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EMERGENCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN A TERTIARY CARE HOSPITAL IN WESTERN UTTAR PRADESH: A THERAPEAUTIC CONCERN

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ABSTRACT **Background:** Antimicobial drug resistance, particularly, the carbapenem resistance, in family Enterobacteriaceae, has emerged globally, thus limiting the therapeutic options and thereby posing serious health issues. This study was done to study the frequency of isolates of Enterobacteriaceae in clinical samples and to detect carbapenem resistance in them. **Methods:** A total of 9211 clinical samples were subjected to culture i) by conventional methods as per standard bacteriological techniques, and ii) by an automated BACTEC System (BD) as per the manufacturer's instructions. Antibiotic susceptibility testing of the obtained isolates was done by Kirby Bauer disc diffusion method as per CLSI guidelines 2020. All the ertapenem resistant isolates were subjected to i) Modified Hodge Test (MHT) for the production of carbapenemase, and ii) Combined Disk Test (CDT), using imipenem and EDTA, for the production of metallo-beta-lactamases. **Results:** The total number of isolates of Enterobacteriaceae obtained were 1425 with frequency of isolation maximum from urine (30.1%), followed by pus (28.3%), blood (20.8%) and broncho-alveolar lavage (11.6%). The most frequent isolates were of Escherichia coli (26.6%), followed by Klebsiella pneumoniae(24.0%), Klebsiella oxytoca (14.1%), Proteus vulgaris (9.8%), Proteus mirabilis (8.6%), Citrobacter freundii (8.4%), Citrobacter koseri (6.2%). Carbapenem-screen positive were 993 (69.7%). Carbapenem resistance was detected most commonly in isolates of Escherichia coli (34.4%), followed by Klebsiella pneumonia (30.1%) and Citrobacter freundii (9.0%). Carbapenem resistant isolates were recovered predominantly from urine, followed by blood and pus. Among the carbapenem resistant isolates, carbapenemase producers, metallo-beta-lactamase producers and co-producers were 39.6%, 29.9% and 23.0%, respectively. Carbapenemase production was maximum among the carbapenem-resistant isolates of Escherichia coli (142/342, 41.5%), of Klebsiella pneumonia (121/299, 40.5%) and Citrobacter freundii (35/89, 39.3%). Metallo-beta-lactamase production was almost same among the carbapenem-resistant isolates of Escherichia coli (108/342, 31.6%), Klebsiella pneumonia (93/299, 31.1%), and of Citrobacter freundii (27/89, 30.3%). Co-production of carbapenemase and metallo-beta-lactamase was most frequently noted among the carbapenem-resistant isolates of Citrobacter koseri (22/74, 29.7 %), Proteus mirabilis (17/59, 28.8%) and Proteus vulgaris (11/46, 23.9%). **Conclusion:** The phenotypic methods, MHT and CDT, are useful to detect carbapenemase producers and metallo- beta-lactamase producers, respectively.

KEYWORDS : Enterobacteriacae, Carbapenem resistance, Combined Disk test, Modified Hodge Test.

INTRODUCTION:

The family Enterobacteriaceae comprises of large number of Gram-negative bacteria that may cause several community-onset or healthcare-associated infections. The infections associated with Enterobacteriaceae may include blood stream, urinary tract, respiratory tract, skin and soft tissue, and genital infections.[1,2] The cell wall active agents, mainly the beta-lactam antibiotics, are used to treat infections caused by Enterobacteriaceae. The resistance to this group of antibiotics, thus, poses difficulties in treatment, thereby challenging the therapeutic options. Beta-lactamase production is the main mechanism of beta-lactam antibiotic resistance in Enterobacteriaceae. These highly diversified enzymes cleave the β-lactam ring, an essential component of β-lactam antibiotics, thus preventing penicillin-binding protein inhibition.[1-5] Carbapenem has been the antibiotic of choice for severe infections associated with Enterobacteriaceae. The development of resistance to carbapenems in Enterobacteriaceae has become a serious global health concern.[1,6] Resistance to carbapenem occurs when an organism acquires an enzyme carbapenemase or when an isolate produces an extended - spectrum cephalosporinase, such as an Amp C beta-lactamase.[7,8,9] The mechanisms of carbapenem resistance include changes in outer membrane proteins, over expression of drug efflux pumps and carbapenem hydrolyzing enzymes.[10] Carbapenem-resistant Enterobacteriaceae (CRE) are usually resistant to all β-lactam antibiotics and may also be associated with non–beta-lactam antibiotic resistance determinants, which give rise to multidrug - resistant superbugs, often leaving behind very limited treatment choices.[5,10,11,12] Although molecular methods are considered most appropriate for the detection of carbapenem resistance, it is difficult to conduct in resource constrained laboratories. Hence, various phenotypic tests are being employed to detect the production of carbapenemases.[11]

