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Carbapenem resistance among *Escherichia coli* in a tertiary care Rural Hospital

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Abstract
The worldwide emergence of resistance to the powerful antibiotic carbapenem constitutes an important growing public health threat. Bacteria having carbapenemase have the potential to spread rapidly within the hospital environment and also across continents. so, the present study was carried out with the Aim to find the prevalence of Carbapenem resistance & Metallo-β-lactamases (MBLs) in *Escherichia coli* in rural hospital. And the antimicrobial resistant pattern of Carbapenem resistance & Carbapenem sensitive *Escherichia coli*.
Methods: The present study was carried out in the Department of Microbiology from the period of January 2012 to August 2013. 294 *Escherichia coli* isolated from various clinical specimens were tested for carbapenem resistance by using meropenem disc [10µg] and by Modified Hodge test. The test was performed on all isolates on Mueller Hinton agar in accordance with guidelines of the CDC & CLSI. Metallo-β-lactamase detection was done. Isolates were tested for antibiotics by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI guidelines.
Result: Of 294 *Escherichia coli* isolated from various clinical specimen, Carbapenem resistance was observed in 31(10.54%) & none of the isolates were positive for Metallo-β-lactamase by phenotypic method. Among 31 Carbapenem resistant isolates, Maximum Carbapenem resistance were from urine (35.48%) followed by stool (19.35%), Pus (19.35%), Misc (16.12%), Sputum (6.45%) &Blood (3.22%). Ward-wise maximum Carbapenem resistance were from medicine (32.25%) followed by ICU (22.58%), Paediatric (19.35%), Surgery (12.9%), Obgy (9.67%) & OPD (3.22%). High antibiotic resistance was seen in carbapenem resistant *E. coli*.
Conclusion: Laboratories should routinely check for carbapenemase production among clinical isolates by phenotypic or genotypic methods.

Keywords: antibiotic resistance, carbapenem resistance, metallo-β-lactamase, modified hodge test, *Escherichia coli*

1. Introduction
Carbapenems are the latest developed molecules that possess the broadest spectrum activity, since 2000s; the spread of community-acquired *E. coli* isolates producing extended-spectrum β-lactamases (ESBLs) capable of hydrolysing almost all antibiotic β lactams except carbapenems has been reported worldwide [1]. The consequence of this emerging phenomenon has been an increased consumption of carbapenems. These antibacterial agents are crucial for treating life-threatening hospital-acquired infections, such as those linked with transplantations, hospitalizations in intensive care units, and surgery. The emergence of carbapenem resistance may jeopardize or stop the development of modern techniques in medicine. It is clear that very few novel antibiotics will be launched in the next few years, making the issue of carbapenem- resistant Enterobacteriaceae of primary importance worldwide [2].
Health officials are watching in horror as bacteria become resistant to powerful carbapenem antibiotics _one of the last drugs on the shelf [3]. Davies, the United Kingdom's chief medical officer, described CREs (Carbapenem resistant Enterobacteriaceae) as a risk as serious as terrorism [4]. Carbapenems were the last β-lactams retaining near-universal anti-Gram-negative activity, but carbapenemase are spreading, conferring resistance. These bacteria have the potential to spread rapidly within the hospital environment and also across continents [5]. Increasing reports on New Delhi metallo β-lactamases producing *Escherichia coli* constitutes a serious threat to global health since it is found to be highly resistant to most of the currently available antibiotics including carbapenem.
Escherichia coli the most common human pathogens, causes infections that range from cystitis to pyelonephritis, septicaemia, pneumonia, peritonitis, meningitis, device-associated infections. They are the most common source of both community-and hospital-acquired infections. They spread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons [1, 6, 7]. Plasmid may also include a number of other antibiotic resistance genes resulting into extensive drug resistance phenotypes "superbugs" [8]. In general, the term superbugs are a colloquial reference to a bacterium that carries resistant genes to many antibiotics [9]. Such bacteria are usually susceptible only to polymyxins & tigecycline [2].
Carbapenem resistance arises from two main mechanisms: (i) acquisition of carbapenemase genes that encode for enzymes capable of degrading carbapenems,
(ii) a decrease in the uptake of antibiotics by a qualitative or/and quantitative deficiency of porin expression in association with overexpression of β-lactamases that possess very weak affinity for carbapenems. The most important

carbapenemase are categorized as three types of enzymes:
(i) the KPC type enzymes first described in the US but now found worldwide;
(ii) the VIM, IMP, and NDM metallo-β-lactamases;
(iii) the OXA-48 type enzymes circulating among Mediterranean countries & progressively disseminating to other geographical areas [2].

The worldwide spread of carbapenemase-producing bacteria is a significant threat to human health: Firstly, the production of carbapenemase, in association with other resistance determinants, confers extensive drug resistance, leaving few or no therapeutic options [2]. Secondly, the association with travel underscores the risk of healthcare in countries where antibiotic-resistant bacteria are endemic [8]. Lastly, studies of patients infected are at increased risk of complications and death [10, 11].

Carbapenem are the most powerful tools against Gram negative bacilli. Due to the extensive misuse of this antibiotic, Carbapenem resistance have also developed in *Escherichia coli*. The bacteria having carbapenemases has the potential to spread rapidly within the hospital environment and also across continents. Timely detection can help in appropriate and aggressive infection control measures, effective antibiotic policy can help in confronting the menace of antibiotic resistance. The studies of Carbapenem resistance are being reported worldwide but there are very few studies from rural areas. Information on the prevalence of carbapenem resistance & Metallo β lactamases (MBLs) in clinical isolates from our area is not known. So, the present study was carried out with the

2. Aim

- To find the prevalence of Carbapenem resistance *Escherichia coli* in rural hospital
- To know the antimicrobial resistant pattern of Carbapenem resistance & Carbapenem sensitive *Escherichia coli*
- To know the prevalence of Metallo β lactamases (MBLs)

3. Material & Methods

The prospective study was carried out in the Department of Microbiology, MIMER Medical College, Pune from the period of January 2012 to August 2013.A total of 294 non duplicate clinical isolates of *Escherichia coli* were randomly selected. Sample size calculation was calculated using Krejcie & Morgan table. Approval was obtained from the Ethical Committee for carrying out the study.

All the confirmed *Escherichia coli* strains (n=294) was subsequently tested for carbapenem resistance by using meropenem disc [10µg]. The Isolates was considered resistant if the zone of inhibition was 19mm or less. *Escherichia coli* were also tested for carbapenemase production by phenotypic method - Modified Hodge test. The test was performed on all isolates on Mueller Hinton agar in accordance with guidelines of the Centers for Disease Control (CDC) and Clinical and Laboratory Standards Institute (CLSI) [12].

A lawn culture was prepared on Mueller Hinton agar (MHA) using an overnight culture suspension of *E. coli* (ATCC 25922) adjusted to 0.5 McFarland's standards. The plate was left for 15 min for drying and then a disc of 10 µg meropenem was applied at the center of plate. The isolates under study were streaked from the edge of the disk to the periphery of the plate. Four isolates were tested in each plate. After an overnight incubation at 37° C, the clover leaf like appearance between the test streaks near the disc was taken as positive for carbapenemase production.

Metallo-β-lactamase detection

Two imipenem disks (10 µg), one containing 10µl of 0.1 M anhydrous EDTA (292 µg), were placed 25 mm apart on a Mueller-Hinton plate [13]. A strain producing a diameter of >4 mm around the disk with IMP-EDTA and not around the disk with IMP alone was considered positive for MBL.

Isolates were tested for antibiotics by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI guidelines [1]. The antibiotic tested were**:** LM-Lomefloxacin (10µg), CIP-Ciprofloxacin (5µg), CF-Cefotaxime (30µg),G-Gentamycin (10µg), NET-Netilmycin (10µg), NO-Norfloxacin (10µg), CPZ-Ceptazidime (30µg), SLB-Ampicillin+sulbactum (20µg), CTX- Ceftriaxone(30µg), CFP- Cefoperazone (75µg), AN- Amikasin(30µg), PF-Pefloxacin (10µg). Antibiotic disc were obtained from Hi-media laboratories Pvt Ltd, Mumbai, India. Statistical analysis was done by using standard normal test (z test).

4. Result

Of 294 *Escherichia coli* isolated from various clinical specimen, Carbapenem resistance was observed in 31(10.54%) & none of the isolates were them were positive for Metallo-β-lactamase by phenotypic method. Among 31 Carbapenem resistance *E. coli*, 30 (96.77%) were Modified Hodge test positive.

