Original Research Article

Rectal Carriage of Carbapenemase Producing Enterobacteriaceae in Intensive Care Units of a Tertiary Care Hospital

Vignesh Kanna Balaji, K. Sudha*, B. Usha, Jeyakumari Durai Pandian, Joshy M. Easow and Swapna Muthuswamy

Annapoorna Medical College and hospital, Salem, Tamilnadu, India

*Corresponding author

Abstract

Antibiotic resistance is observed in both pathogenic and normal commensal flora. Gastrointestinal tract may serve as a reservoir for carbapenem resistant Enterobacteriaceae (CRE), resulting in cross transmission within / among health care settings. Awareness of the fecal carriage of Extended spectrum Beta lactamase (ESBL) and Carbapenemase-Producing Enterobacteriaceae (CPE) bacteria is very important for the clinicians, microbiologists, infection control practitioners and epidemiologists. Forty rectal swabs were collected from the ICU patients were inoculated directly onto MacConkey agar containing 5mg/l Cefotaxime. The grown isolates were subjected to identification and antimicrobial susceptibility testing. The BioMerieux RAPIDEC® CarbaNP test and Modified Carbapenemase inactivation method (mCIM) were performed to detect the Carbapenemase producing Enterobacteriaceae, as per CLSI guidelines. A total of 25 isolates were obtained from rectal swabs of 40 ICU patients. Of 25 isolates, 10 (38%) were E. coli and 15 (62%) were Klebsiella species. CarbaNP test showed positive for 7 isolates (70%) out of 10 E. coli and 13 isolates (41%) out of 15 Klebsiella species. The mCIM test showed positive for 8(80%) isolates out of 10 E. coli and 15(100%) isolates showed positive Klebsiella species. In CarbaNP test and mCIM, 5 and 2 isolates were negative respectively. mCIM is relatively simple and requires good technical skill compared to RAPIDEC® CarbaNP.

Keywords
Rectal carriage, CarbaNP, Modified carbapenemase inactivation method, Carbapenemase-producing enterobacteriaceae

Introduction

Antibiotic resistance is observed in both pathogenic bacteria and normal commensal flora. Current strategy applied for monitoring antibiotic resistance is through examination of pathogenic organisms and only periodic cross-sectional evaluation of resistance is undertaken for commensal flora. Members of family Enterobacteriaceae heavily colonize human gut and are the most frequent cause of bacterial infections in patients of all ages. Humans are regularly exposed to new strains with novel genetic repertoires including antibiotic resistance through food, water, from other animate and inanimate sources in the community and hospital settings.

Resistance amongst the commensal flora is a serious threat because, the highly populated...
ecosystem like the gut, may at a later stage, be the source of extra intestinal infections. Resistant strains may spread to other hosts or transfer resistance elements to other members of micro-biota including pathogens. Extended spectrum Beta lactamase (ESBL) producing Enterobacteriaceae (ESBL-E) are rampantly reported in India and carbapenems are the drug of choice for ESBL-E. Gastrointestinal tract may serve as a reservoir for Carbapenem Resistant Enterobacteriaceae (CRE), resulting in cross transmission within the health care setting.

Infection caused by ESBL-E is associated with antibiotic abuse or lengthy hospital stay and poor compliance with infection control measures. ESBL-E infections are also linked to increased mortality, largely due to lack/delay in effective therapy. The increasing use of carbapenems for empirical therapy of hospital-acquired sepsis has led to a rapid global dissemination of Carbapenemase-Producing Enterobacteriaceae (CPE).

Patients of medical units with high levels of antibiotic consumption are also prone for colonization of Multi drug resistant pathogens includes ESBL and Carbapenemase producers. Intestinal carriage serves as a reservoir of CPE and can promote cross-transmission in health care settings. Awareness of the fecal carriage of ESBL and CPE bacteria is very important for the clinicians, microbiologists, infection control practitioners and epidemiologists. Global data shows that the prevalence of these bacteria poses serious threat to people in both community as well as hospital setting.

Study for the detection of Carbapenemase producing Enterobacteriaceae is pertinent for framing antibiotic and infection control policies. Hence, we intended to screen the CPE in fecal sample of ICU patients.