MATERIALS AND METHOD:

The prospective study was carried out in a tertiary care hospital in western Uttar Pradesh, over a period of one year (from July 2021- June 2022). The study included all the clinical samples received in the Microbiology laboratory, from various in-patient and out-patient departments.

Ethics Approval: Approval from the institutional ethics committee was taken before conducting the study.

Sample Processing:

[1,2,3]Clinical samples such as blood, urine, pus, sputum, endotracheal aspirate, pleural fluid, cerebrospinal fluid and other body fluids, received in the laboratory, were processed for culture and antimicrobial susceptibility testing.[1,2,3] Samples were subjected to culture i) by conventional methods as per standard bacteriological techniques[1,2,3] and ii) by an automated BACTEC System (BD) as per the manufacturer's instructions**.** The isolates grown onto culture media were identified by conventional methods such as colony characteristics, culture smears, motility and the biochemical tests.[1,2,3] Antibiotic susceptibility test was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines2020, by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar.[13]

Screening Of Carbapenem Resistance:[13]

Isolates of Enterobacteriaceae were screened for carbapenem resistance using ertapenem disc (10µg), by Kirby Bauer disc diffusion method, according to CLSI guidelines.[13]

Detection of carbapenemase production:[13]

All the ertapenem resistant isolates were subjected to Modified Hodge Test (MHT) for the production of carbapenemase, as recommended by CLSI guidelines.[13]

A positive test showed a clover leaf like indentation of standard strain of *Escherichia coli* ATCC 25922, growing along the test organism growth streak within the disc diffusion zone.

Detection Of Metallo-beta-lactamase Production:[13]

All the ertapenem resistant isolates were subjected to Combined Disk Test (CDT), using imipenem and ethylene diamine tetra acetic acid (EDTA), for the production of metallo-beta-lactamase, as recommended by CLSI guidelines.[13] The inhibition zones with the imipenem and EDTA disc ≥ 7 mm than the imipenem disc alone, were interpreted as positive for metallo-beta-lactamase (MBL) production.

RESULTS:

