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Original Article

Pattern of Carbapenem Resistance in Enterobacteriaceae in Rajshahi Medical College Hospital

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Abstract

The global emergence and spread of Carbapenem Resistant *Enterobacteriaceae* have been threatening the ability to treat an infection. Hence the present study was carried out with the aim to isolate important members of *Enterobacteriaceae* family with identification of carbapenem resistant isolates among them. The study was done in the Department of Microbiology, Rajshahi Medical College with collaboration of different disciplines of RMCH from January 2019 to December 2019. Samples were collected purposively. Causative organisms were isolated by culture and identified by colonial morphology, gram staining and relevant biochemical tests. Identified *Enterobacteriaceae* those showed resistance to carbapenem (imipenem, meropenem) were tested phenotypically by Modified Hodge Test (MHT) to see carbapenemase production. A total of 97 *Enterobacteriaceae* were isolated from 275 samples. *E. coli* (54.64%) was the most frequent isolate. By Modified Hodge Test, 19(19.59%) bacteria were phenotypically confirmed as Carbapenem Resistant *Enterobacteriaceae (*CRE). This study signifies that carbapenem resistance is increasing at an alarming rate.

Key words: Carbapenem Resistant *Enterobacteriaceae*, Modified Hodge Test.

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Introduction

The family *Enterobacteriaceae* is a very large family of gram-negative bacteria that has been studied and characterized for almost a hundred years. They are ubiquitous in nature. Certain species are part of normal flora in human.[1]

Currently, the family *Enterobacteriaceae* includes more than 100 species and 40 genera and these numbers continue to increase.[2] Important genus include in their family are *Escherichia*, *Shigella*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Proteus*, *Serratia*, *Providencia* and *Morganella.* Members of *Enterobacteriaceae* cause a variety of infections such as intra-abdominal infections, urinary tract infections and respiratory tract infections in both the community and hospital settings, affecting normal hosts and those with pre-existing illness.[3]

Carbapenem are β-lactam antibiotics, presently considered as the most potent agents of treatment for multidrug resistant gram-negative bacterial infections as these agents have stability against majority of β-lactamase and their high rate of permeation through bacterial outer membrane[4] But the increasing use of carbapenem has led to the

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emergence of Carbapenem Resistant *Enterobacteriaceae* (CRE)[5] which are among the top tier of the WHO list of antibiotic-resistant 'priority pathogens' that pose the greatest threat to human health & it was categorized in critical group among the 12 deadliest bacteria.[6,7] Carbapenem resistance may be due to carbapenemase production, efflux pump and AmpC enzyme production. However, carbapenemase production were found to be the predominant mechanism of resistance.

Aims and Objective of the study: To isolate and identify *Enterobacteriaceae* with their resistance pattern to carbapenem in Rajshahi Medical College Hospital.

Materials and methods:

This was a cross-sectional type of descriptive study conducted in the Department of Microbiology of Rajshahi Medical College in collaboration with different disciplines of Rajshahi Medical College Hospital, Rajshahi for a period of one year. A total of 275 samples (wound swab, urine) were collected after taking prior consent using predesigned data sheet. These samples were collected from patients admitted in different disciplines of RMCH and also those coming to OPDs of the hospital.

Inclusion criteria:

- Adult patient of both sexes with surgical wound infections and burn wound infections having the criteria such as sign of inflammation, wound dehiscence, wound discharge and fever etc.
- Patients suspected for urinary tract infections (presence of clinical symptoms like urgency, frequency, burning sensation during micturition).

Exclusion criteria-

Patients refused to be a part of this study, patients of paediatric age group, patients having UTI with other urinary tract diseases were excluded in this study.

Microbiological method:

Collection of specimen:

Wound swab and urine were collected following recommended standard procedure.[8,9]

Culture: Bacterial isolates were aerobically cultured in Blood agar, Nutrient agar and MacConkey's agar media. HI chrome UTI agar was selectively used for uropathogens.

Biochemical test: After observing the colonial morphology, gram staining and microscopy, gram-negative organisms were further tested by biochemical tests such as TSI, Simmon citrate, MIU and ornithine decarboxylase test for identification as a member of family *Enterobacteriaceae*. Isolates with reduced susceptibility to meropenem and imipenem (diameters of zone of inhibition ≤19 mm) by disk diffusion method were further tested by Modified Hodge Test to detect carbapenemase production.

Modified Hodge Test: The clover leaf method or Modified Hodge Test (MHT) has been extensively used as a general phenotypic method for detection of carbapenemase. An inoculum of 5ml *E.coli* (ATCC 25922) was prepared with sterile normal saline and standardized by 0.5 McFarland standard. The inoculum was diluted by adding 4.5ml of sterile normal saline with 0.5ml of standard inoculum. The diluted inoculum of *E.coli* (ATCC 25922) was spread to Mueller-Hinton (MH) agar plate with a cotton swab. After a brief drying at room temperature, an imipenem disk (10µg) was placed at the center of the plate. A straight line was drawn with the help of an inoculating wire loop containing identified test bacteria from margin of imipenem disk of the MH plate and incubated overnight at 35oC in aerobic condition. Positive result was indicated by the presence of clover leaf like indentation of the *E.coli* (ATCC 25922) along the streak line of test bacteria within the zone of inhibition.[10]

Results

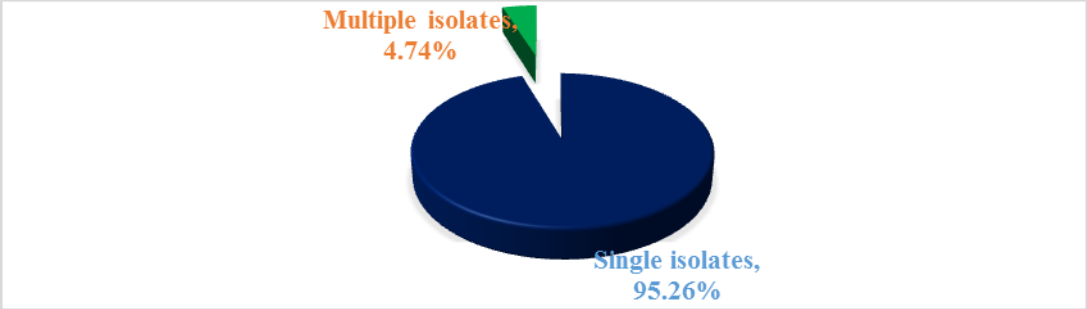
Out of 275 samples, 211(76.73%) were culture positive while 64 (23.27%) were culture negative

(Table-1). Among the 211 culture positive cases, 201(95.26%) were single isolates and 10(4.74%) were multiple isolates giving rise to a total of 221 isolated bacteria (Figure-I). Out of 221 culture positive isolates, 97(43.89%) were identified as a member of the family *Enterobacteriaceae* (Figure-II) where *E. coli* (54.64%) was the most frequent isolate followed by *Klebsiella pneumoniae* (20.62%), *Proteus* species (16.49%) and *Enterobacter* species (8.25%)(Figure-III). Table- 2 shows the distribution of different isolates of Carbapenem Resistant *Enterobacteriaceae*. Among 97 isolated *Enterobacteriaceae*, 19(19.59%) were phenotypically confirmed by Modified Hodge Test as Carbapenem Resistant *Enterobacteriaceae*. *E. coli* and *Klebsiella pneumoniae* were the highest in number accounting to 06(11.32%) and 06(30%) followed by *Proteus* species were 04(25%) and *Enterobacter* species were 03(37.5%) (Table-2).

Table-1: Age and sex distribution of study population with culture positive cases (N=275).

Age (years)	Total cases			Culture positive cases		
	Total	**Male**	**Female**	**Total**	**Male**	**Female**
19-30	97 (35.27%)	35 (12.73%)	62 (22.55%)	80 (29.09%)	30 (10.91%)	50 (18.18%)
31-40	81 (29.45%)	32 (11.64%)	49 (17.82%)	71 (25.82%)	29 (10.55%)	42 (15.27%)
41-50	52 (18.91%)	29 (10.55%)	23 (8.36%)	31 (11.27%)	17 (6.18%)	14 (5.09%)
>50	45 (16.36%)	26 (9.45%)	19 (6.91%)	29 (10.55%)	17 (6.18%)	12 (4.36%)
Total	275 (100%)	122 (44.36%)	153 (55.64%)	211 (76.73%)	93 (33.82%)	118 (42.91%)

Figure- I: Frequency of single and multiple bacterial isolates in culture positive cases (N=211).



Among the 211 culture positive cases, 201(95.26%) were single isolates and 10(4.74%) were multiple isolates giving rise to a total of 221 isolated bacteria.

Figure-II: *Enterobacteriaceae* among culture positive organisms (N=221).

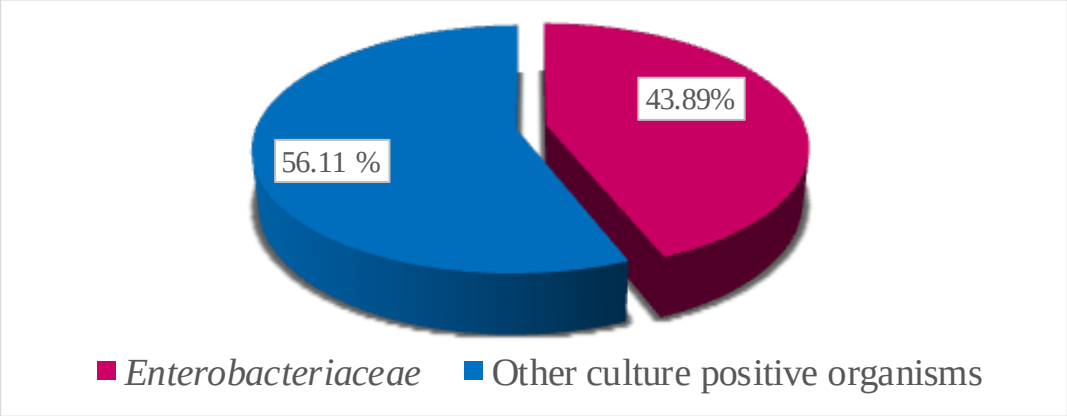


Figure-III: Frequency of different members of *Enterobacteriaceae* (N=97)

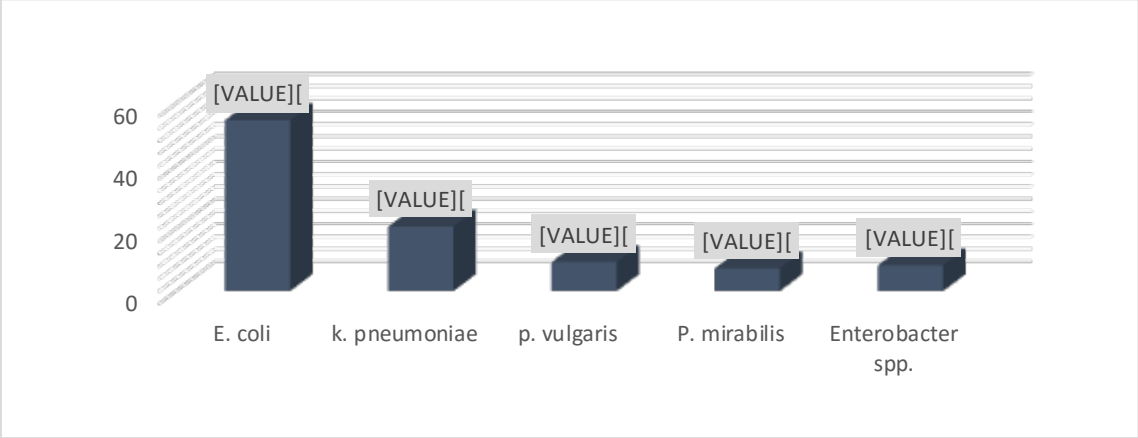


Table- 2: Distribution of Carbapenem Resistant *Enterobacteriaceae* by Modified Hodge Test (MHT) (N=97).