Table 1: Prevalence of Carbapenem resistance in *E. coli* specimen-wise

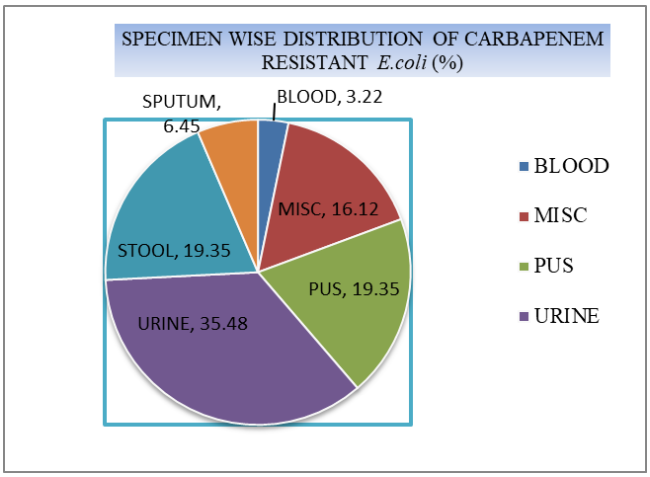
Specimen	Total no of *E. coli*	No of carbapenem resistant isolates	% of carbapenem resistant isolates
Urine	109	11	10.09
Pus	51	6	11.76
Sputum	26	2	7.69
Miscellaneous	60	5	8.33
Stool	33	6	18.18
Blood	15	1	6.66
Total	294	31	10.54

This table depicts higher prevalence of Carbapenem resistance in stool 18.18% followed by Pus 11.76 %

Table 2: Prevalence of Carbapenem resistance in *E. coli* Ward-wise

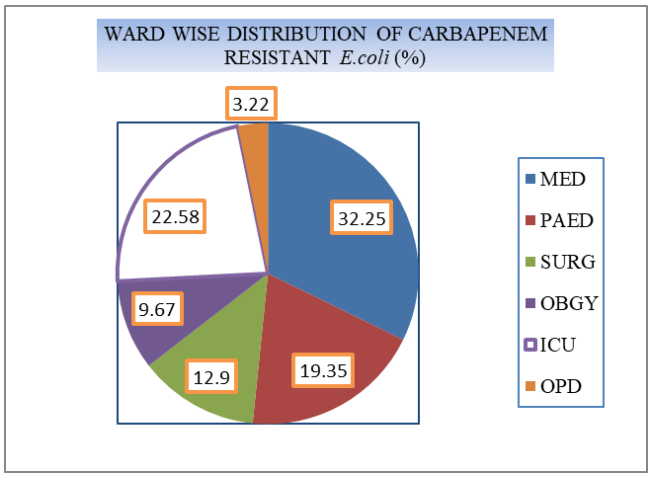
Specimen	Total no of *E. coli*	No of carbapenem resistant isolates	% of carbapenem resistant isolates
Medicine	60	10	16.66
ICU	31	7	22.58
Pediatrics	62	6	9.67
Surgery	37	4	10.81
Obgy	45	3	6.66
TB	10	0	0
Ortho	15	0	0
Skin	10	0	0
OPD	24	1	4.16
Total	294	31	10.54

Table shows higher prevalence of Carbapenem resistance in ICU ie 22.58% & lower Prevalence in OPD 4.16%



This Fig1depicts high Carbapenem resistance isolates from urine 35.48%

Chart 1: Distribution of 31 Carbapenem resistance isolates according to Specimen



This Fig 2 depicts highest Carbapenem resistance isolates from medicine (32.25%) & lowest from OPD (3.22%)

Chart 2: Distribution of 31 Carbapenem resistance isolates according to ward

Table 3: Antibiotic resistant pattern of Carbapenem Resistant *E. coli* & Carbapenem Sensitive *E. coli*

Antibiotic tested	Carbapenem resistance *E. coli* (n=31)	Carbapenem sensitive *E. coli* (n=263)	Z value	P value
Lomefloxacin	96	80	3.72	0.0001**
Ciprofloxacin	90	80	1.68	0.04
Cefotaxime	86	73	1.90	0.02*
Gentamycin	81	71	1.32	0.09
Netilmicin	94	69	4.87	0.0001**
Norfloxacin	94	86	1.68	0.04
Ceftazidime	95	82	2.84	0.002*
Ampicillin+sulbactum	100	100	0	0.5
Ceftriaxone	92	78	2.5	0.005*
Cefoperazone	98	83	4.39	0.00001**
Amikacin	47	14	0.74	0.23
Pefloxacin	100	79	8.36	0.00001**

* Z >1.98, P< 0.05 &** Z > 2.46, P < 0.01 for difference between Carbapenem Resistant *E. coli* & Carbapenem Sensitive *E. coli*

5. Discussion

The emergence of antibiotic resistance is a major concern for both developed and developing countries. Carbapenemases have been prevalent in non-fermentative Gram-negative bacteria since the early 1990s and contribute to carbapenem resistance rates ~ 50% for Acinetobacter baumanni & Pseudomonas aeruginosa. In contrast, carbapenemase resistance was rare in Enterobacteriaceae throughout the 1990s. Hospital outbreaks with carbapenemase-producing K. pneumonaie were first reported in the early 2000s. Since then, carbapenem resistance rates in Enterobacteriaceae have increased, particularly in K. pneumoniae [14-17]. Resistance to imipenem and meropenem in *E. coli* is rare worldwide [15-18].

