby Bauer disc diffusion method 52(59.09%) male patient were sensitive and 12 (63.15%) were resistant to carbapenem whereas 36(40.90%) were sensitive and 7(36.84%) were resistant to carbapenem. The statistical analysis of *p*-value = .00001 for both carbapenem resistant and carbapenem sensitive as shown in Table 1.

Out of 19 carbapenem resistance 14 isolates were show MHT positive results whereas 16 were CDDT positive, 18 were DDST and mCIM positive. Among 107-gram negative bacteria 19 (17.75%) were screened by Kirby Bauer. Among the phenotypic methods for detection of Carbapenem was done by MHT and mCIM. For Metallo beta lactamase detection was done by CDDT and DDST. The sensitivity of MHT, mCIM, CDDT, DDST within CI 95% were 74%, 95%, 84% and 95% respectively shown in Table 2.

Table 1. Bivariate Associations for Demographics presentation of gram-negative carbapenem resistant and gram-negative carbapenem sensitive patient

Characteristic	Frequency N%	Carbapenem Sensitive N%	Carbapenem Resistant N%
Gender			
Male	64(59.8)	52(59.09)	12(63.15)
Female	43(40.1)	36(40.90)	7(36.84)
Residence			
Urban	34(31.7)	29(32.9)	5(26.3)
Rural	73(68.2)	59(67.0)	As
Age group (in year)			
less than10	6 (5.6)	5(4.6)	1(5.2)
11_ 20	17(15.8)	13(14.7)	4(21.0)
21-30	32(29.9)	24(27.2)	8(42.1)
31-40	22(20.5)	20(22.7)	2(10.5)
41-50	14(13.0)	12(13.6)	2(10.5)
> 50	16(14.9)	14(15.9)	2(10.5)
Wards			
Medicine	55(51.4)	49(55.6)	6(6.8)
Surgery	23(21.4)	18(20.4)	5(5.6)
Pediatrics	8(7.4)	6(6.8)	2(2.2)
Obstetrics & Gynecology	6(5.6)	4(4.5)	2(2.2)
Intensive care unit	14(13.0)	11(12.5)	2(2.2)
Previous history of admission			
Yes	32(29.9)	29(32.9)	3(15.7)
No	75(70.0)	59(67.0)	16(84.2)
New antibiotic			
Yes	23(21.4)	15(17.04)	8(42.1)
No	84(78.5)	73(82.9)	11(57.8)
Length of hospital days			
<5 days	65(60.7)	54(61.3)	11(57.8)
5-7 days	37(34.5)	31(35.2)	6(31.5)
> 7 days	5(4.6)	3(3.4)	2(10.5)

Table 2. Comparison between different phenotypic methods of carbapenem resistance among gram negative bacteria

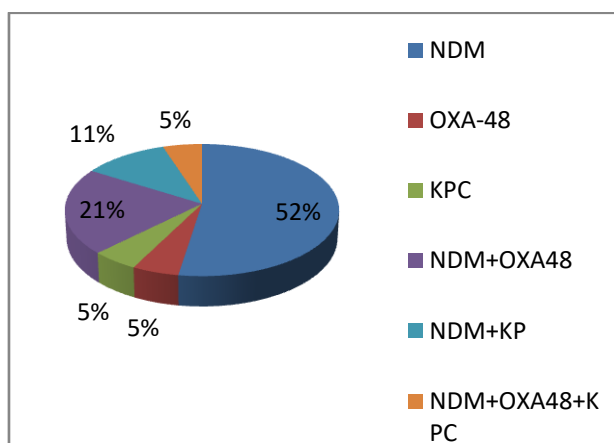| Organism	No. of isolates	Disc Diffusion test	MHT	CDDT	DDST	mCIM
Escherichia coli	42	4	2	2	3	4
Klebseilla pneumoniae	22	4	3	4	4	4
Citrobacter species	11	3	3	3	3	2
Enterobacter species	5	1	0	1	1	1
Proteus species	6	1	1	1	1	1
Pseudomonas aeruginosa	11	3	3	3	3	3
Acientobacter Species	10	3	2	2	3	3
Total	107	19	14	16	18	18

Fig. 1. Distribution of Genes

Several isolates carried more than one gene. About 52% of gene carried bla$_{NDM}$ alone which was followed by 5% of bla$_{OXA-48}$ and bla$_{KPC}$ .The coexisting genes include the highest prevalence of 21% bla$_{NDM+OXA-48}$ followed by bla$_{NDM+KPC}$11% and 5% were found in all three genes. The highest prevalence of gene was seen *Escherichia coli* 26% which is followed by *Klebseilla pneumoniae*21% and 16% of *Citrobacter species*, *Pseudomonas aeruginosa* and *Acientobacter Species* with least prevalence of 5% in *Enterobacter Species* The prevalence of resistance genes in *Enterobacterals* were dominant in *Escherichia coli* bla$_{NDM}$, bla$_{OXA-48}$, co-existence of blaNDM$_{+OXA-48}$ and bla$_{NDM+KPC}$. *Klebseilla pneumoniae* to be second dominant organism consist of bla$_{NDM}$, bla$_{KPC}$ and bla$_{NDM+OXA-48+KPC}$. *Citrobacter species* and *Enterobacter species* consist of only bla$_{NDM}$. Whereas *Proteus species* do not contain any resistant gene. In non-fermenter *Pseudomonas aeruginosa* and *Acientobacter Species* contain bla$_{NDM}$ and bla$_{NDM+OXa-48}$ is mentioned in Fig. 1.

8. DISCUSSION

In a study by Mohamudha R.P et al. [23] it is found Meropenem resistance of 45 isolates, Imipenem and Ertapenem resistance of 33 and 21 respectively and Gupta E et al. [24] found 22.16% of overall, resistance to Meropenem and 17.32% Imipenem. In our study out of 19 carbapenem resistant microorganisms, 14 isolates show MHT positive result whereas 16 were CDDT positive, 18 were DDST and mCIM positive. Among 107-gram-negative bacteria 19 (17.75%) were carbapenem resistant by Kirby Bauer. The phenotypic methods for detection of Carbapenem were done by MHT and mCIM. Metallo beta lactamase detection was done by

CDDT and DDST. The sensitivity of MHT, mCIM, CDDT, DDST within CI 95% were 74%, 95%, 84%, 95% respectively. Similar study reported by Naim H, et al. [25] show CDDT 84.81%, MHT, and DDST 97.41% and 84.81%. A study by Cury et al. [26], reported 35.5% MHT positive *Enterobacterals*. Several isolates carried more than one gene. New Delhi metallo-β-lactamase (52%) was the pre-dominant gene in this study, which was similar to that reported by Naim et al. [25] which is followed by 5% ofbla$_{OXA-48}$ and bla$_{KPC}$. In one of the studies, it is reported highest percentage of bla$_{NDM}$ 83%,bla$_{OXA-48}$ 75%,bla$_{VIM}$ 49% and bla$_{IMP}$ 43%, while reported least percentage of bla KPC [27,28,29].

The coexisting genes include the highest prevalence of 21% bla$_{NDM+OXA-48}$. In contrast to one of the studies, the co-existence of bla$_{VIM}$ and bla$_{NDM}$ was 39.6%, whereas Mohanam et al. [30] reported 14.6% and Ellappan et al. [31] reported 17.3% co-existence genes. In our study it was followed by bla$_{NDM+KPC}$11% and 5% were found in all three genes. The highest prevalence of gene was seen *Escherichia coli* 26% which is followed by *Klebseilla pneumoniae* 21% and 16% of citrobacter species, *Pseudomonas aeruginosa* and *Acientobacter Species* with least prevalence of 5% in *Enterobacter Species*. KPC is endemic in Israel, while bla$_{VIM}$, bla$_{IPM}$, bla$_{NDM}$ and bla$_{OXA-48}$ are endemic in Greece ,Japan , India and Turkey and it is disseminated around the world [32]. The genes in the blood isolated were 1 bla$_{NDM+blaOXA-48}$ and 1 bla$_{NDM}$. The genes in urine were 5bla$_{NDM}$ followed by 1 bla$_{OXA-48}$. The gene in pus the genes were 1 bla$_{NDM+OXA-4}$, 1 bla$_{NDM}$. In sputum 1 bla$_{NDM+KPC}$, 1 bla$_{NDM}$ and 1 bla$_{KPC}$. In Endotracheal aspirate gene were 2 bla$_{NDM +OXA-48}$ followed by 1 bla$_{NDM}$. In other samples from hospital associated infection includes 1 bla$_{NDM}$and 1 bla$_{NDM+OXA-48}$. In

our study none of the non-fermenter consist bla_{OXA-48}. Similar study was reported by Vamsi et al. [33] which also reported no genes of blaOXA - 48 were detected. Several studies reported co-harboring of carbapenemase [34,35]. Coexistence of carbapenemase genes is a therapeutic challenge for clinicians. It is due to restricted treatment options and the potential for world-wide spread by horizontal transfer [36].