Isolates	Total no. of organisms	No. of organism	
		Positive by disk diffusion test (Screening test)	**confirmed by MHT (Phenotypic method)**
E.coli	53(54.64%)	16(30.19%)	06(11.32%)
Klebsiella pneumoniae	20(20.62%)	07(35%)	06(30%)
Proteus species	16(16.49%)	06(37.50%)	04(25%)
Enterobacter species	08(8.25%)	05(62.50%)	03(37.5%)
Total	97(100%)	34(35.05%)	19(19.59%)

Discussion

In Bangladesh, there is limited data regarding Carbapenem Resistant *Enterobacteriaceae* as few studies have been previously conducted. Therefore, the present study was designed to find out Carbapenem Resistant *Enterobacteriaceae* by phenotypic method. In the present study, 275 samples (wound swab, urine) were collected and 211(76.73%) samples yielded a positive culture (Table-1). In Bangladesh, nearly similar findings of 77.13% culture positive were observed in a study done by Dutta *et al*. (2013).[11] Saha *et al.* (2017)[12] in India and Mangesha *et al*. (2014)[13] in Ethiopia also reported 74.5% and 75% culture positivity respectively in their study which were in accordance with the present study The reason for such high occurrence of culture positivity in the present study may be due to spread of resistant pathogen in hospital environment, irrational use of antimicrobial agents, improper knowledge of patients about personal hygiene, poor sanitation, prolonged hospital stay and overcrowding of patients in hospital that contribute to high rate of cross infection.[14,15] Highest number of culture positive cases 80 (29.09%) were seen within the age group of 19 to 30 years (Table-1). The highest positive cases were observed within this age group (19-30 years) might be due to the active participation of people in different physical and mechanical works and during this, they may get injured.

In the present study, among all culture positive cases, mono-microbial growth was observed in 201 (95.26%) cases and poly-microbial growth was 10 (4.74%) (Figure- I). This corresponds with the study of Nahar *et al.* (2016)[16] in Bangladesh and Kaur *et al*. (2017)[17] in India, who reported 91.6% and 95.64% for mono-microbial isolates whereas 8.4% and 4.36% for poly-microbial isolates respectively. Open wounds can get easily colonized and invaded by numerous bacteria as they provide a warm and moist environment for bacterial colonization and proliferation. This might be the reason for polymicrobial growth in few samples.

A total of 97(43.89%) *Enterobacteriaceae* were identified among 221culture positive isolates (Figure- II). This finding was in conformity with the study of Gowda *et al.* (2018)[18] in India, Lohani *et al.* (2019)[19] in Nepal which were 51.8% and 45.57% respectively.

E.coli was found to be the most predominant bacteria accounting to 53 (54.64%) (Figure-III). In other studies, *E.coli* was also found to be the most prevalent isolates but the percentage varies. In Bangladesh, Biswas *et al*. (2015)[20] and Farzana (2013)[21] found a quite similar percentage of 55.45% and 53% respectively in their study.

The isolated *Enterobacteriaceae* those were resistant to carbapenem by disk diffusion test (either imipenem and meropenem or both) were further detected by Modified Hodge Test (MHT) and found 19.59% isolates were MHT positive for carbapenem resistance (Table- 2). This was nearly similar with the study of Haque (2019)[22] and Begum (2015)[23] in Bangladesh and Kaur *et al.* (2017)[17] in India where the prevalence of CRE was 17%, 14.5% and 17%, respectively.

Conclusion

From the above mentioned study it can be concluded that the proportion of CRE is gradually increasing. So, rational use of antibiotics, strong infection control strategies and regular update of antibiotic panel should be implemented in hospital settings of the country to combat with this vulnerable situation of antimicrobial resistance.

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