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Prevalence of Carbapenemase Producing *Escherichia coli* Isolates among Various Wards of a Tertiary Care Hospital in South India.

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ABSTRACT

To detect carbapenemases in *Escherichia coli* (E. coli) and to study its prevalence in various wards of a tertiary care hospital. The study was done from March 2013 to February 2014. A total of 98 E. coli from different clinical samples were taken. Those isolates which showed intermediate or resistant zones to meropenem by disc diffusion were included in the study. These isolates were then subjected to Modified Hodge test antimicrobial susceptibility to those resistant isolates were performed. Out of 98 *E. coli* isolates, 15 (15.3%) were positive for carbapenemase production by Modified Hodge test. Out of 15 MHT positive E. coli isolates, carbapenemase production was detected in 32%, 18.18%, 12.9% and 5% from ICU, orthopaedics, general medicine and general surgery wards respectively. This study demonstrates the growing prevalence of carbapenemase producing E. coli isolates among various wards in the hospital thereby requiring immediate implementation of nationwide antibiotic policy to limit further spread.

Keywords: Carbapenemase, *Escherichia coli*, multi-drug resistance, Modified Hodge test

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INTRODUCTION

Gram-negative bacilli can cause a variety of infections in humans which may be either community-acquired or hospital-acquired. Gram-negative bacilli have the intrinsic ability to produce various resistance mechanisms and to transfer the resistance determinants to other bacteria with the help of plasmids. [1] Carbapenems, especially the broad-spectrum variants, are an extremely important part of our ability to control severe Gram-negative infections, particularly those caused by multidrug-resistant (MDR) bacteria. However, resistance is emerging in the form of new beta-lactamases. [2] Carbapenemases are defined as beta-lactamases that significantly hydrolyze atleast imipenem or/and meropenem. Based on amino-acid homology, carbapenemases are classified into Ambler class A, B, D. [3] Of particular concern is emergence of carbapenemases in E. coli, as they are the most common pathogens causing Gram-negative bacterial infections. There are several carbapenemases such as *imipenemase* (IMP), Verona integron-encoded *metallo-β-lactamases* (VIM), *São Paulo* MBL (SPM-1), *German imipenemase* (GIM-1), *Klebsiella pneumoniae* carbapenemase (KPC), *New Delhi Metallo β-lactamases* (NDM-1). [4] Other mechanisms of resistance to carbapenems are due to increased efflux systems, decreased outer membrane permeability and alteration of penicillin-binding proteins. [5] This study was done to detect the prevalence of carbapenemase producing *E. coli* and to prevent its dissemination by proper hospital infection control measures and also to alarm the clinicians on judicious use of antimicrobials.

MATERIALS AND METHODS

This study was conducted in a tertiary care hospital from March 2013 to February 2014. A total of 98 *E. coli* isolated from various clinical samples such as pus, urine, blood, sputum and broncho-alveolar lavage were taken. The samples were processed and identified by standard bacteriological techniques and antimicrobial susceptibility to carbapenems was done by Kirby-Bauer disc diffusion method. Zone sizes were interpreted according to CLSI guidelines. The isolates which showed intermediate or resistant zones for meropenem, i.e 16mm-21mm, were tested for carbapenemase production by Modified Hodge test (MHT). A lawn culture (0.5 McFarland) of the *Escherichia coli* 25922 was streaked on Mueller Hinton agar plate. A 10 µg meropenem disc was placed in the center of the agar plate and the test organism along with positive and negative control organisms were streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 37°C. Quality control of the carbapenem disks were performed according to CLSI recommendations. After 24 hrs, MHT positive test showed a clover leaf-shaped indentation of the *Escherichia coli* 25922 along the test strain whereas a MHT negative test showed no indentation along the test organism. [6]

