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Detection of Carbapenemase Producing E. Coli by Phenotypic Method (Modified Carba NP Test)

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Abstract: ***Objective: Carbapenems are important antibiotics for multidrug - resistant Enterobacteriaceae. Resistance to carbapenem is increasing at an alarming rate worldwide leading to major therapeutic failures and increased mortality rate. Easy and effective detection of carbapenemase producing carbapenem - resistant Enterobacteriaceae the key to control dissemination of carbapenem resistance in community - acquired infection. The aim of present study was to evaluate efficacy of Modified Carba NP (CNP) for early detection of carbapenemase producing E. coli. Material and Methods: Study was performed in the Microbiology Department in Muzaffarnagar medical college, Muzaffarnagar. In this study clinical specimens like pus, blood, sputum, throat swab, endotracheal tube aspirate, CSF, urine, body fluids, etc. obtained from various ICU & OPD and emergencyA total of 95 CRE were subjected to Modified CNP test for the detection of CPE. Statistical Analysis: It was done on Statistical Package for the Social Sciences (SPSS) software, Results: A total of 95 CRE isolates were included in this study. Majority of CRE was isolated from age group 20 to 30 years (33%) followed by 41 to 60 years (20%) with male preponderance over female patients. Maximum number of CRE were isolated from samples received from wards 50 (48%) followed by intensive care unit (ICU) 27 (25%) and outdoor units 20 (19%). CRE was isolated from urine samples also 25 (27%). Out of 95 CRE isolates, 46 (43%) isolates were phenotypically were confirmed by Modified CNP test. Conclusion: Modified strip CNP test is simple and inexpensive test which is easy to perform and interpret and gives rapid results in less than 5 minutes. It has high degree of sensitivity and specificity. Modified strip CNP test shows significantly higher detection capacity for carbapenemase producers.***

Keywords: Carbapenem - resistant E. coli, Carbapenemase producing E. coli, Detection by phenotypic method, Modified Carba NP test (CNP), Carbapenemase producing Enterobacteriaceae.

1. Introduction

Members of Enterobacteriaceae family all are gram negative, rod shaped facultative anaerobes mainly colonizing the intestinal tracts of humans and animals and are a common cause of community - associated and health care - associated infections[1].

In the past, most of the first line and low - cost antimicrobial drugs such as penicillin and first and second generation cephalosorins have been used effectively for the management of gram - negative bacterial infections. However, drug - resistant organisms acquire resistance to these first - line antibiotics, thereby necessitating the need of second - line drugs like the third and fourth generation cephalosporins. Further emergence of β lactamases, extended spectrum β lactamases (ESBL) [2]

Carbapenems such as ertapenem, imipenem, meropenem, and doripenem have proven efficacy in severe infections caused by these MDR Enterobacteriaceae;

Thats why they are frequently used as last resort therapeutic options. Carbapenems have the widest spectrum of antibacterial activities[3] and are also active against the chromosomal cephalosporinases and ESBL, therefore are preferred antibiotics in case of invasive or life - threatening infections. However, in recent years, carbapenem - resistant Enterobacteriaceae (CRE) has emerged in the community as a major health threat.

Carbapenem resistance was first reported in center of - 1990s from the United States and then reports forcarbapenem resistance outbreaks are increasing worldwide at an alarming rate[4]. In addition to carbapenem resistance, CRE often carry genes that confer high levels of resistance to many other antimicrobials, therefore these bacteria are difficult to treat and are associated with very high mortality rate[5]. The mechanisms of development of carbapenem resistance in Enterobacteriaceae are complex because they involve a broad range of organisms and are mediated by different mechanisms.) [6]

Currently, CRE are a problem not confined to individual facilities but are affecting entire communities and nations. High incidence of treatment failures, morbidity, and mortality in many infectious diseases are strongly correlated with carbapenem resistance. There are limited antimicrobials effective against carbapenemase - producing bacteria. This is the need of hour to have a laboratory test which is easy to perform, cost effective, and at the same time is highly sensitive and specific. Genotypic methods like real time polymerase chain reaction - restricted fragment length polymorphism (PCR - RFLP) [7, 8] and whole genome sequencing, and other techniques like matrix assisted laser desorption ionization - time of flight - mass spectrometry (MALDI - TOF - MS) [9, 10] have overall good sensitivities and specificities but require trained microbiologists,

expensive equipment, and are time consuming. In addition, it may fail to detect unknown novel carbapenemase genes[11].