9. CONCLUSION

In our study it is found that phenotypic determinant has sensitivity of MHT, mCIM, CDDT, DDST within CI 95% was 74%, 95%, 84%, 95% respectively. It is important to perform genotypic detection to avoid false positive results as there is a call of carbapenem resistance in hospital settings. In our study 52% of gene was carried by bla_{NDM} alone which is followed by 5% of $bla_{OXA-48}$and bla_{KPC}. The coexisting genes include the highest prevalence of 21% $bla_{NDM+OXA-48}$followed by $bla_{NDM+KPC}$11% and 5% were found in all three genes. The highest prevalence of gene was seen *Escherichia coli* 26% which is followed by *Klebseilla pneumoniae* 21% and 16% of *citrobacter species*, *Pseudomonas aeruginosa* and *Acientobacter Species* with least prevalence of 5% in *Enterobacter Species* The carbapenem nosocomial infection can be stopped by regular update of antimicrobial susceptibility surveillance, If the measures followed are continuously updated and upgraded the infection level can be fur every hospital should have infection control meeting and release antibiogram for the clinician to get updated about the antimicrobial resistance pattern, which will also help the society. Hand hygiene is the key to break the spread of infection and control most of the multidrug resistant organism.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by ethics committee MU/MM/BNS/2020/51(a).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Revelas A. Healthcare - associated infections: A public health problem. Niger Med J. 2012;53(2):59–64.
2. Nuvials X, Palomar M, Alvarez-Lerma F, et al. Health-care associated infections. Patient characteristics and influence on the clinical outcome of patients admitted to ICU. Envin-Helics registry data. Intensive Care Med Exp. 2015;3(Suppl 1): A82.
3. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: No ESKAPE. Journal of Infectious Disease. 2008;197(8):1079–1081.
4. Bush K and Jacoby GA. Updated functional classification of beta - lactamases, Antimicrobial Agents and Chemotherapy. 2010;54(3):969–976.
5. Rice LB, Progress and challenges in implementing the research on ESKAPE pathogens, Infection Control and Hospital Epidemiology. 2010;31(supplement 1):S7–S10.
6. Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS One. 2017;Dec 21;12(12) :e0189621.
   DOI:10.1371/journal.pone.0189621Tacconelli
7. E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018; 18:318-327.
8. O'Neill J. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations.
   Available:http://amr-review.org/Publications
   (Accessed: April 2019)
9. Bourafa N, et al. Molecular characterization of carbapenem-resistant Gram-negative bacilli clinical isolates in Algeria. Infection and drug resistance. 2018;11:735.
10. Karuniawati A, Saharman YR, Lestari DC. Detection of carbapenemase encoding genes in Enterobacteriace, Pseudomonas aeruginosa, and Acinetobacter baumanii isolated from patients at Intensive Care Unit CiptoMangunkusumo Hospital in

2011. Acta Med Indones. 2013;45(2): 101–6.

11. McKibben L, et al. Guidance on public reporting of healthcare-associated infections:recommendations of the Healthcare Infection Control Practices Advisory Committee. American journal of infection control. 2005;33(4):217–226. Available:https://doi.org/10.1086/502585

12. Okoche D, Asiimwe BB, Katabazi AF, Kato L, Najjuka FC. Prevalence and characterization of carbapenem - resistant enterobacteriaceae isolated from Mulago national referral hospital, Uganda. PLOS One. 2015;10(8):e0135745.

13. Deldar Abad Paskeh M, Mehdipour Moghaddam MJ, and Salehi Z. Prevalence of plasmid-encoded carbapenemases in multi-drug resistant *Escherichia coli* from patients with urinary tract infection in northern Iran. Iranian Journal of Basic Medical Sciences. 2020;23(5):586–593.
Available:http://ijbms.mums.
ac.ir/article_14823.html
https://doi.org/10.22038/ijbms.2020.34563.8199

14. Sheikh AF, et al. Emerge of NDM-1-producing multidrug-resistant pseudomonas aeruginosa and coharboring of carbapenemase genes in South of Iran. Iranian Journal of Public Health. 2020; 49(5):959.