RESULTS

The overall prevalence of carbapenemases in E. coli was found to be 15.3%. It was also found that out of the Escherichia coli isolates from ICU, orthopaedics, general medicine wards and general surgery, 8(32%), 2(18.18%), 4(12.9%) and 1(5%) respectively were found to produce carbapenemases. (Table 1) (Figure 1)

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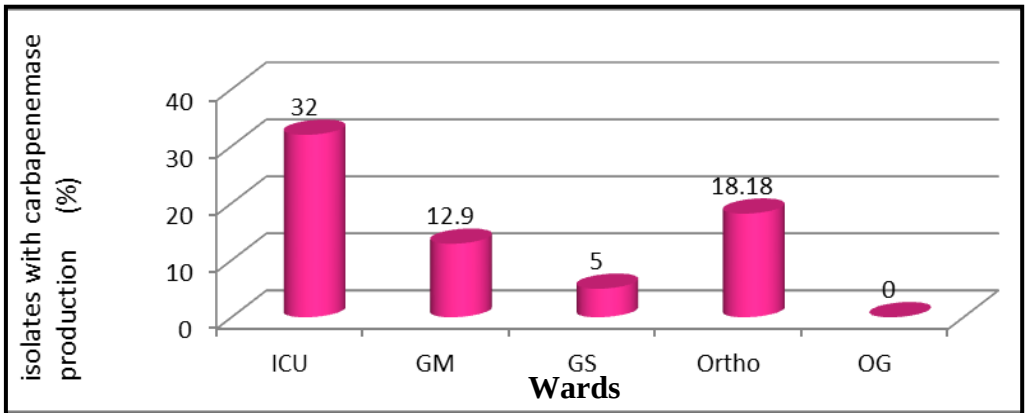
It was found that among the carbapenemase producing E. coli, all the isolates (100%) were susceptible to polymyxin B; susceptibility to ciprofloxacin and amikacin were exhibited by 75% of the organisms; resistance to cefotaxime, cefdinir, cefixime, gentamicin and piperacillin-tazobactam were exhibited by 50% of the organisms, resistance to ceftriaxone and aztreonam were exhibited by 75% of the organisms and all were resistant to ceftazidime and cefuroxime.

Table 1: Distribution of carbapenemase producing Escherichia coli isolates among the different wards

Wards	No. of *E. coli* isolates	Carbapenemase producers (%)
ICU	25	8 (32)
GM	31	4 (12.9)
GS	20	1 (5)
Ortho	11	2 (18.18)
OG	11	0

Figure 1: Distribution of carbapenemase producing Escherichia coli isolates among different wards



DISCUSSION

A total of 25 E. coli were isolated from ICU, out of which 8 (32%) carbapenemase producers were detected. Out of 31 E. coli isolates from general medicine ward, 4 (12.9%) were found to produce carbapenemases. Among the isolates from general surgery ward, 1 out of 20 (5%) and among the isolates from orthopedic ward, 2 out of 11 isolates (18.18%) were found to produce carbapenemase. A study done by Nagaraj S et al. [7] and Parveen et al. [8] showed higher prevalence of carbapenemases in ICU, GM and GS. Among carbapenemase producing E. coli isolates, all were resistant to ceftazidime and cefuroxime, 75% were resistant to ceftriaxone and aztreonam; and 50% were resistant to cefotaxime, cefdinir, cefixime, gentamicin and piperacillin-tazobactam. 75% were susceptible to ciprofloxacin, amikacin and all the isolates (100%) were susceptible to polymyxin B. Mulla et

al. (9) from Gujarat reported a high resistance in E. coli to third generation cephalosporins, fluoroquinolones and aztreonam.

CONCLUSION

Carbapenem resistance constitutes a serious threat to the antibiotics available to deal with increasing resistance in Gram negative pathogens infecting neonates, infants, and compromised children with nosocomial infection caused by carbapenemase and ESBL producing bacteria. The ability to limit the spread of these pathogens will require effective laboratory screening methods to rapidly identify patients infected with these organisms thereby minimizing adverse clinical outcomes.

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