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Phenotypic method for detection of carbapenemaseproducers in Gram negative bacteria isolated from Diabetic patients with urinary tract infection

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ABSTRACT

Background- The overall escalation of antimicrobial/antibacterial resistance has taken on extreme and greater importance from a public health/community health perspective. The emergence of MDRO poses a major challenge to global health. Antimicrobial resistance poses a major threat to the treatment of various hospital-acquired infections, and this study investigated the emergence of carbapenem resistance in clinical isolates. The aim of the current study was to investigate the prevalence and antibiotic susceptibility of carbapenem-resistant (CR) uropathogens of the family Enterobacteriaceae in diabetic patients. For bacteria that produce ESBL, carbapenems are the drug of choice. However, treatment failures due to carbapenamase producers remain a concern.

Purpose: Several phenotypic methods are available for the detection of carbapenemase producers. Antimicrobial susceptibility and phenotypic characterization of ESBL, MBLS, and KPC were determined using methods recommended by CLSI. The easy-to-perform modified Hodge assay for the detection of carbapenemase producers in Enterobacteriaceae isolated from clinical specimens.

Materials and Methods: A total of 14 Gram-negative CRE specimens were collected from January to March 2023 . Processing was done in Microbiology laboratory. MHT was performed on all isolates according to CDC and CLSI guidelines.

Result – The carabapenamase activity was detected from different isolates. E.coli 86% and Klebsiella pneumonia 14%.

Conclusion

In the present study, the incidence of multidrug resistance, including carbapenem resistance, was relatively high among clinical isolates of Enterobacteriaceae such as Klebsiella pneumonia and E. coli from urine samples of hospital patients.

To conclude our study, comparison of phenotypic methods such as MHT & Double disk synergy test with VITEK 2 Automated system showed carbapenem producers**.**

Key words – Carbapenem-resistance, Gram-negative bacteria, Enterobacteriaceae

I. INTRODUCTION

The increasing incidence of hospital acquired infections caused by multidrug-resistant microbes/organisms is severely limiting the options for treating infections. [1] Antibiotic resistance in bacterial pathogens is a widespread and critical challenge in clinical management, resulting in high morbidity and mortality rates worldwide [2] Diabetes mellitus is one of the leading non-communicable diseases (NCDs) and ranks 5th among NCDs globally [3,4] A surge in diabetes cases has been observed over the last three to four decades due to a less established health sector, with the situation being worse in developing countries. A large proportion of the population remains at risk of developing diabetes-related complications. Uncontrolled and elevated blood glucose levels increase damage to numerous organs, including the blood vessels, retina and kidneys, leading to various complications. [5,6] The burden of urinary tract infections and other complications in diabetics is putting pressure on healthcare facilities even in developed countries. [7,8] Urinary tract infections in diabetics are reported to have more severe symptoms such as emphysematous cystitis, pyelonephritis, renal abscesses and papillary necrosis [9] and lead to prolonged hospitalisation. Treatment of UTI is usually associated with worse outcomes in diabetics than in non-diabetics [10] Bacteria commonly found in urinary tract infections [11] include E. coli and Klebsiella pneumonia, as well as other Enterobacteriaceae [12,13,14] Several members of the bacterial family Enterobacteriaceae are normally present in the harmless human gut flora. However, these bacteria are the main cause of a wide range of opportunistic infections [15] Gram-negative bacteria can lead to severe complications or even treatment failure when caused by MDR strains, which are becoming

