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Original Research Article

Fecal carriage of carbapenem resistant *Enterobacteriaceae* among the intensive care unit patients

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ABSTRACT

Background: The war against multidrug-resistant bacteria is challenging and of global concern. Hospitals are increasingly plagued by resistant gram negative pathogens. Bacteria of the family Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* are part of the normal human intestinal flora but are also often responsible for community- and healthcare-associated infections. These bacteria are prone to acquiring resistance genes.
Methods: Rectal swabs/swabs from the peri-anal area of the patients who were admitted in the Intensive Care Unit (ICU) of the accident and emergency department of this teaching hospital. Swabs were collected first on day 1 of admission, then day 4, and thereafter weekly during the period of stay in the ICU. All the swabs were immediately inoculated into trypticase soy broth with one 10µg meropenem disc and were incubated overnight at 35±2ºC, ambient air. Next day, the broth was vortexed, and then sub-cultured onto a MacConkey agar plate. On the third day, MacConkey agar plates were examined for lactose fermenting (pink-coloured) colonies. The representative isolated colonies were subjected to conventional antimicrobial susceptibility testing by the Kirby Bauer Disc diffusion method following the CLSI guidelines to know the susceptibility to carbapenem and other antimicrobial agents. Carbapenemase production was done by a Modified Hodge Test (MHT) and Imipenem-EDTA test.
Results: Out of 89 patients, carbapenem resistant *Klebsiella pneumoniae* and *E. coli* isolates were recovered from 35 (39.3%) patients i.e. *Klebsiella pneumoniae* isolates from fifteen patients and carbapenem resistant *E. coli* isolates from twenty patients. Prevalence of carbapenemase producing isolates was found to be 1.42%.
Conclusions: Surveillance for CRE can definitely help reduce rates of healthcare associated infections.

Keywords: Carbapenemase producers, Carbapenem resistant enterobacteriaceae, Faecal carriage, Intensive care unit patients, Imipenem- ethylenediaminetetraacetic acid test, Modified Hodge test

INTRODUCTION

For the last couple of decades, globally, the fight against the multidrug-resistant bacteria, still remains a challenge. There have been focused and concerted efforts in the direction of the development of novel antimicrobial molecules, more so against the Gram positive organisms, because of the problem of drug resistance in case of *Staphylococcus aureus* (*S. aureus*) and the *Enterococcus* species. A call for development of new antimicrobial molecules was rightly highlighted by "The Infectious Disease Society of America (IDSA)," with the proposing of the slogan, "Bad Bugs no Drugs," and issuing the acronym, "ESKAPE" pathogens (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* species, *Pseudomonas aeruginosa* and *Enterobacter* species).[1,2] The bacteria belonging to the family Enterobacteriaceae, that form part of the normal

human gut flora, such as *Escherichia coli* (*E. coli*), and *Klebsiella pneumoniae*, are prone to acquiring the resistance genes. There is growing evidence now that extra-intestinal pathogenic *E. coli* may be transmitted to humans via the food supply from a food animal source.

A predominant mechanism by which the gram-negative bacteria acquire resistance to the carbapenems is by acquiring carbapenemases, the enzymes that hydrolyse these antibiotics. The problem of antibiotic resistance among the Enterobacteriaceae gets compounded when it is seen in carbapenemases. This resistance can develop either as a result of acquiring enzyme carbapenemases or production of cephalosporinases, combine with mutations that decrease the permeability of bacterial cell membrane towards the entry of the drug. Just as better diagnostic tools and awareness for surveillance of CRE is gathering momentum, the problem of rapid global spread of these organisms is becoming increasingly evident.[3,4]

METHODS

The study was carried out in the Department of Microbiology, Pt. BD Sharma, Post Graduate Institute of Medical Sciences, Rohtak, in association with the Department of Pulmonary and Critical Care Medicine, over a period of one year.

The patients that were included in the study were those, in whom the either the rectal swab/peri-anal swab revealed, the commonest members of the family Enterobacteriaceae, i.e. *E. coli* or *Klebsiella* spp. that were resistant to the carbapenems. Rectal swabs/swabs from the peri-anal area of the patients who were admitted in the Intensive Care Unit (ICU) of the accident and emergency department of this teaching hospital, a tertiary level health care providing facility were collected. Swabs were collected first on day 1 of admission, then day 4, and thereafter weekly during the period of stay in the ICU.