In our study Carbapenem resistance was observed in 10.54%, similar were the finding of K V Ramana *et al*. who reported 11.3% whereas Sachinkumar reported 5% carbapenem resistant in their study [19, 20].

Deshpande *et al*. in their study tested 24 carbapenem resistant Enterobacteriaceae members and found 22 (91.6%) were MHT positive and later confirmed by PCR [21]. Whereas in our study 30(96.77%) were Modified Hodge test positive. the limitation of our study is not comparing the results with a molecular method due to cost constringent.

CLSI recommends MHT to detect carbapenemase production in Enterobacteriaceae isolates though the sensitivity &specificity of the test for detecting low-level metallo-β-lactamase not known [12].

The Prevalence of Carbapenem resistance *E. coli* isolates according to Specimen was observed to the tune of 10.09% in Urine, 11.76% in Pus, 7.69% in Sputum, 8.33% in Miscellaneous,18.18% in Stool, 6.66% in Blood. In was observed that the Prevalence of Carbapenem resistance *E. coli* from Community acquired infections was 4.16% but for the true extent of resistance the larger group has to be studied

Among the 31 isolates, maximum Carbapenem resistance were from urine (35.48%) followed by stool (19.35%), Pus (19.35%), Misc. (16.12%), Sputum (6.45%) and Blood (3.22%). Ward- wise maximum Carbapenem resistance were from medicine (32.25%) followed by ICU (22.58%), Paediatric (19.35%), Surgery (12.9%), Obgy (9.67%) and OPD (3.22%).

Genotypic method such as PCR can be used as for detection, but it is a real challenge for the routine clinical microbiology laboratories since molecular methods are not available in most medical institutions, capital costs are higher; this is limitation of our study. Carbapenem resistance and carbapenemase production conferred by bla $_{NDM\ 1}$ is detected reliably with phenotypic testing methods recommended by the Clinical and Laboratory Standards Institute including disk diffusion testing and the Modified Hodge test [12, 21]. though the sensitivity and specificity of the test for detecting low-level metallo-β-lactamase not known [12].

The Power of the study is the findings can be useful in developing strategies to control the spread of such bacteria as we still have no guidelines to treat infections caused by carbapenemase producing bacteria. Timely detection, appropriate and aggressive infection control measures, effective antibiotic policy can help in confronting the menace of antibiotic. So that a uniform protocol is maintained as the modern concept is that primary caretakers have to use antibiotics as little as possible and as short as possible.

Antibiotic resistance pattern of carbapenem resistant *E. coli* & carbapenem sensitive *E. coli* was studied. High antibiotic resistance was seen in carbapenem resistant *E. coli*.

In this study none of the isolates were positive for Metallo-β-lactamase by phenotypic method. Rawat *et al*. in their study reported 15.3% of the isolates of *E. coli* were positive for Metallo-β-lactamases [22]. it can be due to geographical variation.

NDM-1 was first detected in a clinical isolate of K. pneumoniae derived from a patient transferred from an Indian hospital to Sweden in 2008 [23]. Since then, NDM-1-producing Enterobacteriaceae have been detected worldwide in patients that typically have a history of recent hospitalization in the Indian subcontinent, recent travel to South Asia, or originate from these regions [8, 24]. MBLs inactivate all beta-lactam antibiotics except for aztreonam and strains harboring these enzymes are considered a major clinical threat because coresistance to multiple antibiotic subclasses is frequent and severely limits therapeutic options [23, 24].

In a recent Pakistan study fecal colonization with NDM-1-producing Enterobacteriaceae was detected in 13.8% of outpatients and 27.1% of inpatients, indicating a large reservoir of resistance within the community in this area [25].

Physicians who treat patients unlucky enough to be caught up in these outbreaks have no better medicines than they did when CREs first emerged. Some organisms respond to two drugs, tigecycline and colistin (also known as polymyxin E). Neither works in every patient, and colistin is notorious for damaging the kidneys. Physicians find themselves caught between using bad drugs or using no drugs at all [3].

6. Conclusion

Carbapenem resistance was observed in 10.54% of *E. coli*, so Laboratories should routinely check for carbapenemase production among clinical isolates by phenotypic or genotypic methods. This can be useful in developing strategies to control the spread of such bacteria as we still have no guidelines to treat infections caused by carbapenemase producing bacteria [26]. Thus Timely detection, appropriate and aggressive infection control measures, effective antibiotic policy can help in confronting the menace of antibiotic resistance.

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