Materials and Methods

The prospective cohort study was conducted in the department of Microbiology. Institutional Ethical clearance was obtained and the samples were collected from April 2017 to September 2017. A total of 40 rectal swabs were obtained from ICU patients after getting informed consent from the patients or their attenders. All the ICU patients who stayed for more than 3 days were included in the study. Exclusion criteria include previously hospitalized patients, elimination of other than Enterobacteriaceae organisms.

Rectal swabs were inoculated onto MacConkey agar with 5mg/l of cefotaxime and incubated at 37°C for 24 hrs. Identification was made by following standard microbiological methods used for identification of Enterobacteriaceae.

Antimicrobial susceptibility testing

On Muller-Hinton agar, lawn culture of test organism was made and antibiotic disc were placed on it amikacin (30µg), gentamicin (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), meropenem (10µg), imipenem (10µg), co-trimoxazole (1.25/23.75µg), and piperacillin/tazobactam (100/10µg) and incubated at 37°C for 24hrs; interpretation was based on CLSI guidelines 2017.

Carbapenemase detection

RAPIDEC® Carba NP

Carbapenemase producing Enterobacteriaceae was detected by using BioMerieux RAPIDEC® Carba NP kit. The tests were performed according to the manufacturers recommendation. A loop of the bacterial colony was picked up from overnight-incubated Muller-Hinton agar plate and mixed
into API suspension medium (provided with kit); the bacterial suspension was then transferred to wells in the test strip and incubated at 37°C. Visual readings of the test strip was done after 30 min and also after 2 hrs. A positive test corresponded to a color change from red to yellow-orange, whereas a red color indicated a negative result.

**mCIM (Modified Carbapenemase inactivation method)**

Isolate was emulsified in 2ml of Trypticase soy broth and 10µg meropenem disk was added to each tube, incubated at 35°C in ambient air for 4 hrs.

*E. coli* ATCC 25922 was inoculated as lawn culture onto Muller Hinton agar medium, and the incubated meropenem disk was placed on the MHA, after which the MHA was incubated at 35°C for 18-24 hrs.

The zone of inhibition was measured and reported as positive if zone diameter is 6-15mm, and negative if >19mm. If the test isolate produces a Carbapenemase, the meropenem in the disk will be hydrolysed and there will be no inhibition or limited growth inhibition of the meropenem susceptible *E.coli* ATCC 25922.

**Results and Discussion**

A total of 25 isolates were obtained from rectal swabs of 40 ICU patients. Out of 25 isolates, ten were (10/25, 22%) *E. coli* and fifteen were (15/25, 42%) *Klebsiella* species. All the isolates were evaluated for antimicrobial susceptibility to various antimicrobials. Maximum sensitivity was found against piperacillin/tazobactam (61.53%). All the isolates (100%) were resistant to cotrimoxazole, imipenem and meropenem. whereas 60-90% isolates were highly resistant to the other antimicrobials tested.

All the isolates were subjected for the detection of Carbapenemase production by BioMerieux RAPIDEC® CarbaNP kit, a colorimetric assay. Out of 25 isolates 20 were positive for Carbapenemase production out of which, seven were (7/20, 36%) *E.coli* and thirteen were (13/20, 64%) *Klebsiella* species.

Modified Carbapenemase inactivation test is performed for all the isolates. Out of 25 isolates, 23 were positive. Eight were (8/23, 34%) *E.coli* and fifteen were (15/23, 66%) *Klebsiella* species. In CarbaNP test and mCIM, 5 and 2 isolates were negative respectively (Fig. 1 and 2; Table 1).