Processing of samples

All the swabs, after collection of the sample from the patient, were immediately inoculated into trypticase soy broth with one 10 µg meropenem disc and were incubated overnight at 35±2ºC, ambient air. Next day, the broth was vortexed, and then sub-cultured onto a MacConkey agar plate. Streaking was done for obtaining isolated colonies and the inoculated culture plates were incubated overnight at 37±2ºC, ambient air. On the third day, MacConkey agar plates were examined for lactose fermenting (pink-coloured) colonies. The representative isolated colonies were subjected to conventional antimicrobial susceptibility testing by the Kirby Bauer Disc diffusion method following the CLSI guidelines to know the susceptibility to carbapenem and other antimicrobial agents. Screening for carbapenemase production was carried out by modified hodge test (MHT).

Interpretation/Results

- After 16-24 hours of incubation, the plate was examined for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disc.
- MHT Positive test showed a clover leaf-like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disc diffusion zone.
- MHT Negative test showed no growth of the *E.coli* 25922 along the test organism growth streak within the disc diffusion.

Expected values

- A positive MHT indicates that the isolate is producing a carbapenemase.
- A negative MHT indicates that the isolate is not producing a carbapenemase.
- For the organisms turning out to be CRE and/or MHT positive isolates, the species level identification was done.

Method limitations

The class of carbapenemase cannot be determined by the results of the MHT. Some isolates show a slight indentation but do not produce carbapenemase.[5]

Imipenem-EDTA Disc Method

A 0.5 McFarland standard suspension of test organism was prepared and was inoculated onto plates of Mueller-Hinton agar. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. $2H_2O$ in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. Two 10µg imipenem discs were placed on to the inoculated plate, and 10µl of EDTA solution was added to one of them to obtain the desired concentration (750µg). The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16-18 hours of incubation in air at 35°C. In the combined disc test, if the increase in inhibition zone with the imipenem and EDTA disc is greater than or equal to 7mm than imipenem disc alone, it was considered as MBL positive. A positive imipenem-EDTA disc method indicates that the isolate is producing a metallo-betalactamase. A negative imipenem-EDTA disc method indicates that the isolate is not producing a metallo-betalactamase. *Pseudomonas aeroginosa* ATCC 27853 was used as the control strain.[6]

Disposal of waste

All the biomedical waste generated during this study in the laboratory was discarded as per the Biomedical Waste Management and Handling Rules, 2017 guideline.[7]

Statistical analysis

The data was collected using Microsoft Excel spread sheet and doubly checked for errors. Qualitative data was presented as mean and standard deviation. Quantitative data was expressed in proportions.

Prevalence of carbapenem resistant Enterobacteriaceae was calculated by the following formula:

Total number of patients showing carbapenemase production in a given year (1 year) x 100 Total number of patients examined during the same period (1 year) A 'p` value ≤0.05 was considered as statistically significant. SPSS 20.0 software was used for analysis.

A total of 89 patients formed part of the study. A total of 100 rectal/peri-anal swabs were collected from these patients. A total of 89 patients were screened. Out of these 89 patients, growth of carbapenem resistant Enterobacteriaceae (CRE), i.e. *E. coli* or *Klebsiella* species was obtained from 35 patients. Samples were collected on day 1, day 4, and day 7 of admission from all these 35 patients.

Swabs on day 7 could not be collected from four patients, as these patients had died in the intervening period. Similarly, one more patient was lost in the study, before his rectal/peri-anal swab on day 4 could be collected.

RESULTS

From a total of 89 patients, hundred rectal swabs were taken over a period of one year (Figure 1).

