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Original Research Article

Detection of Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital in South IndiaPrachi Saban^{*1} and R Gopal²¹Department of Microbiology, Sri Aurobindo Medical College and Pg Institute, Indore, India²Department of Microbiology, Sri Manakula Vinayagar Medical College, Puducherry, India

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Department of Microbiology,
Sri Aurobindo Medical College and Pg Institute, Indore, India***Article History:****Received:** 11/08/2017**Revised:** 24/08/2017**Accepted:** 26/08/2017**DOI:** <https://doi.org/10.7439/ijbr.v8i8.4338>**Abstract****Introduction:** Carbapenems are a class of β -lactam antibiotics with a broad spectrum of activity and greatest potency against Gram positive and Gram negative bacteria and their use has markedly increased in the past few decades. The development of carbapenem resistance has now become a grave concern in the treatment of diseases.**Materials and Methods:** The study was a laboratory based cross sectional study done with the main aim to detect the presence of carbapenem resistance among *E. coli* and *K. pneumoniae* isolates. A total of 550 samples (pus, urine, sputum, etc) were subjected to culture. The antibiotic susceptibility for various antibiotics was studied by Kirby Bauer's Disk Diffusion Test. Carbapenem resistance and Metallo-beta-lactamase (MBL) production was detected using Modified Hodge Test and EDTA Disk Synergy Test respectively.**Result:** A total of 129 isolates (43%) were meropenem resistant out of which 42 isolates (14%) were MBL producing. Carbapenase production was noted in 42.1% of *E. coli* and 45.8% of *K. pneumoniae*. Twelve percent of the *E. coli* and 20.8% of the *K. pneumoniae* were found to have prevalence of MBL producers respectively.**Conclusion:** High incidence of carbapenem resistance and MBL producers was noted in the isolates. Given the emerging pattern of resistance to these third line antimicrobials and limited new molecules it is imperative to frame a new antibiotic policy and its strict implementation.**Keywords:** Antimicrobial resistance, Carbapenem resistance, MBL Producers, *E. coli* and *K. pneumoniae*.**1. Introduction**

Antimicrobial resistance today poses a serious problem in the management of infectious diseases from both community and hospitals. It not results in adverse outcomes but also adds to the cost of treatment directly and indirectly. Emerging resistance to these third line antimicrobials (carbapenems) [1-3] and limited availability of new molecules today, virtually has the health care system with its back to the walls. It is therefore pertinent to study the incidence of carbapenem resistance in our region and hospital setting to be able to frame a rational antibiotic policy based on sound knowledge of the locally prevalent resistance pattern. Hence we studied the antibiotic

susceptibility pattern of various isolates to look for the incidence of carbapenem resistance and metallo-beta-lactamase (MBL) producers among the isolates of *E. coli* and *K. pneumoniae* from various samples at our tertiary care centre.

2. Materials and methods

The study was carried out at Department of Microbiology, Sri Manakula Vinayagar Medical College & Hospital as a cross sectional laboratory based study from Dec 2014 to May 2015. A total of 550 samples of sputum,

pus, urine, swabs and blood were subjected to culture. All the samples were processed and cultured on Blood agar, MacConkey agar and all the isolates of *E. coli* and *K. pneumoniae* were identified by the standard biochemical reactions as per CLSI guidelines.[4] Duplications of isolate from a same patient were excluded. Patient's demographic data, ward in which the patient stayed, duration of stay and clinical diagnosis were noted.

2.1 Antimicrobial susceptibility testing

The inoculums of testing strain of *E. coli* and *K. pneumoniae* was prepared in sterile Nutrient broth up to 0.5McFarland standard by standard techniques for antibiotic susceptibility test. Antimicrobial susceptibility of all the isolates was performed on Mueller Hinton agar (MHA) plates by the standard Kirby Bauer disk diffusion method. The diameter of the zones of inhibition of growth were interpreted as per as CLSI guidelines. *Escherichia coli* ATCC 25922 was used as control organism.

The antibiotics disks used were: Ampicillin (10mcg), Ampicillin sulbactam (10/10mcg), Amikacin (30mcg), Amoxicillin clavulanic acid (50/10mcg), Cefuroxime (30mcg), Cefotaxime (30mcg), Cefotaxime clavulanic acid (30/10mcg) Cefoxitin (30mcg), Cefoxitin-cloxacillin (30/200mcg), Ceftazidime (30mcg), Ceftazidime Clavulanic Acid (30/10mcg), Cefotetan (30mcg), Cefepime(30mcg), Gentamicin (10mcg), Netilmicin(30mcg), Ciprofloxacin(5mcg), Piperacillin (10 units), Piperacillin + Tazobactam (100/10mcg) and Meropenem (10mcg).

2.2 Modified Hodge Test: [5]

This is a phenotypic test, used to determine if reduced susceptibility to carbapenems is mediated by carbapenemase production. An overnight culture suspension of *E. coli* adjusted to McFarland standard was inoculated using a sterile cotton swab on the surface of MHA plate. After drying, 10 mcg meropenem disks was placed at the centre of the plate and the test strain was streaked from the disk to the periphery of the plate in four different directions. The plate was incubated overnight at 37°C. The results were observed and compared with the standards.

2.3 EDTA Disk Synergy (EDS Test) [6]

EDTA disk synergy test was done with simultaneous testing of two different β lactams for detection of metallo β lactamases. A 0.5M EDTA solution was prepared by dissolving 186.1gm of disodium EDTA in 1000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH. An overnight liquid culture of test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of MHA plate. A 10mcg Meropenem disk or 30mcg ceftazidime disk was placed on the agar. A blank disc

was kept on the inner surface of the lid of the MHA plate and 10 μ l of 0.5M EDTA was added. This EDTA disc was transferred to the surface of the agar and was kept 10mm edge to edge apart from Meropenem or ceftazidime disc. The culture was incubated overnight at 37°C. The results was observed and compared with the standards.

3. Results

Among the 332 isolates tested, 228 isolates were *E. coli* and 72 isolates were *K. pneumoniae* adding to 300. (Table 1) The maximum numbers of samples were from urine and least from blood. Out of the 300 isolates, *E. coli* was isolated in 182 and 33 samples while *K. pneumoniae* was isolated in 24 and 17 samples of urine and swabs respectively.

Table 1: Distribution of *E. coli* and *K. pneumoniae* in isolates from various samples

Samples (N= 300)	<i>E. coli</i> (228) n (%)	<i>K. pneumoniae</i> (72) n (%)
Urine (206)	182 (80.1%)	24 (10.5%)
Swab (50)	33 (60.0%)	17 (30.9%)
Blood (15)	6 (30%)	9 (45%)
Sputum (14)	0 (-)	14 (63.3%)
Pus (15)	7 (26.9%)	8 (30.7%)

A total of 129 isolates (43%) were meropenem resistant by Modified Hodge test (Table 4) out of which 42 isolates (14%) were MBL producing (Table 2). *E. coli* and *K. pneumoniae* contributed to 32% and 11% of carbapenem resistance respectively while 9% and 5% of the *E. coli* and *K. pneumoniae* were MBL producers respectively, among the total isolates. Carbapenemase production was noted in 96/228 (42.1%) of *E. coli* and 33/72 (45.8%) of *K. pneumoniae*. Twelve percent of the *E. coli* and 20.8% of the *K. pneumoniae* were found to have prevalence of MBL producers respectively.

Table 2: Incidence of carbapenem resistance and MBL producers among the isolates of *E. coli* and *K. pneumoniae*

Isolates (N= 300)	n	%
Carbapenem resistance (129) (43%)	<i>E. coli</i>	96 (32%)
	<i>K. pneumoniae</i>	33 (11%)
MBL Producers (42) (14%)	<i>E. coli</i>	27 (9%)
	<i>K. pneumoniae</i>	15 (5%)

4. Discussion

Based on the results obtained from the previous studies regarding carbapenemases producing *Enterobacteriaceae*, there is clear indication of increasing number in the last few years. The incidence of carbapenem resistance reported in the index study is 43% in the