On the other hand, phenotypic tests Carba NP (CNP) test are inexpensive, easier to perform, and require minimal training. MHT is highly sensitive test for detecting KPC and OXA - 48 producers but has low sensitivity for MBL producers[12, 13]and low specificity due to false positives from ESBL or AmpC β - lactamase producers with porin loss[14, 15]. Also, it requires long incubation period of 24 hours. CNP test is a rapid chromogenic test which gives results in 2 hours with approximately 100% sensitivity and specificity for detecting carbapenemases in Enterobacteriaceae

2. Materials and Methods

Present study was performed in the Microbiology Department in Muzaffarnagar medical college, Muzaffarnagar. In this study clinical specimens like pus, blood, sputum, throat swab, endotracheal tube aspirate, CSF, urine, body fluids, etc. obtained from various ICU&OPD and emergency. These samples were further processed for identification of organism as per routine laboratory protocols. Organisms identified as members of Enterobacteriaceae were screened for carbapenem resistance by Kirby Bauer disk diffusion method following CLSI 2018 guidelines using Meropenem 10 µg disk. Isolates which show inhibition zone diameter of ≤19 mm were considered as screening test positive and named as carbapenem - resistant E. coli.95 E. coli isolates were further evaluated for carbapenemase production Modified CNP test.

Modified CNP test[16] is a colorimetric test based on detection of hydrolysis of the β - lactam ring of a carbapenem (imipenem) by carbapenemase enzyme produce by bacteria. Hydrolysis of imipenem acidifies the medium, changing the color of the pH indicator (phenol red solution) from red to yellow.

It is a phenol red - based rapid method for the detection of carbapenemases in E. coli in less than 2 hours. In this test, a bacterial culture is mixed with lysis solution. After lysis, the cell - free supernatant is mixed with a solution of phenol red, zinc sulphate, and imipenem. In carbapenemase - producing bacteria, the colour of the reaction changes from red to yellow

- Modified Carba NP test was performed with 4 - 5 loops (10 µL) of a pure bacterial culture was taken from blood agar plate, suspended into 400 µL TrisHCl bacterial lysis buffer, and then vortexed it for 1 minute.
- One hundred microliters of this bacterial suspension was added in TWO (2) microcentrifuge tubes (0.5 mL capacity) each.
- Tube 1 (control tube) contained 100 µL of diluted phenol red and 0.1 mmol/L ZnSO4
- Tube 2 contained 100 µL of diluted phenol red, 0.1 mmol/L ZnSO4, and 6 mg/mL imipenem - cilastatin powder (equivalent to 3 mg/mL of imipenem)
- Test strain turning yellow color from original red color.

3. Results

A total of 95 CRE isolates were included in this study. Majority of CRE was isolated from age group 20 to 30 years (33%) followed by 41 to 60 years (20%) with male preponderance over female patients. Maximum number of CRE were isolated from samples received from wards 50 (48%) followed by intensive care unit (ICU) 27 (25%) and outdoor units 20 (19%). CRE was isolated from urine samples also 25 (27%).

Out of 95 CRE isolates, 46 (43%) isolates were phenotypically were confirmed by Modified CNP test,

4. Discussion

Carbapenems are commonly used to treat infections caused by ESBL and/or AmpC β - lactamases producing and other MDR Enterobacteriaceae. Although they are stable to hydrolysis by most β - lactamases, their usage as the last resort antibiotics was seriously compromised by the appearance of class of bacterial enzymes of inactivating carbapenems, called as carbapenemases.1[7] Resistance to carbapenem antimicrobials is either due to expression of carbapenemases or due to combined mechanisms of resistance (overexpression of broad - spectrum β - lactamases together with efflux pumps, impermeability); however, carbapenemases production represents the most important mechanism of resistance in Enterobacteriaceae, associated with multi - or pan - drug resistance.

In present study, resistance to carbapenem was seen in all age groups and in both the genders. Though in less number, pediatric and geriatric populations were also found to be affected. CRE was isolated from both nosocomial as well community - acquired infection; however, nosocomial spread seems to be the main mode of dissemination (78% CRE was isolated from IPD and 22% from OPD). Similar findings were seen in study by Nair and Vaz29 in which most of the CRE isolates were detected in patient samples from the wards (42%) and the ICU (26%) followed by OPD patients (19%). Chauhan etal[18] and Panduranganet al[19]also have same observation. Thus, to prevent CRE spread in hospital settings, measures like hand hygiene, contact precautions, minimizing the use of invasive devices, patient and staff cohorting should be given due importance.

Modified strip CNP test shows promising results. It is simple, inexpensive, reproductive test which is easy to perform and interpret and give rapid results in less than 5 minutes. It has high degree of sensitivity and specificity. Its sensitivity is low especially for MBL producers and also has low specificity.

5. Conclusion

CP - CRE have high tendency to spread in the community and are associated with high morbidity and mortality. Modified CarbaNP test is easy solution for early detection of CP - CRE particularly in low resource countries especially in India which are large reservoir of carbapenemase producers. Contribute to formulate better treatment plan to

avoid therapeutic failures and to design logical infection control policies to prevent further dissemination of carbapenemase producers in the community thus shifting the paradigm of carbapenem resistance.

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