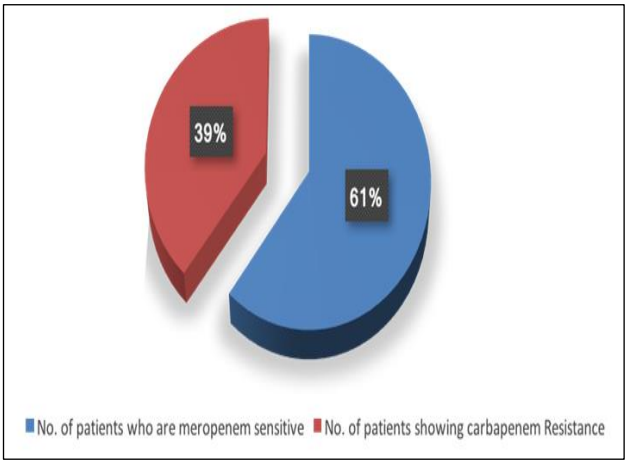

Figure 1: Distribution of organisms isolated from culture of rectal swabs.

Out of these, carbapenem resistant *Klebsiella pneumoniae* and *E. coli* isolates were recovered from 35 (39.3%) patients i.e *Klebsiella pneumoniae* isolates from fifteen patients and carbapenem resistant *E. coli* isolates from twenty patients.

The antimicrobial susceptibility findings of *Klebsiella pneumoniae* isolates is documented in Tables 1 (Table 1). Among the β-lactam group, 73.3% of the isolates were sensitive to ceftazidime, followed by ceftriaxone (66.7%), piperacillin-tazobactam and cefipime (60%), amoxyclav (33.3%), ampicillin and cefoperazone (26.7%). Among the fluoroquinolone group, 6.7% of the isolates were sensitive to levofloxacin and ciprofloxacin. Among the aminoglycoside group, 46.7% of the isolates were sensitive to amikacin, followed by gentamicin (26.7%).

Table 1: Antimicrobial susceptibility pattern of K. pneumoniae isolates (n=15).

Antibiotics	Sensitive	Percentage
Ampicillin	4	26.7
Ceftazidime	11	73.3
Cefepime	9	60
Ceftriaxone	10	66.7
Cefoperazone	4	26.7
Amoxyclav	5	33.3
Piperacillin tazobactam	9	60
Levofloxacin	1	6.7
Ciprofloxacin	1	6.7
Amikacin	7	46.7
Gentamicin	4	26.7
Cotrimoxazole	1	6.7
Doxycycline	5	33.3
Clindamycin	11	73.3
Tigecycline	14	93.3

Among the other drugs, 6.7% of the isolates were sensitive to cotrimoxazole, 33.3% were sensitive to doxycycline, 73.3% sensitive to clindamycin and 93.3% were sensitive to tigecycline. The antimicrobial susceptibility findings of *E. coli* isolates is documented in Tables 2 (Table 2).

Among the β-lactam group, 70% of the isolates were sensitive to ceftazidime, followed by ceftriaxone (65%), cefipime (60%), amoxyclav (35%), piperacillin-tazobactam (30%), ampicillin and cefoperazone (10%). Among the fluoroquinolone group, 5% of the isolates were sensitive to levofloxacin and ciprofloxacin.

Among the aminoglycoside group, 30% of the isolates were sensitive to amikacin, followed by gentamicin (15%). Among the other drugs, 5% of the isolates were sensitive to cotrimoxazole, 15% were sensitive to doxycycline, 85% sensitive to clindamycin and 75% were sensitive to tigecycline.

Out of 100 isolates from 35 patients, five isolates were carbapenemase producer (Table 3). Among these carbapenemase producers, one *E. coli* isolate was MHT positive and one was imipenem EDTA positive (Figure 2).

Table 2: Antimicrobial susceptibility pattern of *E. coli* isolates (n=20).

Antibiotics	Sensitive	% sensitivity
Ampicillin	2	10
Ceftazidime	14	70
Cefepime	12	60
Ceftriaxone	13	65
Cefoperazone	2	10
Amoxyclav	7	35
Piperacillin tazobactam	6	30
Levofloxacin	1	5
Ciprofloxacin	1	5
Amikacin	6	30
Gentamicin	3	15
Cotrimoxazole	1	5
Doxycycline	3	15
Clindamycin	17	85
Tigecycline	15	75

Table 3: Carbapenemase production among isolates.