A total of 9211 clinical samples were received in the laboratory. The

number of isolates of Enterobacteriaceae obtained from various clinical samples were found to be 1425. The frequency of isolation was maximum from urine (30.1%), followed by pus (28.3%), blood (20.8%), broncho-alveolar lavage (11.6%), endotracheal aspirate (4.4%), sputum (2.0%), pleural fluid (1.8%), menstrual blood (0.5%), placental membrane (0.3%), synovial fluid (0.2%), and ascitic fluid (0.1%). Out of total of 1425 clinical isolates, most frequent were of *Escherichia coli* (26.6%%), followed by *Klebsiella pneumonia* (24.0%), *Klebsiella oxytoca* (14.1%), *Proteus vulgaris* (9.8%), *Proteus mirabilis* (8.6%), *Citrobacter freundii* (8.4%), *Citrobacter koseri* (6.2%), *Salmonella* Typhi (0.7%), *Morganella morganii* (0.6%), *Providencla rettgeri* (0.4%), *Providencla stuartii* (0.3%), *Enterobacter aerogenes* (0.2%) and *Serratia marcescens* (0.1%). Out of total of 1425 clinical isolates, carbapenem-screen positive isolates were 993 (69.7%) and the remaining 432 (30.3%) were carbapenem-screen negative. Among the carbapenem-screen positive isolates, majority (724/993, 72.9%), were recovered from in-door patients, thus indicating the association of these isolates with nosocomial infections. Carbapenem resistance was seen most frequent in *Escherichia coli* (34.4%), followed by *Klebsiella pneumonia* (30.1%), *Citrobacter freundii* (9.0%), *Klebsiella oxytoca* (7.7%), *Citrobacter koseri* (7.5%), *Proteus mirabilis* (5.9%), *Proteus vulgaris* (4.6%), *Morganella morganii* (0.4%), *Providencia rettgeri* (0.3%) and *Enterobacter aerogenes* (0.1%) (Table-1). Carbapenem resistant isolates of Enterobacteriaceae were recovered predominantly from urine (32.7%), followed by blood (28.8%), pus (19.8%), endotracheal aspirate (8.2%), broncho-alveolar lavage (5.4%), sputum (2.8%), pleural fluid (1.4%) and ascitic fluid (0.8%) (Table-2). Out of 993 carbapenem-screen positive isolates, 393 (39.6%) were only MHT positive, 297 (29.9%) isolates were only CDT positive, 228 (23.0%) were both MHT and CDT positive, and the remaining 75 (7.6%) were both MHT and CDT negative (Table-3). Hence, among the carbapenem-resistant isolates, carbapenemase producers, metallo-beta-lactamase producers and co-producers were 39.6%, 29.9% and 23.0%, respectively, Based on the phenotypic tests, carbapenemase production was maximum among the carbapenem-resistant isolates of *Escherichia coli* (142/342, 41.5%), followed by those of *Klebsiella pneumonia* (121/299, 40.5%) and *Citrobacter freundii* (35/89, 39.3%) (Table-4). Metallo-beta-lactamase production was almost same among the carbapenem- resistant isolates of *Escherichia coli* (108/342, 31.6%), and those of *Klebsiella pneumonia* (93/299, 31.1%), followed by those of *Citrobacter freundii* (27/89, 30.3%) (Table-4). Co-production of serine carbapenemase and metallo-beta-lactamase was most frequently noted among the carbapenem- resistant isolates of *Citrobacter koseri* (22/74, 29.7 %), followed by those of *Proteus mirabilis* (17/59, 28.8%) and *Proteus vulgaris* (11/46, 23.9%) (Table-4).

1: Frequency Of Carbapenem-screen Positive Isolates Of Enterobacteriaceae (n=993) :

Carbapenem-screen positive isolates	Number (n=993)	Percentage %
Escherichia coli	342	34.4%
Klebsiella pneumoniae	299	30.1%
Citrobacter freundii	89	9.0%
Klebsiella oxytoca	76	7.7%
Citrobacter koseri	74	7.5%
Proteus mirabilis	59	5.9%
Proteus vulgaris	46	4.6%
Morganella morganii	4	0.4%
Providencla rettgeri	3	0.3%
Enterobacter aerogenes	1	0.1%

Table 2: Frequency Of Isolation Of Carbapenem-resistant Enterobacteriaceae (n=993) From Various Clinical Samples:

Clinical samples	Number Of Isolates (n=993)	Percentage (%)
Urine	325	32.7%
Blood	286	28.8%
Pus	197	19.8%
Endotracheal aspirate	81	8.2%
Broncho-alveolar lavage	54	5.4%
Sputum	28	2.8%
Pleural fluid	14	1.4%
Ascitic fluid	8	0.8%

Table 3: Phenotypic Tests-wise Positivity Of Carbapenem-resistant Enterobacteriaceae (n=993):

Total number of Carbapenem-screen positive isolates of Enterobacteriaceae (n=993)	Only MHT Positive		Only CDT Positive		Both MHT & CDT Positive		Both MHT & CDT Negative	
	No.	%	No.	%	No.	%	No.	%
	393	39.6	297	29.9	228	23.0	75	7.6

Table 4: Distribution Of Carbapenem-screen Positive Isolates Of Enterobacteriaceae (n=993), Based On Phenotypic Tests:

