Section: Microbiology

Original Research Article

PHENOTYPIC CHARACTERIZATION OF CARBAPENEM RESISTANT ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE AT A TERTIARY CARE HOSPITAL IN INDORE, INDIA

Manish Kumar Tiwari[1], Ramanath Karicheri[2], Himanshu Bhim Khatri[3]

[1]PhD scholar, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[2]Professor, Department of Microbiology, Index Medical College Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India
[3]Associate Professor, Department of Microbiology, Surabhi Institute of Medical Sciences, Siddipet, Telangana, India.

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Corresponding Author:
Dr. Ramanath Karicheri,
Email: ramanath.karicheri@gmail.com

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Abstract

Background: Carbapenem is one of the last resort antibiotics used for infections caused by multidrug-resistant Enterobacteriaceae. But Enterobacteriaceae can produce the enzyme carbapenemase and become carbapenem resistant. The presence of carbapenemase can be detected by using methods like the Carba-NP test and the modified carbapenem inactivation method (mCIM). The aim of the study was to detect carbapenemase production in Escherichia coli and Klebsiella pneumoniae isolates by the CarbaNP and modified carbapenem inactivation method (mCIM). **Materials and Methods:** Fifty dry human radii were collected from the Department of Anatomy at Rajah Muthiah Medical College, Chidambaram, and Government Erode Medical College, Perundurai, for this study. The number, position, and direction of the foramen were observed in each bone. **Result:** A total of 156 Escherichia coli and Klebsiella pneumoniae isolates which were detected for carbapenem resistance by the Kirby Bauer disc diffusion method were subjected to CarbaNP and mCIM tests. The positivity for mCIM was slightly higher (94.88 %) when compared with the Carba NP test (91.02%). The results of the present study did not reveal any statistical difference in the detection of carbapenemase production by both methods. (p-value> 0.05). **Conclusion:** The Carba NP and mCIM tests were equally effective in detection of the carbapenamase production.

INTRODUCTION

There is an increasing risk to public health due to the global spread of carbapenem resistance in Enterobacteriaceae.[1] It is imperative to identify these drug-resistant organisms promptly in order to treat the patient effectively. In both the hospital and community settings, resistant bacteria are always prevalent, but in a hospital situation, they are more frequent.[2] Carbapenems are one of the last-resort antimicrobials used to treat infections caused by multidrug-resistant Enterobacteriaceae.[3,4] Enterobacteriaceae were once susceptible to carbapenems but they are now quickly developing resistance to them. This is a cause for worry.[5,6] Numerous reports show a rise in the number of healthcare associated infections caused by multidrug resistant (MDR) organisms.[7] In order to overcome various classes of antibiotics, bacteria have evolved various drug resistance mechanisms, including the production of enzymes,[8] alteration of the target site of action, an antimicrobial efflux pump system, alteration of diffusion barriers, and modified metabolic activity.[9,10] Carbapenems have the broadest antimicrobial spectrum of all the β- lactam antibiotics currently available.[11] The reason for this is that they have a higher affinity for penicillin binding proteins (PBPs), are usually stable against serine-based β- lactamases, and have unprecedented outer membrane permeability.[12] However, the widespread use of CREs has put the use of carbapenems in danger. The CDC defines CREs as bacteria that screen positive for resistance to at least one carbapenem antibiotic (ertapenem, meropenem, doripenem, or imipenem) or that produce a carbapenemase (an enzyme that makes them resistant to carbapenem antibiotics).[13,14] The ability of these organisms to multiply and their capacity to horizontally transfer plasmid carrying resistant genes to other organisms have led to a rise in the spread of carbapenem-resistant organisms.[15] Carbapenemases belong to various classes: A (KPC), B (IMP, VIM,

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NDM), and D (OXA-48, OXA-181).[16] Infections brought on by CRE have worse outcomes.[17] For the treatment of these virulent organisms, there are very few antibiotics available but these antibiotics have higher side effects and are more expensive.[18] Currently, antibiotics like polymyxins, tigecycline, fosfomycin, aminoglycosides, and temocillin are used to treat CRE infections.[19] The function of carbapenem-containing regimes in combination with other antibiotics is not yet clear.[20] Expeditious and accurate identification of carbapenemase-producing carbapenem-resistant Enterobacteriaceae (CP-CRE) is critical for halting the spread of these pathogens.[21] There are tests that can be used to identify carbapenemase production in cultured isolates by using both phenotypic and molecular methods. There are two main types of phenotypic tests that are used nowadays: (i) growth-based assays that determine how well a standard microorganism grows when exposed to an antibiotic disc that is previously exposed to a test organism (such as the modified carbapenem inactivation method [mCIM]); (ii) hydrolysis methods, by detecting the product of hydrolysis catalyzed by carbapenemases (such as Carba NP).[22] The present study aimed at the detection of carbapenemase production by mCIM and CarbaNP test.