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increasingly common worldwide. MDR infections are being used at an increasing rate and, as a result, resistance in Gram-negative bacteria has now become a worldwide problem. [16] Although carbapenemase activity has been detected mainly in clinical isolates of Pseudomonas and Acinetobacter, recent studies have shown the emergence of carbapenem resistance in Enterobacteriaceae members in different geographical regions, which is of great concern, as these bacteria can be easily transmitted by patients, leading to hospital-acquired infections (HAIs), but can also spread in the community, leading to community-acquired cases [17] Carbapenem drugs have been used as treatment therapy for these organisms. Enterobacteriaceae (ertapenem, meropenem / imipenem), Pseudomonas aeruginosa (meropenem or imipenem) and A. baumannii (meropenem or imipenem) that show resistance to at least one of the carbapenems are referred to as carbapenem- resistant Enterobacterales (CRE), carbapenem-resistant Pseudomonas aeruginosa (CRPA) and carbapenem-resistant A. bauamannii (CRAB)[18] According to the most recent report of the ICMR AMR surveillance network, resistance to imipenem was found in 28 % of E. coli, 55 % of Klebsiella pneumonia and 80 % of A. bauamannii isolates. [19] Carbapenem resistance in Eneterobacteriaceae may be due to several reasons, including excessive production of Amp-C beta-lactamase, loss of porins, production of metallo-beta-lactamases (MBL) and production of carbapenemases in Klebsiella pneumonia. The major carbapenemase determinants responsible for resistance or reduced susceptibility to antibiotics of the carbapenem group in members of Enterobacteriaceae [20]

II. MATERIALS & METHODS

Sample Collection

The prospective study was conducted at VIMS & RC from January to March 2023.

Ethical approval has been obtained from VIEC.

All samples from outpatients and inpatients were included in the study. All samples were processed on CLED (cysteine lactose electrolyte deficiency) agar after preliminary identification and processed in VITEK 2 compact system for identification of organisms and antibiotic susceptibility testing. Samples that were resistant to the carbapenem group were separated for further investigation..

MIC (Minimum Inhibitory Concentration)

An MIC test was performed for all isolates using different antibiotics (meropenem, PTZ, cefotaxime, colistin... etc.).

MHT (Modified Hodge Test)

All carabapenem-resistant enterobacteria detected in the disc diffusion test were also tested for carbapenemase production in the modified Hodge test

This is a simple phenotypic screening test for the detection of carbapenemases.

PRINCIPLE

The MHT was performed on all isolates regardless of their sensitivity pattern to carbapenems (imipenem, meropenem and ertapenem) in accordance with the Centres for Disease Control (CDC) and Clinical and Laboratory Standards Institute (CLSI) guidelines [21]

REQUIREMENTS

1. Muller – Hinton Agar plate

2. Organisms – a) Escherichia coli ATCC 25922

b) Klebsiella pneumoniae ATCC BAA-1705 (Positive control)

c) Klebsiella pneumoniae ATCC BAA-1706 (Negative control)

d) Test strains (showing reduced carbapenem sucsceptibility)

3. Saline, swabs, forceps, inoculating loop, densicheck.

PROCEDURE

A lawn culture was prepared on MHA with E.coli suspension (ATCC 25922) adjusted to 0.5 McFarland' standards overnight.

The plate was dried for 15 minutes and then a disc containing 10 µg of meropenem was applied to the centre of the plate.

The isolates to be tested were spread from the edge of the disc to the edge of the plate. Four isolates were tested on each plate.

After overnight incubation at 37°C, the cloverleaf-like appearance between the test strips near the plate was considered positive for carbapenemase production.

EVALUATION

Carbapenemase activity was detected by this method. It is much more sensitive than the modified Hodge test. If the zone of inhibition was ≥ 19 mm, it was considered negative. The results were interpreted according to the CLSI guideline [22]

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INTERPRETATION

After 16-24 hours incubation, examine the plate for a cloverleaf-like depression at the interface between the test organism and E.coli ATCC 25922 within the zone of inhibition of the carbapenem susceptibility disc.

MHT positive test - Cloverleaf-like depression of E.coli ATCC 25922 growing along the growth strip of the test organism within the diffusion zone of the disc.

MHT negative test - No growth of E.coli ATCC 25922 along the strip of the test organism within the diffusion zone of the disc.

DETECTION OF MBL AND CARBAPENEMASE PRODUCTION BY ENTEROBACTERIACEAE

1. DOUBLE DISK SYNERGY TEST

Imipenem-EDTA is used for screening metallo-beta-lactamase producers.

PRINCIPLE -

Metallo-beta-lactamase (MBL) activity is inhibited by chelating agents. Imipenem disc (10µg/ml) acts as a chelating agent and inhibits MBL activity.

PROCEDURE -

1. A broth culture of the test strain (turbidity adjusted to 0.5 McFarland standards) was inoculated overnight onto a Mueller-Hinton agar plate.
2. A 10-µg disc of imipenem without EBTA was placed on the agar plate at a distance of 24 mm from an EDTA-containing imipenem disc.
3. After 16 hours of incubation at 35°C, the zone of inhibition around the imipenem-EDTA disc was detected.

INTERPRETATION -

A zone diameter difference of ≥7 mm was detected between the imipenem disc and the imipenem-EDTA disc, the test was considered MBL positive

QUALITY CONTROL

Organisms (ATCC)	**Std. Zone of diameter (mm)**
E.coli (25922)	25-30
Klebsiella pneumonia (ATCC BAA- 1705)	23.8

DOUBLE DIFFUSION DISK

EDTA + Imipenem

Modified Hodge test with a 10µg ertapenem dose. Isolates 1 and 2 produce carbapenemase and are positive in this test. Isolate 2 does not produce carbapenemase and is negative in this test.

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A positive clinical isolate. A cloverleaf-like depression at the interface between the organism and E.coli 25922, within the zone of inhibition of the carbapenem.

III. RESULT

Table no. 1 - Total bacterial isolates from urine of both diabetic & non diabetic patients

Total No. of urine samples (Diabetic & Non-diabetic)	Total no. of growth	**Total no. of no growth**
248	47 (19%)	201 (81%)

Table no. 2 – Bacterial isolates from urine sample of diabetic patients

Total no. of urine isolates from diabetic patients	**No. of isolates**	**NoGrowth**
126	32(26%)	94(74%)

Table no. 3 - Bacterial isolates from urine sample of non - diabetic patients

Total no. of urine isolates non-diabetic patients	**No. of isolates showing Growth**	**No. of isolates showing no Growth**
122	23(19%)	99(81%)

Table no – 4 – Bacterial isolates from urine samples of both diabetic & non- diabetic patients

Name & total no. of isolates from diabetic patients (32)		**Name & total no. of isolates from non-diabetic patients (23)**	
E.coli23		Klebsiella pneumonia5	
Klebsiella pneumonia5		E.coli 13	
Pseudomonas aeruginosa2		Klebsiella aerogenes 1	
Pseudomonas sp1		Pseudomonas aeruginosa 5	
Enterococcus Faecalis2		Enterococcus Faecalis 1	