Carbapenem-screen positive isolates of Enterobacteriaceae (n=993)	Only MHT Positive		Only CDT Positive		Both MHT & CDT Positive		Both MHT & CDT Negative	
	No.	%	No.	%	No.	%	No.	%
Escherichia coli (n=342)	142	41.5	108	31.6	78	22.8	14	4.1
Klebsiella pneumoniae (n=299)	121	40.5	93	31.1	66	22.1	19	6.3
Citrobacter freundii (n=89)	35	39.3	27	30.3	18	20.2	9	10.1
Klebsiella oxytoca (n=76)	29	38.2	21	27.6	16	21.1	10	13.2
Citrobacter koseri (n=74)	27	36.5	18	24.3	22	29.7	7	9.5
Proteus mirabilis (n=59)	21	35.6	14	23.7	17	28.8	7	11.9
Proteus vulgaris (n=46)	16	34.8	13	28.3	11	23.9	6	13.0
Morganella morganii (n=4)	1	25	1	25	0	0	2	50
Providencla rett1eri (n=3)	1	33.3	1	33.3	0	0	1	33.3
Enterobacter aerogenes (n=1)	0	0	1	100	0	0	0	0
Total number (n=993)	393		297		228		75	

DISCUSSION:

The emergence of multidrug resistant Enterobacteriaceae has become a major health issue, worldwide, that requires appropriate action and measures.[6,8,14,15] Of special concern, within the family Enterobacteriaceae, is an escalation in recognition of isolates producing carbapenemases that cause resistance to the carbapenems. These enzymes include the class A carbapenemases, the class B metallo-beta-lactamases, and the class D oxacillinases.[4,7,8] A notable increase in the frequency of carbapenem-resistant Enterobacteriaceae (CRE) has become an alarming matter, posing hindrance in the treatment of severe infections caused by these nosocomial organisms.[11,12,16]

In our study, the number of isolates of Enterobacteriaceae obtained from various clinical samples were found to be 1425. The frequency of isolation was maximum from urine (30.1%), followed by pus (28.3%), blood (20.8%) and broncho-alveolar lavage (11.6%).

Enterobacteriaceae represent the major group of bacteria recovered from clinical samples and are the common causes of community-acquired and health-care acquired infections, such as urinary tract, bloodstream, and lower respiratory tract infections.[1,2,16,17]

In the present study, out of 1425 clinical isolates of Enterobacteriaceae, most frequent were of *Escherichia coli* (26.6%), followed by *Klebsiella pneumonia* (24.0%), *Klebsiella oxytoca* (14.1%), *Proteus vulgaris* (9.8%), *Proteus mirabilis* (8.6%), *Citrobacter freundii* (8.4%) and *Citrobacter koseri* (6.2%). Ruppé E et al.[6] reported that *Escherichia coli* and *Klebsiella pneumonia* were the most common isolates recovered from the clinical samples. The Enterobacteriaceae, most notably *Escherichia coli* and *Klebsiella pneumoniae*, are among the most important causes of serious bacterial infections[17-19] Various studies,[20,21,22] have shown that the members of Enterobacteriaceae are the common causes of urinary tract infections, which has been observed in our study also.

In our set up, amongst the clinical isolates of Enterobacteriaceae,

carbapenem resistance was found to be 69.7%, which is higher than the study carried out by in Etawah, Uttar Padesh, in 2017, by Diwakar J et al.[23] who had detected carbapenem resistance in 43.4% isolates. Akshaya R et al.,[11] in 2016, reported carbapenem resistance in 13.95% isolates. In 2011, study done by Manoharan et al.[24] reported 17% resistance to carbapenem in Enterobacteriaceae . From north India, studies carried out by Priya dutta et al. (2012),[25] Wattal C et al. (2011),[26] and Gupta E et al. (2006),[27] showed 7.87%, 13-57% and 17-22% resistance to carbapenems, respectively.

These findings indicates the rising trends of carbapenem resistance, pinpointing the indiscriminate use of carbapenems, In the present study,among the CRE isolates, majority (72.9%), were recovered from in-door patients, thus indicating the potential of these isolates to cause nosocomial infections. Alarming rates of carbapenem resistance in Gram-negative bacteria is being encountered in healthcare-associated infections, worldwide including India.[15,23,24] Nosocomial infections, particularly the blood stream infections, due to these resistant organisms are associated with significant mortality.[28]

In our study, carbapenem resistance was found frequently in *Escherichia coli* (34.4%), followed by *Klebsiella pneumonia* (30.1%), *Citrobacter freundii* (9.0%), *Klebsiella oxytoca* (7.7%) (Table-1). The detection of carbapenemases in *Escherichia coli* and *Klebsiella* species in the majority of in-door patients should be considered as an infection control emergency, therefore, continued antimicrobial resistance surveillance and detection of these enzymes becomes vital in light of these findings.[11,19,29]

In our set up, CRE isolates were recovered predominantly from urine (32.7%), followed by blood (28.8%), pus (19.8%) and endotracheal aspirate (8.2%) (Table-2). Diwakar J et al.[23] also found carbapenem-resistant isolates from urine, pus and blood.

In the present study, among the carbapenem - screen positive isolates, 39.6% were only MHT positive (carbapenemase producers), 29.9% isolates were only CDT positive (metallo-beta-lactamase producers), 23.0% were both MHT and CDT positive (co- producers) (Table-3). Diwakar et al.[23] showed that 81.81% isolates were positive for carbapenemase production by MHT, which is much higher as compared to our study (39.6%).

In our study, carbapenemase production was maximum among the isolates of *Escherichia coli* (41.5%), followed by those of *Klebsiella pneumonia* (40.5%) and *Citrobacter freundii* (39.3%) (Table-6). However, Diwakar et al. [23] reported carbapenemase production predominantly in *Klebsiella pneumoniae* (27.27%), followed by *Escherichia coli* (23.63%). A study done in north India, in 2018, by Gupta V et al.[30] also reported that 78% isolates of *Klebsiella pneumonia* were MHT positive.

In this present study, among the carbapenem-resistant isolates, 29.9% were CDT positive. Metallo-beta-lactamase production was almost similar in the isolates of *Escherichia coli* (31.6%), and those of *Klebsiella pneumonia* (31.1%), followed by those of *Citrobacter freundii* (30.3%) (Table-6). Gupta V et al.[30] reported that 64% isolates were positive by CDT (metallo-beta-lactamase producers),[28] which is much more than that detected in our set up (29.9%). Diwakar J et al.[23] detected MBL production in 47.27% isolates by using Meropenem with and without EDTA Ezy MIC Strips and CDT. The above data gives us an insight of the widespread presence of carbapenemase and MBL production among the members of Enterobacteriaceae.

In the present study, on comparing the phenotypic tests, it was found that MHT is more promising in detecting carbapenem resistance (39.6%) as compared to CDT (29.9%), which is similar to the observations made by other studies.[23,28,30] However, Kaur M et al.[29] observed that non molecular tests for detection of carbapenemases have variable results for MHT, EDTA disc synergy test and MIC by Agar Dilution Test, and showed that out of these three tests, the MHT lacks specificity and sensitivity.

In our study, among the carbapenem resistant isolates, co-production of serine carbapenemases and MBL (both MHT and CDT positive) was seen in 23.0%. Co-production was most frequently detected in *Citrobacter koseri* (29.7%), followed by *Proteus mirabilis* (28.8%) and *Proteus vulgaris* (23.9%) (Table-4). However, the remaining 7.6% isolates were both MHT and CDT negative (Table-5), which could be due to porin loss, over expression of drug efflux pumps and other mechanisms of drug resistance.[1,9,10]

The drug resistance genes are often carried on mobile genetic elements and can be transmitted from one person to another often via the hands of healthcare giver or via contaminated medical equipment.[11] The spread of CRE justify the implementation of core infection control measures and judicious antibiotic usage.[11,19,29]

CONCLUSIONS:

The spread of Carbapenem-resistant Enterobacteriaceae is a therapeautic threat. Therefore, it becomes crucial to identify class A and class B carbapenamase producers. The phenotypic methodologies, the Modified Hodge Test and the Combined Disk Test, have been considered as gold standard techniques to detect serine carbapenemase and metallo-beta-lactamase producers, respectively. The MHT and CDT are simple, easy to perform and cost-effective techniques used to indicate carbapenem resistance. A combined effort of continuous antimicrobial resistance surveillance and strict adherence to infection prevention and control practices becomes essential to combat and limit the burden of multidrug-resistant bugs.

Limitation:

Due to limited resources, molecular tests for detection of genes responsible for carbapenem resistance could not be carried out.

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Conflict Of Interest: nil

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