MATERIALS AND METHODS

Study design: Prospective and observational study.
Study setting: Department of Microbiology at a tertiary care hospital in Indore, India.
Study period: December 2020 to December 2022

Ethical Consideration

Before the commencement of the study, clearance from the institutional ethics committee (IEC) was taken (IEC approval letter No: MU/Research/EC/Ph.D./2020/57). The study subjects were explained in detail the purpose of the study and were assured confidentiality of their identity. Written informed consent was taken from all the patients before collecting their samples.

Study Population

All patients admitted in the hospital wards and ICUs or visiting the outpatient department of the hospital.

Sampling

All consecutive, non-duplicate samples were included till the sample size was met.

Inclusion Criteria

Isolates of Escherichia coli and Klebsiella pneumoniae that was resistant to either ertapenem or meropenem or both. The breakpoint for determining resistance was equal to or less than 18 mm and 19 mm for ertapenem (10 µg) and meropenem (10 µg), respectively.

Exclusion Criteria

Isolates of Escherichia coli and Klebsiella pneumoniae that were intermediate or susceptible to ertapenem and meropenem, and other Gram negative bacteria.

Methodology

Clinical samples such as urine, pus, sputum, endotracheal aspirate (ETA), bronchoalveolar lavage (BAL), and blood were collected aseptically as per the standard operating procedure (SOP). They were aseptically inoculated onto Blood and MacConkey agar plates and incubated at 370C for 16-18 hours. Escherichia coli and Klebsiella pneumoniae were identified based on their culture characteristics and conventional biochemical testing. Patients of all age groups were included in the study. The isolates that were resistant to either meropenem or ertapenem or both as per CLSI M100 2021 standards by the Kirby-Bauer disk diffusion method were included in the study. These isolates were further subjected to modified Carba NP and carbapenem inactivation method (mCIM) test to detect CRE and the result was analyzed. The Carba NP and mCIM tests was done as per CLSI M100 2021 guidelines.[23]

Carba NP Test[23]

The meropenem resistant Klebsiella pneumoniae and Escherichia coli isolates were grown overnight on Mueller-Hinton agar (MHA). The bacterial mass was scraped off with a 1-µl loop and suspended in a 1.5-ml Eppendorf tube containing 100 µl of 20m MTris-HCl lysis buffer and mixed using a vortex device for 5 sec. This lysate was mixed with 100 µl of an aqueous indicator solution consisting of (3 ml 0.5% phenol red + 16.6 ml of distilled water + 180 µl of 10mM ZnSO4.7H2O previously adjusted to pH 7.8 by 0.1N NaOH) and 12 mg/ml imipenem-cilastatin injectable form (equivalent to 6 mg of imipenem standard powder) (reaction tube). A control tube was also set that did not contain the antibiotic imipenem-cilastatin. Tubes were vigorously mixed for 5 to 10 sec. using a vortex device before incubation. Tubes were incubated at 35°C for 2hrs.

Interpretation

The tubes were monitored throughout 2 h for colour change from red to orange/yellow in the antibiotic-containing tube, which was interpreted as a positive result. [Figure 1].

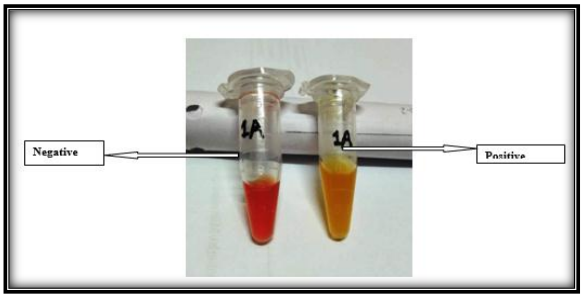

Figure 1. Interpretation of CarbaNP test.

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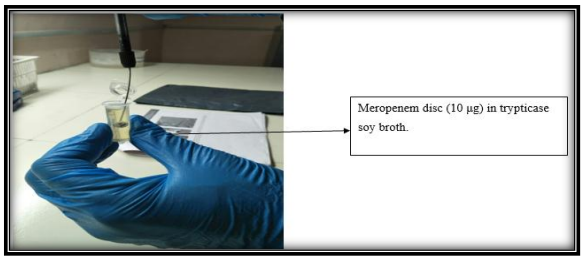

Figure 2. Tubes with 2 mL of trypticase soy broth (TSB) with 1 µL bacterial inoculum and 10 µg Meropenem Disc.

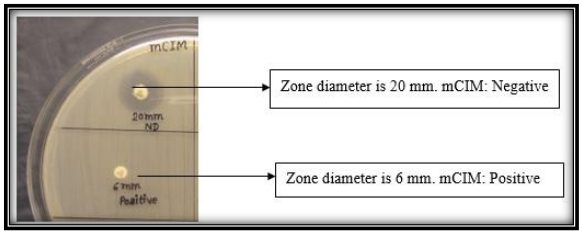

Figure 3. Escherichia coli ATCC 25922 Overnight Lawn cultured with a Meropenem (10 µg) Disc in Position.

Modified Carbapenem Inactivation Method (mCIM):[23]

Escherichia coli and Klebsiella pneumoniae isolates found resistant to carbapenem by the Kirby Bauer disc diffusion were sub-cultured on a blood agar plate and incubated at 350 C for 18 to 24 hours. From the sub-cultured plate, 1 µl loopful of the isolated colony was taken and suspended in 2 ml of trypticase soy broth (TSB). The mixture was vortexed, 10 µg meropenem (carbapenem) discs were added and incubated for 4 hours at 350 C [Figure 2]. Just prior to the completion of 4 hours of incubation, a 0.5 McFarland suspension of Escherichia coli ATCC 25922 was prepared and lawn cultured onto a Mueller Hinton Agar (MHA) plate. After the completion of 4 hours the meropenem disc was removed from the mixture using a 10 µl loop, taking care to remove excess liquid from the disc and it was immediately placed on Escherichia coli ATCC 25922 prepared MHA plate. This plate was then incubated overnight (18 – 24 hrs.) at 350 C. The next day the size of the zone of inhibition was measured. Zone size ≥ 19 mm was taken as negative and a zone size of 6 – 15 mm or the presence of pinpoint colonies within a 16 - 18 mm zone was taken as positive for carbapenemase producing Enterobacteriaceae. [Figure 3].

RESULTS

A total of 865 Escherichia coli and Klebsiella pneumoniae were isolated during the study period.156 isolates of the total 865 isolates were found to be carbapenem resistant. The prevalence rate of carbapenem resistant Escherichia coli and Klebsiella pneumoniae was estimated to be 18%. We have included 96 samples of Escherichia coli isolates and 60 samples of Klebsiella pneumoniae isolates in our study [Table 1]. Sample distribution based on sample type was as follows: the number of endotracheal tubes (ET) samples was 04, bronchoalveolar lavage (BAL) samples were 2, blood samples were 27, pus samples were 23, sputum samples were 13, and urine samples was 87. Urine was the most frequent sample type in our study. [Table 2] An effort to seek any meaningful correlation between demographic variables and CRE by mCIM was also made. [Table 3] shows the age group distribution of isolates. The patients' ages were taken from 01 years old to up to 90 years old and subjects were categorized into 9 groups with a ten-year age difference. According to our observation, the age group 31 – 40 years had the highest number of samples than other groups whereas the age group of >90 years had the least number of samples. [Table 4] shows the gender wise distribution of isolates. In our study, there was a preponderance of males of females.

All these 156 bacterial isolates (100 %) were meropenem resistant as per disk diffusion method using CLSI 2021 guidelines. The chi-square test was used to check for the difference in the percentage of positivity by both methods for all isolates [Table 5], for E.coli [Table 6] and for Klebsiella pneumoniae [Table 7]. All the differences were not statistically significant (p> 0.05).

Table 1: Subject distribution based on E. coli and K. pneumoniae.

Isolate	No of subjects (total =156)	Percentage (%)
Escherichia coli	96	61.53%
Klebsiella pneumoniae	60	38.46 %

Table 2: Sample distribution based on sample type.

Sample type	No of the sample (total =156)	Percentage (%)
Endotracheal tube (ET)	4	2.5 %
Bronchoalveolar lavage (BAL)	2	1.25 %
Blood	27	17.30 %
Pus	23	14.74 %
Sputum	13	8.33 %
Urine	87	55.76 %

Table 3: Subjects distribution based on age group

Age group	No of subjects (total =156)	Percentage (%)
<20 years	4	2.5 %
21-30 years	35	22.43 %

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31-40 years	36	23.07 %
41-50 years	26	16.66 %
51-60 years	26	16.66 %
61-70 years	15	9.6 %
71-80 years	8	5.0 %
81-90 years	5	3.2 %
>90 years	1	0.64 %

Table 4: Subjects distribution based on gender

Gender	No of subjects (total =156)	Percentage (%)
Male	85	54.48 %
Female	71	45.51 %

Table 5: Comparison of Carba-NP and mCIM results of total isolates.

Result	Total isolates				Statistical significance	
	Carba-NP	Percentage	mCIM	Percentage	Chi- Square test	p value
Positive	142	91.02%	148	94.88 %	1.76	0.18 (>0.05- Not significant)
Negative	14	08.98%	08	5.12 %		

Table 6: Comparison of Carba-NP and mCIM results of Escherichia coli isolates.

Result	E. coli isolates				Statistical significance	
	Carba-NP	%	mCIM	%	Chi-Square test	p-value
Positive	87	90.62%	91	94.80 %	1.23	0.26 (>0.05- Not significant)
Negative	09	09.38%	05	5.20 %		

Table 7: Comparison of Carba-NP and mCIM results of Klebsiella pneumoniae isolates.

Result	K. pneumoniae isolates				Statistical significance	
	Carba-NP	%	mCIM	%	Chi- Square test	p value
Positive	55	91.66 %	57	95 %	0.53	0.46 (>0.05- Not significant)
Negative	05	8.44 %	03	5 %		

DISCUSSION

When comparing the number of isolates we had a higher number of E. coli isolates (61.53%) as compared to K. pneumoniae (38.46%). This was consistent with many studies like the one by Thomas et al. (E. coli=63.75% and K. pneumoniae=21.25%), Srivastava et al. (E. coli=60.93% and K. pneumoniae=26.92%), and Binod et al. (E. coli=63.9% and K. pneumoniae=12.3%).[24-26]

In our study, urine was the most common sample (55.76%) and endotracheal aspirate was the least common sample (2.5%). Many studies had urine as the most common sample. Pawar et al. (31.76%), Pravin et al,[27,28] (46%), Srivastava et al,[26] (56.86%), and Dwomoh et al,[29] (68.8%) had urine as the most common sample.

In this study patient's ages ranged from 0 to 90 years old. This wide range was because the place of study was a tertiary care hospital where a population of all age groups report for treatment. In our study, the most number of isolates were from the age group 31-40 years (23.07%) and the least number of isolates from the age group < 20 years (2.5%). But a study conducted by Pawar et al,[27] had the highest number of isolates in the age group 41-60 years (37.05%) and the least number of isolates from the age group >80 years (4.7%).

In our study, isolates from males accounted for 54.48 % of the total isolates whereas those from females accounted for 45.51%. Thus, there was a preponderance of males over females. These findings were consistent with a study by Thomas et al.[24]

The latest CLSI guidelines (M100-ED31:2021) with revised zone diameter for resistance and sensitive criteria do not make it compulsory for conducting phenotypic methods for the detection of CRE on a routine basis on a patient's sample.[23]

In our study, the sensitivity of CarbaNP was 91.02%. It was similar to studies conducted by Kour et al. & Sreeja et al,[30,31] where the Sensitivity of carbaNP was (92.3%). and (94%), respectively.

In this study, the sensitivity of the mCIM test was 94.88%. It was similar to studies conducted by Giri et al & Kour et al,[32] where the sensitivity of mCIM was 98.66% and 100%, respectively.

CONCLUSION

Although phenotypic methods CarbaNP and mCIM have a high sensitivity and specificity, it does not detect non-CP-CRE mechanisms of resistance in the isolates. Moreover, mCIM has high reproducibility, erroneous results can be obtained if there is any deviation from the standardized protocol of conducting the test. On the other hand, the Kirby-Bauer disk diffusion test is much simpler to perform as compared to mCIM. Hence, when using the breakpoints for the Kirby-Bauer disk diffusion test as given in the latest CLSI document (M100-ED31:2021) for CRE, it is not mandated to conduct supplementary testing by phenotypic methods for guiding antibiotic treatment decisions in patients.

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Moreover, in the present study, the difference between the positivity of both phenotypic methods was not statistically significant.

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