Isolate	Carbapenemase producer		Non-carbapenemase producer
	MHT Positive	Imipenem-EDTA Positive	
E. Coli	01	01	18
Klebsiella spp.	00	03	12

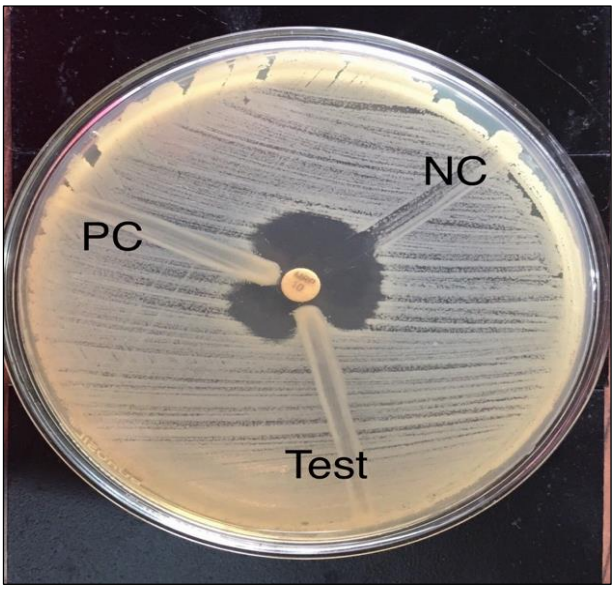

Figure 2: Modified Hodge test showing clover leaf-like indentation.

Also, three *K. pneumoniae* were imipenem EDTA positive. Hence, the prevalence of carbapenemase producing isolates was found to be 1.42%.

DISCUSSION

The study showed the prevalence of carbapenemase producing isolates to be 1.42%. The maximum sensitivity among carbapenem resistant *E. coli* and *Klebsiella* species isolates was towards ceftazidime.

Resistance amongst the commensal flora is a serious threat because a very highly populated ecosystem like the gut, at a later stage, could be a source of extra intestinal infections. As a part of the human intestinal flora, *Enterobacteriaceae* are easily spread and difficult to eliminate, especially in countries with low levels of hygiene. The production of acquired carbapenemases makes the choice of antibiotic treatment of infections caused by Gram-negative bacteria very limited.

In the present study, rectal swabs were taken from 89 patients admitted in the ICU of Accident and Emergency Department in a teaching tertiary care hospital. Out of these, CRE, i.e. *E. coli* and *K. pneumoniae* were recovered from 35 patients. Hence, the prevalence of carbapenem resistance is found to be 39.3%. A similar study by Zhou et al, on carbapenem-non-susceptible Enterobacteriaceae from a teaching hospital in Wenzhou, Southern China shows the rates of imipenem, meropenem, and ertapenem resistance as 59.2%, 40.8%, and 96.0% respectively.[8]

The present study shows that the carbapenem resistant *K. pneumoniae* isolates cultured from rectal swabs were showing markedly reduced susceptibility to all major classes of antimicrobial agents. Among the β-lactam group, only 26.7% isolates were susceptible to ampicillin and cefoperazone, followed by amoxyclav (33.3%), cefipime (60%), piperacillin- tazobactam (60%), ceftriaxone (66.7%), ceftazidime (73.3%). Among the fluoroquinolone group, only 6.7% isolates were resistant to levofloxacin and ciprofloxacin. Among the aminoglycoside group, only 26.7% isolates were resistant to gentamicin, followed by amikacin (46.7%). Among the other drugs, the sensitivity towards cotrimoxazole was 6.7%, followed by 33.3% towards doxycycline and 73.3% and 93.3% for clindamycin and tigecycline respectively.

Hariharan et al, studied the antimicrobial susceptibility pattern of *Klebsiella* spp. isolates and it showed a high level of resistance to ampicillin (93.1%) which is greater than the resistance found in the present study, followed by resistance to gentamicin (50%) which is less than present study percentage. The resistance to ceftazidime was more than compared to the present study whereas resistance to ciprofloxacin was less. There was a marginal resistance to piperacillin/Tazobactam i.e1.7% which is very less as compared to present study.[9] Also a study by Khan et al, on frequency of carbapenemase producing *K. pneumoniae* in Makkah, Saudi Arabia showed the drug resistance to cephalosporin ranging from 29.2% to 42.7%. Among cephalosporins, ceftriaxone and

ceftazidime showed 42.7% and 29.2% resistance which is more than the resistance seen in present study, whereas cefepime showed 30.9% resistance which is less than the present study. Among aminoglycosides, resistance to amikacin was 41.9% and 51.6% to gentamicin which are less than the resistance observed in the present study.[10]

The present study reveals high degree of antimicrobial resistance towards all major classes of antimicrobial agents in *E. coli* isolates recovered from rectal swabs. Among the β-lactam group, 90% isolates were resistant to ampicillin and cefoperazone, followed by piperacillin-tazobactam (70%), amoxyclav (65%), cefipime (40%), ceftriaxone (35%), ceftazidime (30%). Among the fluoroquinolone group, 95% isolates were resistant to levofloxacin and ciprofloxacin. Among the aminoglycoside group, 85% isolates were resistant to gentamicin, followed by amikacin (70%). Among the other drugs, 95% isolates were resistant to cotrimoxazole, 85% were resistant to doxycycline, 15% resistant to clindamycin and 25% were resistant to tigecycline. Hariharan et al, studied the antimicrobial susceptibility pattern of *E. coli* isolates and resistance to ampicillin, ciprofloxacin and gentamicin was 82.8%, 77.6% and 48.3 which are less than the resistance seen in the present study. Whereas, resistance to ceftazidime was 34.5% which is more than the resistance observed in the present study. This difference in the antimicrobial susceptibility could be due to different antibiotic policies followed in the different geographical areas.[9]

According to WHO Global report on antimicrobial resistance surveillance, only 71 (37%) WHO Member States could provide data on carbapenem resistance in *K. pneumoniae*. Hence, it shall be beneficial to conduct such studies to know the resistance pattern among CPE in rest all countries also so that the exact data for the prevalence of CPE could be documented.

Since, CPE readily spread and colonize the patients in healthcare environments, preventing the transmission of these organisms should be a major public health initiative and coordinated international efforts are needed in this direction.

The infection control strategies for CRE based on the importance of active surveillance, contact precautions and patient isolation have to be implemented, such as

- Determine whether CRE have been isolated
- Determine a selected wards and occurrence of intrafacility transmission
- Implement early CRE detection and containment measures
- Enhance existing infection control requirements (i.e. education, decontamination, minimize patient transfers and use of invasive devices)
- Develop a regional strategy
- Investigate community CRE spread.

Moreover, the detection of CRE can often be missed, if the Microbiology Laboratory personnel are not suspicious about its presence and subsequently its testing, since many carbapenemase producing organisms might be exhibiting low-level resistance, and hence, appear as susceptible, e.g. in case of group 2 carbapenems, i.e. meropenem and imipenem.[11] The identified risk factors of CRE colonisation include critical care illness, recent exposure to health care setting/ organ or stem cell transplant, mechanical ventilation, prior exposure to extended spectrum antimicrobial agents etc.[12] Now, there are very few option left, like colistin and polymixin B for the treatment of these patients infected with CPE which are among the last resort for the treatment and having many systemic side effects. The screening of faecal carriage of CPE should be done on regular basis in all the health care settings.[13]

CONCLUSION

Since, the prevalence of MDROs fast increasing among patients admitted in the hospitals, there are very few antimicrobial agents available for the treatment of such patients. Due to presence of many carbapenemase producing organisms exhibiting low-level resistance which appear as susceptible, especially group 2 carbapenems, i.e. meropenem and imipenem, CRE becomes difficult to detect. CRE colonisation is associated with various risk factors including critical care illness, recent exposure to health care setting/ organ or stem cell transplant, mechanical ventilation, prior exposure to extended spectrum antimicrobial agents etc. Hence, regular screening of patients for drug resistance becomes the most important part in the clinical practice. This study, directed towards the detection of prevalence of faecal carriage of carbapenem resistant Enterobacteriaceae among patients admitted in an intensive care unit, will help in prevention of excessive use antimicrobial agents and will provide the adequate treatment to such patients. Also, implementation of multi-faceted infection control measures, including contact precautions, performing adequate hand hygiene on all occasions, introducing and adhering to bundled prevention strategies and customised antimicrobial stewardship programmes can go a long way in containing the spread of such multidrug resistant organisms.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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