Enterobacteriaceae isolates of which *E. coli* and *K. pneumoniae* account for 32% and 11% respectively. Carbapenase production was noted in 96/228 (42.1%) of *E. coli* and 33/72 (45.8%) of *K. pneumoniae*. Twelve percent of the *E. coli* and 20.8% of the *K. pneumoniae* were found to have prevalence of MBL producers respectively. Nagaraj *et al* reported NDM-1 positivity in 75% of *K. pneumoniae* and 66% in *E. coli* in a tertiary care hospital in South India. [7] In addition Deshpande *et al* reported that all isolates showed carbapenem resistance by Modified Hodge Test and 22 out of 24 isolates were MBL producing out of which 10 were *Klebsiella*, 9 were *E. coli*, 2 were *Enterobacter* spp and 1 was *Morganella morganii*. [8] In contrast in a study done by Mohamudha Parveen *et al* reported a similar finding at JIPMER in which out of 134 isolates of *K. pneumoniae*, 6 were Modified Hodge Test positive and none were MBL producing [9]. Similar study was done by Gupta *et al* in a tertiary care hospital in North India showed that out of 569 isolates of *E. coli*, 32 were carbapenem resistant and out of 343 isolates of *Klebsiella* spp., 39 were carbapenem resistant. [10] Another study done by Drew RJ *et al* tertiary referral children's hospital in the UK found carbapenem-resistant Enterobacteriaceae which were recovered from 24 patients. Of the 24 isolates, seven (all *Klebsiella* spp.) harboured carbapenemases: three had blaKPC and four blaNDM, whereas 17 had resistance due to combinations of Amp C or extended-spectrum β -lactamase activity plus impermeability. [11] In the United States, a recent study by Pannaraj PS *et al* in the pediatric population included 11 CRE isolates from 10 patients, of which 4 had clinical infection with CRE in Los Angeles, California. Carbapenemases were identified in 5 isolates, including two NDM-1 type carbapenemases and three KPCs.

The difference in incidence of carbapenem resistance and MBL producers may be explained on the basis of the regional variation in the prevailing flora and its susceptibility pattern as well as the clonal selection pressure exerted by use of antimicrobials at various centres. Formulation and strict adherence to a rational antibiotic policy is likely to bring down the incidence of the latter.

5. Conclusion

The carbapenem resistant organisms are emerging as a major cause of nosocomial infections resulting in new challenges faced by the physicians and also increased morbidity, mortality and health care costs. The prevention of spread of carbapenemase producers relies on early detection of the isolates and rational use of antimicrobials.

References

- [1]. Walsh TR. Emerging Carbapenemases: A global perspective. *Int J Antimicrob Agents* 2010; 36 Suppl 3:S8 -14.
- [2]. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009; 9:228-36.
- [3]. Cornaglia G, Rossolini GM. The emerging threat of acquired Carbapenemases in Gram negative bacteria. *Clin Microbiol Infect* 2010; 16:99-101.
- [4]. Clinical and Laboratory Standards Institute Performance Standard for Antimicrobial Susceptibility Testing : Twentieth Informational Supplement M100-S20-U .Wayne, PA:CLSI ;2010.
- [5]. Lee K, Chong Y, Shin HB, et al. Modified Hodge and EDTA- disk synergy test to screen metallo- β -lactamase producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001; 7:88-91.
- [6]. Nirav P Pandya, Sweta B, et al. Evaluation of various methods for detection of metallo beta lactamase (MBL) production in gram negative bacilli. *Int J Biol Med Res.* 2011; 2(3): 775-777.
- [7]. Nagaraj S, Chandran SP, Shamanna P, Macaden R. Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital in south India. *Indian J of Med Microbiol* 2012;30:93-5
- [8]. Deshpande P, Rodrigues C *et al*. New Delhi Metallo-beta lactamase (NDM-1) in *Enterobacteriaceae*: Treatment options with carbapenems compromised, *J Assoc Physicians India* 2010; 58:147-9.
- [9]. Parveen RM, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of *Klebsiella pneumoniae* in South India. *Inter J Pharm Bio Sci.*2010; 1(2).
- [10]. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to carbapenems in a tertiary care hospital in north India. *Indian J Med Res* 2006; 124:95-8.
- [11]. Drew RJ, Turton JF, Hill RL, Livermore DM, Woodford N, Paulus S, Cunliffe NA. Emergence of carbapenem-resistant *Enterobacteriaceae* in a UK paediatric hospital. *J Hosp Infect* 2013; 84(4):300-4.
- [12]. Pannaraj PS, Bard JD, Cerini C, Weissman SJ. Pediatric carbapenem-resistant Enterobacteriaceae in Los Angeles, California, a high-prevalence region in the United States. *Pediatr Infect Dis J.* 2015; 34(1):11–16.