ORIGINAL ARTICLE

Emerging Carbapenem Resistance in *Enterobacteriaceae*

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ABSTRACT

Objective: To determine the frequency of carbapenem resistance in *Enteriobacteriaceae*.
Study design: Cross-sectional study.
Setting: Study was carried out from January 2011 to December 2011, in the Department of Microbiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), where 500 samples were collected from patients admitted in wards and ICUs of JPMC.
Method: Specimens were collected from patients admitted in wards and ICUs of JPMC and processed for the diagnosis in the department of Microbiology, BMSI. The nature of samples was Urine, Pus, Respiratory secretions and Blood. The samples were collected after taking all the necessary aseptic measures and transported to the Department and processed by standard methods. The culture positive samples were analyzed for further identification and antimicrobial sensitivity was done according to CLSI 2009.
Result: In this study out of (373) positive cases, (402) organisms were isolated. Out of that 200 (49.75%) were *Enterobacteriaceae*, others were Gram-positive and Gram-negative rods other than *Enterobacteriaceae*. *Escherichia coli* were leading pathogen (65%) among *Enterobacteriaceae.* Out of 200 only 12 (6%) were identified as CRE by Imipenem and Meropenem disc diffusion test. The sensitivity pattern of CRE and Non-CRE shows a significant difference. All the CRE were resistant to commonly used antibiotics. Non-CRE was resistant to Ampicillin, Amoxicillin-clavulanic acid Cephalosporins and Quinolones. Sensitivity against Amikacin, Carbapenems, Colistin and Tigecycline was high.
Conclusion There is an alarming increase of infections caused by Carbapenem and other antibiotics resistant *Enterobacteriaceae.*
Keywords: Carbapenem, CRE, Enterobacteriaceae, Meropenem, Imipenem

INTRODUCTION

Enterobacteriaceae contains more than 100 species of bacteria that normally are inhabitants of human and animal intestine. Various species of the *Enterobacteriaceae* are pathogenic and cause pneumonia, cystitis, pyelonephritis, septicemia, peritonitis, meningitis and device-associated infections[1,2]. Medically important species of family *Enterobacteriacae* are *E.coli, K.pneumoniae, C. frenundii, E. cloacae, E. aerogenes, Salmonella enteric*[3].

Antimicrobials are among the most commonly prescribed drugs in both hospitals and the community. There is a relationship between antimicrobial use and the emergence of bacterial resistance and represents a major public health problem with increasing and alarming frequency[4].

Carbapenems are the last resort of antimicrobial agents against many MDR Gram-negative bacteria. The carbapenems are β-lactam antibiotics with broad spectrum activity involving coverage of Gram-positive and Gram-negative aerobes and anaerobes, and are stable to almost all bacterial β-lactamases[5].

Carbapenemases are capable of hydrolyzing the carbapenem. There is increase incidence and diversity of Carbapenem Resistant strains and infection with Carbapenem-resistant *Enterobacteriaceae* (CRE) is emerging as an important challenge in health-care settings.

The resistance to carbapenem is result from i) alteration of penicillin binding proteins (PBPs), ii) diminished expression of outer membrane proteins (OMPs), iii) active efflux pumps promoting transport of the antibiotic from within the cell to the external environment, resulting in an intermediate level of resistance and iv) production of β-lactamases[6] .

Enterobacteriaceae members have the tendency to spread easily between humans (hand carriage, contaminated food and water) and to acquire genetic material through horizontal gene transfer, mediated by plasmids and transposons[2]. These bacteria are responsible for approximately 100,000 deaths each year in the US and account for about 50% of all the clinically significant bacteria isolated in the hospital laboratories[1]. Among these, *E. coli* and *K. pneumonia*

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are the most prevalent causes of nosocomial infection[7].

Carbapenems in the 1980s provided a new treatment option for serious bacterial infections; but now carbapenem resistance is being observed in *Enterobacteriaceae*, and *Acinetobacter spp*. is becoming common place in *P. aeruginosa*[8]. The National Healthcare Safety Network (NHSN) reported, 5% of *K. pneumoniae* in the United States between 2006 and 2007 that were resistant to carbapenem[9].

METHODS

This study was conducted in the Dept. of Microbiology, BMSI, JPMC, Karachi. In this study 500 samples were collected from the patients admitted in in wards and ICUs of JPMC from January 2011 to December 2011. The nature of samples was urine, pus, respiratory secretions and blood. The samples were collected after taking all the necessary aseptic measures and transported to the Department and processed by standard methods. The culture positive samples were analyzed for further identification and antimicrobial sensitivity was done according to CLSI 2009.

Inclusion/ Exclusion criteria: Irrespective of age or sex 500 samples were collected from the patients admitted in various units of JPMC, Karachi, and no exclusion criterion was adapted. These 500 specimens were divided into four groups: (a) 200 samples of urine were taken from UTI suspected Patients, (b) 150 samples of pus were taken from wounds, irrespective of its site, (c) 100 samples were taken from respiratory tract, Tracheal aspirates and sputum and (d) 50 samples of blood were taken from suspected patients of septicemia.

Microbiological methods: All samples were inoculated on Blood agar, MacConkey agar except urine and Blood. The respiratory secretions were additionally inoculated on Chocolate agar and Sabouraud Dextrose Agar (SDA). The urine was inoculated on Cysteine lactose electrolyte deficient (CLED) agar. These plates were incubated aerobically at 35±2ºC for 24 hrs. Inoculated blood culture bottles were incubated for 24 to 48 hours (if needed up to one week) at 37ºC and then examined for turbidity (showing positive growth). After overnight incubation, established microbiological methods, which include colonial morphology, Gram's staining and biochemical characteristics were used for identification[10].

Control strains: Phenotypically β-lactamase-negative *E. coli* ATCC 25922 was used as negative control.

Clinical isolates: 200 isolates of family *Enterobacteriaceae K. pneumoniae*, *Escherichia coli, Enterobacter spp., Proteus mirabilis, Proteus vulgaris, Citrobacter freundii, Providencia spp. and Serratia spp.* were preceded.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolated organisms was performed by the disk diffusion technique according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI)[11].

Selection protocol for carbapenemase producing *Enterobacteriaceae* strains (Screening Test): According to CLSI guide lines[11], isolates showing following inhibition zone size (Meropenem and/or Imipenem ≤21mm) of antimicrobial agents were identified as Carbapenem resistant. Potentially Carbapenem resistant *Escherichia coli* and *K. pneumoniae* was screen by disc diffusion test.

Statistical analysis: Data was analyzed using SPSS version 11.

RESULT

A total of 500 samples were collected from patients, admitted in different wards and ICUs of Jinnah Postgraduate Medical Centre (JPMC), Karachi. The subjects included in this study were of both sexes with their age ranging from 15 to 80 years. Out of 500 samples 200(40%) samples were taken from UTI patients (Urine), 150(30%) samples of pus were taken from patients wounds, 100(20%) samples of respiratory secretions (Sputum and Tracheal aspirates) were collected from patients of Lower respiratory tract infections (LRTIs) and 50(10%) samples of blood were taken from patients with suspected septicemia.

The nature of samples was urine, pus, respiratory secretions and blood. The culture positive samples were analyzed for further identification and antimicrobial sensitivity was done. In this study out of 500 samples, (402) organisms were isolated. Out of those isolates, 200(49.75%) were *Enterobacteriaceae* and 202(50.25%) were other than *Enterobacteriaceae* which included 116 (28.86%) Gram-positive cocci and 76(18.90%) Gram-negative rods and 10(2.49%) yeast. *Escherichia coli* were leading pathogen (65%) among *Enterobacteriaceae.*

Table 1: Distribution of different groups of microorganisms (n=402)

Organism	=n	%age
Enteriobacteriaceae	200	49.75
Gram-ve bacteria*	76	18.90
Gram-positive	116	28.86
Yeast	10	2.49
Total	402	100

*Other than *Enterobacteriaceae*

Table 2: Frequency of *enterobacteriaceae* species from positive specimens (n=200)

Enteriobacteriaceae species	Nature of specimen				Total (%)
	Urine (157)	Pus (132)	Respiratory secretions (95)	Blood (18)	
Escherichia coli	98 (62.42)	20(15.15)	10(10.5)	3(16.66)	131(65.5)
Klebsiella pneumoniae	15(9.55)	10(7.57)	15(15.78)	2(11.11)	42(21.0)
Klebsiella oxytoca	2(1.27)				2(1.0)
Proteus mirabilis	7(4.45)	2(1.51)			9(4.5)
Proteus vulgaris	1(0.63)				1(0.5)
Citrobacter freundii,		2(1.51)			2((1.0)
Enterobacter cloacae	4(2.54)	6(4.54)		1(5.55)	11(5.5)
Provendicia stuartii	1(0.63)				1(0.5)
Serratia marcescens	1(0.63)				1(0.5)
Total (%)	129(82.16)	40(30.30)	25(26.31)	6(33.33)	200(49.75)

Table 3: Distribution of cabapenem resistant *enterobacteriaceae* by imipenem disc diffusion method (n=200)

Total Organisms	Imipenem Disc(10 µg)		Total positive (%)
	Resistant(≤21mm)	Sensitive	
200	12(6.0%)	188(94%)	12(6.0%)

Table 4: Frequency of carbapenem resistant *enterobacteriaceae* (n=12)

Organisms	=n	%age
Escherichia coli (131)	6	4.58
K. pneumoniae (42)	4	9.52
E. cloacae (11)	01	9.0
Proteus mirabilis (9)	01	11.0

Frequency of *Enterobacteriaceae* species among 200 total *Enterobacteriaceae* isolates were from total positive specimens. According to that *Escherichia coli* were 131(65%), 42(21%) were *Klebsiella Pneumoniae*, 2(1%) were *Klebsiella oxytoca*, 9(4.5%) were *Proteus mirabilis*, 1(0.5%) was *Proteus vulgaris*, 2 (1%) were *Citrobacter freundii*, 11(5.5%) were *Enterobacter cloacae*, 1(0.5%) was *Providencia sturatii* and *Serratia marcescens* was 1 (0.5%). *Escherichia coli* were the most common species isolated from these specimens, comprising 131*/402* (32.58%). This organism was the major isolate recovered from urine samples, representing 98*/157* (62.42%) of the total isolates.

Carbapenem (Imipenem and Meropenem) resistant *Enterobacteriaceae* (CRE) were detected by disc diffusion method. Out of 200 *Enterobacteriaceae* species, 12 (6.0%) were resistant and 188 (94.0%) were sensitive. This shows the low prevalence of CRE in our population and still the carbapenem is the choice of antibiotics against *Enterobacteriaceae*.

Sensitivity pattern of non-CRE and CRE to most common antibiotics was TZP (82%), AK (90%), IPM (99.5%), MEM (99.5%), Tigecycline (TGC) (97%) CT and PB were (100%) in non-CRE. In CRE most sensitive drug was Colistin and Polymyxin B (100%) and Tigecycline (91%).

DISCUSSION

The increasing frequency of carbapenem resistant *Enterobacteriaceae* is a major concern in human health, as this significantly limits treatment options for life-threatening infections. Therefore detailed understanding of the molecular basis and epidemiology of carbapenem-resistance is needed. This study is designed to assess, the current prevalence of carbapenem resistant *Enterobacteriaceae.*

Nazir *et al*[12] has conducted a study showing *Escherichia coli* 9% and *Klebsiella pneumoniae* 21% were resistant to Meropenem isolated from urine

samples at Islamabad. The prevalence of CRE gets higher from 0.4% (2007) to 9% (2011). This shows that the carbapenem resistance is emerging in Pakistan and needs more attention from our health society. In the present study 500 samples were included. Out of the total, 373 (74.6%) were positive while 127(25.4%) were found negative. In our study 402 organisms were isolated from 373 culture positive samples. Most common isolated groups of bacteria was *Enterobacteriaceae,* which were 200 (49.75%), other Gram-negative bacteria were 76 (18.9%), Gram-positive 116 (28.86%) and yeast 10 (2.49%) that were detected. This is consistent with the study of Akhtar *et al*[13].

In our present study *Enterobacteriaceae* were (49.75%) of total clinical isolates. Out of these 12(6.0%) were Carbapenem Resistant *Entero-bacteriaceae* (CRE). The frequency of CRE is lower in present study as compared to the other countries but higher in comparison to the local studies. This may be due to the following factors: the most common is that the carbapenem is not irrationally used in our country because of its cost. Still in Pakistan cephalosporins are the first choice for empirical therapy and there is limited use of carbapenem group. Secondly the Carbapenem resistance in *Pseudomonas* is lower in our country (14%) as compared to other countries reported by Irfan *et al*[14]. Lastly limited work has been done on carbapenem resistance in Pakistan regarding *Enterobacteriaceae.*

Behera *et al*[15] from India who reported that carbapenems are highly active against *Enterobacteriaceae*. The overall susceptibility to Imipenem (IPM), Meropenem (MEM) and Ertapenem (ETP) was 96%, 95% and 93%, respectively. They believe that IPM susceptibility can be used as a surrogate marker for susceptibility in CRE isolates. IPM resistance was noted in 13 isolates of *Enterobacteriaceae*. These results are in accordance to our study.

Another study from three tertiary care hospitals of Islamabad, (Pakistan Institute of Medical Sciences; the Government Services Hospital and Capital Development Authority Hospital Islamabad) was conducted by Nazir *et al*[12]. Their results showed 9% CRE, which too is likewise comparable to our study.

Goswami *et* al[16] from India showed sensitivity pattern of *Escherichia coli* against Meropenem were 51%. This is in contrast with our findings due to high prevalence of Meropenem/Imipenem resistance in *Enterobacteriaceae.* In our study the frequency of CRE was 6%, out of that *Escherichia coli*, (4.58%), *K. pneumoniae* (9.52%), *Enterobacter cloacae* 9% and *Proteus mirabilis* 11%. This is supported by the study from India conducted by Deshpande *et al*[17] on the emerging resistance to carbapenem in a tertiary care Hospital of Northern India. Their findings were *Pseudomonas spp*. 29%, *Acinatobacter spp*, 26%, *Klebsiella pneumoniae* 6.9%, *Escherichia coli* 3.5%, Proteus 8.3% and *Enterobacter spp* being 6%.

Another study conducted by Kidwai *et al*[18] showed the percentage of Meropenem resistance in *Escherichia coli* that was (16%) which is not in agreement with our study. Another study from Oman conducted by Prakash *et al*[19] showed Imipenem resistance was 28%, 17% and 14% in *Enterobacter spp*, *Escherichia coli* and *Klebsiella pneumoniae* respectively which is in higher percentage as compared to our study. This is because the resistance is dependant on different risk factors like ICU stay, exposure to invasive medical devices, antibiotic exposure, specifically cephalosporin, carbapenem and previous hospital stay described by Teo *et al*[20]. Therefore results may vary from center to center and country to country. Even within India, a wide range of Carbapenem Resistant *Enterobacteriaceae* (CRE) (2.7-69%) has been reported.

A study on co-resistance in CRE from Italy by Ambretti *et al*[21]*. K. pneumoniae* were isolated from UTI patients which showed resistance to all beta-lactam (Cefotaxime, Ceftazidime, Piperacillin/ tazobactam) antibiotics including Meropenem, Aminoglycosides, Sulphonamides, and Fluoroquinolones which is consistent with our study. Urban *et al*[22] also reported similar findings.

Colistin and Tigecycline is the core of therapy for carbapenem-resistant *Enterobacteriaceae* and last available active antimicrobial agents. In our study CRE were 100% sensitive to colistin which is in strong agreement with the findings of Souli *et al*[23], Urban *et al*[22] and Arnold *et al*[24] who reported that in vitro susceptibility to Ploymyxins among clinical CRE isolates ranges from 90-100%.

CONCLUSION

Lack of uniform antibiotic policy and indiscriminate use of antibiotics may have lead to emergence of resistant strains of *Enterobacteriaceae*. There is an alarming increase of infections caused by Carbapenem and other antibiotics resistant *Enterobacteriaceae.*

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