15. Workneh M, Yee R, and Simner PJ. Phenotypic methods for detection of carbapenemase production in carbapenem-resistant organisms: What method should your laboratory choose? Clinical microbiology newsletter, 2019; 41(2):11–22.
Available:10.1016/j.clinmicnews.2019.01.001

16. Andre B, Philippe B, Nathalie G, Catherine D, Dominique D, Guillaume A, et al. Phenotypic screening of carbapenemases and associated β-lactamases in carbapenem-resistant enterobacteriaceae. J Clin Microbiol. 2012;50(4):1295-302.

17. Datta P, Gupta V, Garg S, Chander J. Phenotypic method for differentiation of [9] carbapenemases in Enterobacteriaceae: Study from north India. Indian J PatholMicrobiol. 2012;55:357-60.

18. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney's Practical medical microbiology, 14th ed. Churchill Livingstone; 2006.

19. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; CLSI document M100-S27. Wayne PA: Clinical and Laboratory Standards Institute; 2017.

20. Pournaras S, Zarkotou O, Poulou A, Kristo I, Vrioni G, Themeli-Digalaki K, et al. A combined disk test for direct differentiation of carbapenemase-producing enterobacteriaceae in surveillance rectal swabs. J Clin Microbiol. 2013;51(9):2986-90.

21. Dheepa Muthusamy, Appalraju Boppe. Phenotypic Methods for the detection of various Betalactamases in Carbapenamase resistant isolates of A.baumannii in a Tertiary care Hospital in a south India. India. J of Clin Diagnostic Research. 2012;6(6):970-73.

22. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant gram-negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. Biomed Res Int. 2014; 2014:303104.
DOI: 10.1155/2014/303104.

23. Mohamudha RP, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of Klebsiella pneumoniae in South India. Inter Journal Pharma and Bio Sci. 2010;6(2).

24. Gupta E, Mohanty S, Sood S, et al. Emerging resistance to carbapenems in a tertiary care hospital in north India. Indian J Med Res. 2006;124(1):95– 9.

25. Naim H, Rizvi M, Gupta R, Azam M, Taneja N, Shukla I, et al. Drug resistance and molecular epidemiology of carbapenem resistant gram-negative bacilli isolates. J Global Infect Dis 2018; 10:13

26. Cury AP, Andreazzi D, Maffucci M, Caiaffa-Junior HH, Rossi F. The modified Hodge test is a useful tool for ruling out *Klebsiella pneumoniae* carbapenemase. Clinics (Sao Paulo) 2012;67:1427-31.

27. Codjoe, FS, Detection and characterization of carbapenem-resistant gram-negative bacilli infections in Ghana; 2016, Sheffield Hallam University.
Available:http://shura.shu.ac.uk/id/eprint/15577

28. Nachimuthu R. et al. Characterization of carbapenem-resistant Gram-negative bacteria from Tamil Nadu. J. Chemother. 2016;28:371–374.

29. Kazi M, *et al.* Molecular characterization of carbapenem-resistant Enterobacteriaceae at a tertiary care laboratory in Mumbai. Eur. J. Clin. Microbiol. Infect. Dis. 2015; 34:467–472.

30. Mohanam L, Menon T. Coexistence of metallo-beta-lactamase-encoding genes in Pseudomonas aeruginosa. Indian J Med Res. 2017;146:S46-S52.

31. Ellappan K, Belgode Narasimha H, Kumar S. Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant *Pseudomonas aeruginosa* strains from a tertiary care hospital in South India. J Glob Antimicrob Resist. 2018;12:37-43.

32. Canton R, Akova M, Carmeli Y, etal Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clinical Microbiology and Infection. 2012; 18:413-431.

33. Sreeja K Vamsi Rama S, Moorthy, Mary N, Hemiliamma, Rama B, Chandra Reddy, Deepak J chanderakant. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. Saudi Med J. 2022;43(3):236-243.

34. Karabay O, et al. The carbapenem-resistant Enterobacteriaceae threat is growing: NDM-1 epidemic at a training hospital in Turkey. Annals of clinical microbiology and antimicrobials, 2016; 15(1):1–6.

35. Sadeghi MR, et al. Molecular characterization of extended-spectrum β-lactamase, plasmid-mediated AmpC cephalosporinase and carbapenemase genes among Enterobacteriaceae isolates in five medical centres of East and West Azerbaijan, Iran. Journal of medical microbiology. 2016;65(11):1322–1331.

36. Solgi H, et al. Molecular characterization of intestinal carriage of carbapenem-resistant Enterobacteriaceae among inpatients at two Iranian university hospitals: First report of co-production of bla NDM-7 and bla OXA-48. European Journal of Clinical Microbiology & Infectious Diseases. 2017; 36(11):2127–2135.

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