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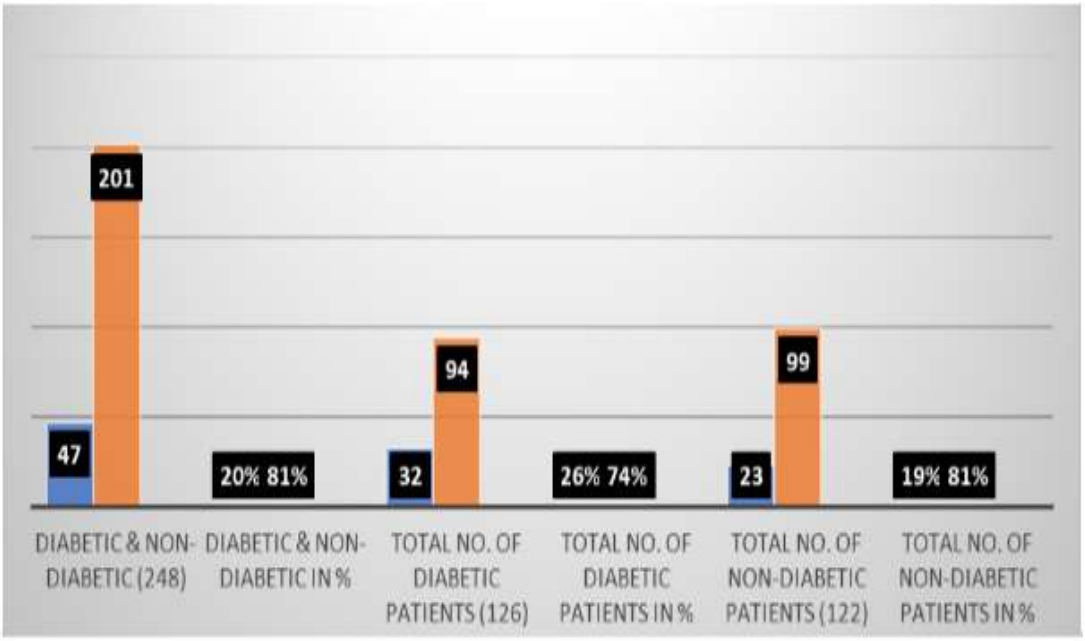
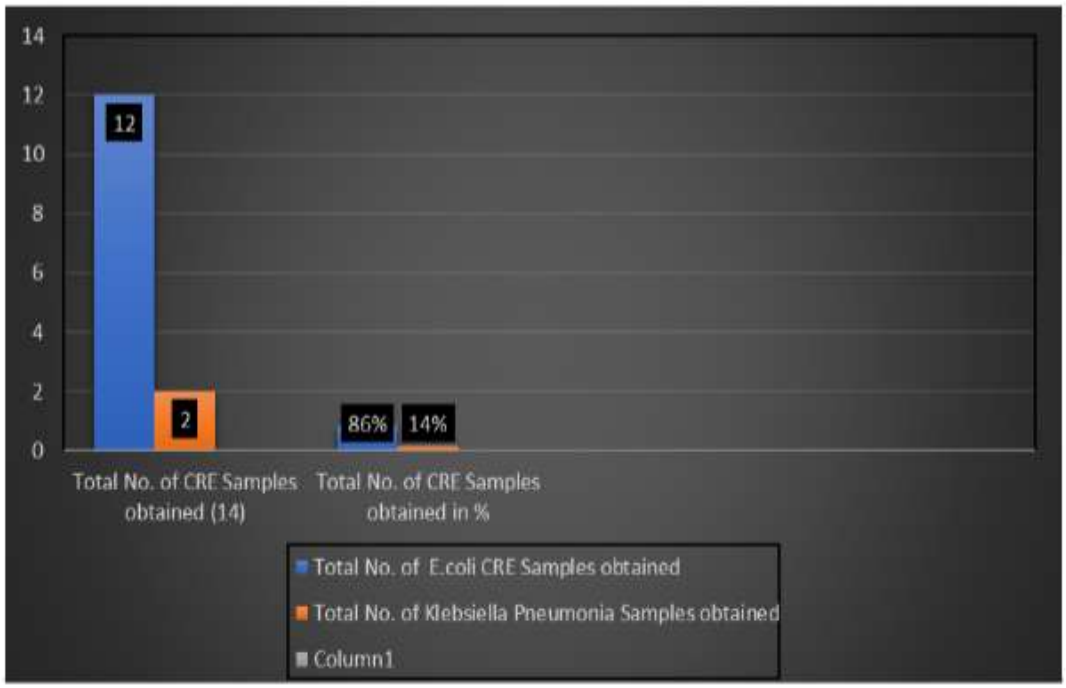
Table no. 5

Total No. of CRE Samples obtained	Total No. of E.coli CRE Samples obtained	Total No. of Klebsiella Pneumonia Samples obtained
14	12(86%)	2(14%)



Graphical representation of total diabetic & non diabetic patients



Graphical representation of total no. of CRE Samples

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Table no. 7

Strains obtained	Antibiotic resistant
E.coli CRE	Ertapenem, Meropenem,
E.coli CRE	Ertapenem, Meropenem, Amikacin
E.coli CRE	Meropenem, Ertapenem, Fosfomycin
E.coli CRE	Ciprofloxacin, Meropenem, Trimethoprim/Sulfamethoxazole
E.coli CRE	Amoxicillin/Clavulanic acid, Imipenem
E.coli CRE	Gentamicin, Cefepime, Ceftriaxone
E.coli CRE	Cefoperazone/Sulbactam, Piperacillin/Tazobactam
Klebsiella pneumonia	Imipenem, meropenem
E.coli CRE	Amikacin, Cefuroxime, Meropenem
E.coli CRE	Cefuroxime/Axetil, Ertapenem, Imipenem
E.coli CRE	Ertapenem, Imipenem, Meropenem
E.coli CRE	Piperacillin/Tazobactam, ciprofloxacin, Imipenem
Klebsiella pneumonia	Amoxicillin/Clavulanic acid, Ceftriaxone, Cefuroxime, Meropenem
E.coli CRE	Cefepime, Ertapenem , Amikacin, Gentamicin

IV. DISCUSSION

The current global emergence of resistance to the most effective antibiotic, carbapenem, in Enterobacteriaceae represents an important and growing public health threat. Our findings illustrate the emergence of carbapenem resistance in Gramme-negative bacteria.

In India, the prevalence of carbapenem-resistant bacterial infections is very high. There are several phenotypic methods for carbapenemase detection, of which MHT is recommended by both CDC and CLSI [23,24] Studies have compared MHT and combined disc tests and found different results. A few studies have raised concerns about the false positivity of the MHT test [25]

A study from Greece included 117 ESBL negative Enterobacteriaceae members that revealed a MHT positivity of 41.8% [26]

Our findings illustrate the emergence of carbapenem resistance among Gram-negative bacteria in the north-eastern region of India. In India, the prevalence of carbapenem-resistant bacterial infections is extensive, especially in the southern& northern regions, where the population density is high. [27] Other tests including the combination disc tests have been in use by many laboratories.[28] Different studies have compared MHT & combination disc tests & found variable results. Other than this few studieshave raised concern over false positivity in the MHT test. [29] Few other research works have proved combined disk synergy tests are more effective in detecting the carbapenemase activity.

The increased incidence of carbapenem resistance in Enterobacteriaceae is a public health concern. In the study, a high percentage 35.9% (385/1072) of potential carbapenemase activity was detected in a collection of Enterobacteriaceae isolates from different clinical samples in India. Among the different Enterobacteriaceae members tested in the present study, Klesiella spp. Showed th highest percentage of carbapenem resistance (30%), whereas Proteus spp. And Citrobacter spp revealed comparatively low carbapenem resistance of (17%) and (12%), respectively.

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The recent study from South India done as a part of antimicrobial surveillance program (SENTRY) that tested 39 Enterobacteriaceae isolates collected between 2006 & 2007 that showed reduced susceptibility to carbapenem antibiotics revealed 26(66.6%) were found MHT positive. [30] Studies have come to a contrasting conclusions about use of MHT, one finding it as inadequate in detecting the metallo-beta-lactamase & others proving that MHT produces false positive carbapenemase. [31]

V. CONCLUSION

In the present study, the incidence of multidrug resistance, including carbapenem resistance, was relatively high among clinical isolates of Enterobacteriaceae such as Klebsiella pneumonia and E. coli from urine samples of hospital patients.

To conclude our study, comparison of phenotypic methods such as MHT & Double disk synergy test with VITEK 2 Automated system showed carbapenem producers.

This study results clearly demonstrate the presence of the carbapenemase activity in high percentage of Enterobacteriaceae members detected by the MHT that has proven to be easily done in any tertiary care setting with minimal infrastructure and is cost effective.

The finding of the present research study shows the presence of carbapenemases in hospitalized diabetic patients with urinary tract infection of our tertiary care center, and future studies can be carried out in one or more number of samples.

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