**Table 1** Comparison of CarbaNP and mCIM test

<table>
<thead>
<tr>
<th>Isolate</th>
<th>CarbaNP (20)</th>
<th>mCIM (23)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.coli</em></td>
<td>7 (36%)</td>
<td>8 (34%)</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp</td>
<td>13 (64%)</td>
<td>15 (66%)</td>
</tr>
</tbody>
</table>
**Figure.1** BioMerieux Rapidec CarbaNP test

**Figure.2** Modified carbapenemase inactivation method
Carbapenems are the most effective antimicrobial agent against Gram-negative bacteria and have a broad spectrum of antibacterial activity against members of Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter baumannii complex. They are generally used as last-resort drugs for the treatment of infection caused by multidrug resistant bacteria. Mechanism of resistance to Carbapenems include production of Beta-lactamase, efflux pumps, and mutation that alter the expression and/or function of porins and penicillin-binding proteins. The alarming increase of CRE prevalence worldwide is worrisome. In many endemic settings of UAE and Iran, prevalence as high as 24.7%–29.8% have been noted. Mohan et al. in 2017, documented the prevalence rate of CRE as 18.7% in India. The faecal carriage of CRE acts as a reservoir for dissemination of these multidrug-resistant pathogens through cross-transmission. These highly drug-resistant organisms may stay for prolonged periods in the intestinal tract without causing any infections or may serve as a source of endogenous urinary tract infections, intra-abdominal infections or even translocate through the gut epithelium to cause sepsis.

Preventing the transmission of CP-CRE is a high priority for all the institutions. Screening the contacts of CP-CRE infected patients is essential to curb transmissions and control outbreaks. However, approaches to routine screening for CRE carriage vary depending on institutional epidemiology, resources and policies. Baseline surveillance is recommended to determine the prevalence and types of Carbapenemase enzymes circulating in an institution. Although molecular assays are expensive method for CP-CRE screening, the fast turnaround time, high sensitivity, specific genetic information, and availability of FDA-approved or CE-marked assays are appealing. Culture-based methods are yet to be approved, less sensitive than molecular methods, labour intensive and slow, but have a lower reagent cost.

In our study 22% E.coli and 42% Klebsiella spp were resistant to carbapenems, which was similar to the results shown in the study conducted by Xu et al. The Carba NP test has multiple benefits. It is inexpensive, rapid, reproducible, and highly sensitive and specific. It eliminates the need for using other techniques to identify carbapenemase producers that are time-consuming and less sensitive or specific. Using this accurate test would improve detection of patients infected or colonized with Carbapenemase producers. A study conducted by Atul Garg et al. in India, showed the sensitivity and specificity of BioMerieux RAPIDEC® CarbaNP test as 92.6% and 96.2% which is in concordance with our study. A study from the National Reference Center for Antibiotic Resistance, France, Dortet et al. documented a sensitivity of 99% and specificity of 100%. Poirel and Nordmann tested this kit with pre-characterized strains and documented a sensitivity and specificity of 96%. In this study the Carba NP test showed positive for 20 isolates of 25 in which, seven were (7/20, 36%) E. coli and thirteen were (13/20, 64%) Klebsiella species.

Clinical and Laboratory Standards Institute (CLSI), 2017 included the “modified CIM (mCIM)” as a Carbapenemase screening test for Enterobacteriaceae (CLSI M100-S27) which is relatively simple with the sensitivity of >99% and specificity of >99% for detection of CPE. Our study showed 23 isolates positive out of 25 by Modified Carbapenemase inactivation test. In this eight were (8/23, 34%) E.coli and fifteen were (15/23, 66%) Klebsiella species. In CarbaNP test and mCIM, 5 and 2 isolates were negative.
respectively. These results concordance with the study conducted by Goel N et al. Wherein, organisms producing low Carbapenemase activity enzymes, thickness of the inoculum and the disc potency are likely to influence the test results of mCIM with an overnight delay⁶.

**Limitation**

A major drawback of our current study is that it was performed as a pilot study over a short duration. Due to time and resource constrains, molecular characterization and other non-enzymatic mechanisms mediating carbapenem resistance, such as upregulated efflux pumps, porin defects, and hyper production of AmpC beta-lactamase were not performed in this study.

Hence concluded that, overall both the methods showed similar results. The mCIM is relatively simple and reliable, but requires good technical skill compared to RAPIDEC® CarbaNP. Early screening is needed for prevention of transmission in hospitalised patients. Surveillance studies might help in the implementation of infection control measures to curtail the spread of these isolates, both in the hospital and in the community.

**Acknowledgement**

We would like to express my gratitude to late Dr Lavina Lilly Francis in her absence, who came up with the idea of this research topic.

**References**

9. Pragasam AK, Veeraraghavan B,


How to cite this article: