

Research Article**Detection of carbapenemase producing enterobacterales using the Modified Hodge Test from clinical isolates in Colombo South Teaching Hospital and Sri Jayewardenepura General Hospital, Sri Lanka in 2017**Jayathilaka SH<sup>1</sup>, Jayatissa AH<sup>1</sup>, Chandrasiri SN<sup>2</sup>, Jayatilleke K<sup>3</sup>, Kottahachchi J<sup>4</sup>*Sri Lankan Journal of Infectious Diseases 2024 Vol.14(1): E56:1-7*DOI: <http://dx.doi.org/>**Abstract**

**Introduction:** Enterobacterales is a large family of Gram-negative bacilli including many pathogens. Carbapenemase producing Enterobacterales (CPEs) have emerged as a global threat. This study was conducted to detect carbapenemase production by Enterobacterales isolates from clinical specimens and to correlate the occurrence of CPE with age, gender, and duration of hospital stay of the patients included in the study.

**Methods:** A descriptive cross-sectional study was carried out using 120 consecutive, non-repetitive clinical isolates identified as Enterobacterales by the microbiology laboratories of Colombo South Teaching Hospital and Sri Jayewardenepura General Hospital. The demographic data of the patients was gathered and used to analyse the correlation with CPE occurrence. All isolates were identified up to species level using API 20E kits. Screening for detection of carbapenemase was carried out using meropenem, imipenem, and ertapenem disks. The Modified Hodge Test (MHT), which is one of the suggested procedures to determine carbapenemase production, was performed to identify carbapenemase producing isolates.

**Results:** Of the 120 isolates, 14 (11.7%) were resistant to at least one of the carbapenems tested. MHT detected carbapenemase production in ten (8.3%) isolates. The majority of these isolates were *Klebsiella* sp. (6; 60.0%). Of carbapenemase producing isolates, six were from urine specimens (60%). Eight (80.0%) of the CPE harbouring patients were males and eight (80.0%) were aged above 50 years. The mean duration of hospital stay of the patients was 7.2 days ( $\pm$ SD 5.65 days).

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**Conclusion:** The proportion of CPE was 8.3% according to this study and emphasises the importance of conducting more studies on the prevalence of CPE in Sri Lanka.

**Keywords:** *Carbapenemase, Enterobacterales, Modified Hodge Test*

## **Introduction**

Enterobacterales is a large family of Gram-negative bacilli which includes pathogens. Both community-acquired and healthcare-associated infections of the urinary tract, gastrointestinal tract, pneumonia, meningitis, sepsis, and medical device-associated infections are commonly caused by Enterobacterales. In the healthcare environment, they can be transmitted via contaminated hands of healthcare workers, air, direct contact between patients, and objects contaminated by patients, visitors, or other environmental sources.<sup>1</sup>

Carbapenems are a class of broad-spectrum  $\beta$ -lactam antibiotics with imipenem, meropenem, ertapenem, and doripenem used widely in clinical practice. In many cases carbapenems are the last effective antibiotic of choice against multidrug resistant (MDR) Gram-negative bacilli, including Enterobacterales.<sup>2</sup>

Carbapenemases belong to molecular classes A, B and D of  $\beta$ -lactamases. Class A includes KPC, IMI, SME, NMC and GES. Class B are metallo- $\beta$ -lactamases such as NDM, VIM and IMP, whereas class D consists of oxacillinase type  $\beta$ -lactamases. The MHT could be used as a phenotypic test to detect whether resistance to carbapenems is mediated by carbapenemase production.<sup>3</sup>

Infections caused by CPE pose a grave threat to the world, with higher associated mortality rates ranging from 48.0% to 71.0%. MDR organisms such as CPE have been labelled as a “serious threat to public health” by the Centres of Disease Control and Prevention, and “one of the greatest threats to human health” by the World Health Organization.<sup>4</sup>

Most pathogens resistant to carbapenems show high resistance to other antibiotic agents as well, such as cephalosporins, quinolones, and aminoglycosides, leaving some patients with no optimal therapeutic options. This leads to prolonged hospital stays, huge medical expenses, and higher mortality in CPE infected patients.<sup>5</sup> The use of carbapenems as an empirical treatment has dramatically increased due to the emergence of extended-spectrum  $\beta$ -lactamase producers.<sup>6</sup> This in turn leads to the rise of carbapenem resistance in pathogenic bacteria.

Although CPE have emerged as a global threat, information on the prevalence of CPE in Sri Lanka is limited. However, an increasing trend of carbapenem resistant Enterobacterales (CRE) prevalence has been reported.<sup>6,7,8</sup> Several studies conducted in Sri Lanka have reported the occurrence of metallo- $\beta$ -lactamases NDM-1, KPC, and oxacillinase OXA-181 carbapenemases.<sup>7,9,10</sup>

The present study was designed with the aim to screen and detect the proportion of CPE and correlate carbapenemase production with the demographic data of patients in two tertiary care hospitals in Sri Lanka.

## Methods

This descriptive cross-sectional study was conducted from 22<sup>nd</sup> to 30<sup>th</sup> November 2017 from clinical isolates collected from the microbiology laboratories of Colombo South Teaching Hospital and Sri Jayewardenepura General Hospital, Sri Lanka. The study was carried out in the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. Information on the age and gender, site or type of specimen, and duration of hospital stay of patients was obtained.

A total of 120 consecutive, non-repetitive Enterobacterales isolates collected from Colombo South Teaching Hospital and Sri Jayewardenepura General Hospital from different clinical specimens were subcultured on blood agar and incubated at 35±2 °C for 24 hours to achieve optimum performance. The isolates were identified by Gram stain, oxidase test, motility test, and API (Analytical Profile Index) 20E. Antibiotic susceptibility testing was performed for all the isolates using the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines, 2017.<sup>11</sup> We used the carbapenem drugs (imipenem, meropenem and ertapenem) to screen each isolate for resistance to carbapenems. Imipenem, meropenem and ertapenem were used to screen for production of carbapenemase.

All isolates resistant to any of the above-mentioned antibiotics were tested for carbapenemase production using the MHT. A 0.5 McFarland standard suspension of the indicator organism

*E. coli* American Type Culture Collection (ATCC) 25922 was prepared and diluted 1:10 in sterile normal saline. The suspension was inoculated on a Mueller Hinton Agar (MHA) plate as per the routine disk diffusion method. An ertapenem disk was placed in the middle of the plate. Three to five colonies of test and quality control organisms (MHT positive-*K. pneumoniae* ATCC BAA-1705 and MHT negative *K. pneumoniae* ATCC BAA-1706) grown overnight were inoculated in a straight line out from the edge of the disk as shown in Figure 1. The plates were incubated at 37 °C for 16-20 hours and observed for enhanced or inhibited growth.



**Figure 1: Detection of carbapenemase production using the Modified Hodge Test**

Data analysis was performed using Minitab version 17. The data was initially prepared in Microsoft Excel and then imported to Minitab. Frequencies were worked out and expressed along with percentages. The t-test and Fisher's exact test were applied to determine the significance of selected risk factors with CPE. A two-sided *P* value of less than 0.05 was considered statistically significant.

## Results

**Table 2: Resistance/Intermediate susceptibility of tested isolates**

Isolate number	Meropenem	Imipenem	Ertapenem
1	R	S	R
2	R	S	R
3	R	S	R
4	R	S	R
5	R	S	R
6	IS	S	R
7	R	IS	R
8	R	S	R
9	R	S	R
10	S	S	IS
11	R	R	R
12	R	R	R
13	R	R	R
14	S	S	R

S-sensitive; R-resistant; I-intermediate susceptible

*cloacae* (n=1; 10.0%) and *Proteus mirabilis* (n=1; 10.0%).

Of the carbapenemase producing isolates, six were from urine specimens (60.0%), 2 were from indwelling catheter tips (20.0%), and one each from a wound swab (10.0%) and a bronchial aspirate (10.0%).

Eight (80.0%) of the carbapenemase producing Enterobacterales harbouring patients were males and eight (80.0%) were aged above 50 years. The mean duration of the stay in the hospital of CPE harbouring patients was 7.2 days ( $\pm$ SD 5.65days). The *P* values of the t-tests performed to determine the association of gender and age with CPE occurrence were 0.253 and 0.955 respectively, whereas the *P* value of the Fisher's exact t-test for the duration of stay in the hospital was 0.0594.

## Discussion

This prospective epidemiological study was conducted to determine the proportion of CPE using 120 clinical isolates identified as Enterobacterales and collected in a single week during November 2017 in two tertiary care hospitals in Colombo, Sri Lanka. The proportion of CPE detected by MHT in this study was 8.3%. This correlates with several other studies conducted in Sri Lanka, as well as in neighbouring countries.<sup>7,8,12,13</sup>

Of ten patients harbouring CPE, eight (80.0%) were male and 80% were aged above 50 years. The gender or the age of the patients had no statistically significant association with the

Of the 120 isolates, 14 (11.7%) were resistant to at least one of the carbapenems tested. Of them, 11 were resistant and one showed intermediate susceptibility to meropenem. Of the 14 resistant isolates, three showed resistance and only one isolate showed intermediate susceptibility to imipenem. Thirteen of the 14 resistant isolates were also resistant to ertapenem while one isolate showed intermediate susceptibility (Table 2).

Ten of the 14 carbapenem resistant isolates (71.4%) were positive by the MHT. Therefore, 10 of the 120 samples (8.3%) were identified as carbapenemase-producers.

The majority of MHT-positive isolates were *K. pneumoniae* (n=4; 40.0%) followed by *Klebsiella oxytoca* (n=2; 20.0%), *E. coli* (n=1; 10.0%), *Serratia marcescens* (n=1; 10.0%), *Enterobacter*

occurrence of CPE infections ( $P=0.253$  and  $0.955$  respectively). The highest age-specific proportion was found in the 51-60 age group (30.0%).

The highest proportion of CPE harbouring patients (80.0%) had stayed in the hospital for 11-20 days. The rest had stayed for 0-10 days. CPE-infected patients had a relatively longer hospital stay compared to the rest of the study population (7.2 days and 5.0 days, respectively.  $P=0.0594$ ). Although this result is not statistically significant, multiple previous studies show that longer duration of hospital stay and stay in an ICU are risk factors for infections with carbapenem resistant Enterobacterales.<sup>13,14</sup> However, the limited duration of the study (one week), coupled with the relatively small sample size of 120 isolates poses challenges in establishing statistically significant findings. Further investigations involving larger sample sizes and extended observation periods are required to enhance the reliability and validity of the study outcome.

*K. pneumoniae* was the predominant pathogen producing carbapenemases (40.0%), followed by *K. oxytoca* (20.0%) in this study. *K. pneumoniae* is the most common Enterobacterales species harbouring carbapenem resistance genes globally.<sup>15</sup> Previous studies conducted in South Asia detected *K. pneumoniae* and *E. coli* as the predominant organisms conferring resistance to carbapenems.<sup>7,12</sup>

Ertapenem is described as the most suitable carbapenem for the detection of NDM-1 and KPC producers with low-level resistance to carbapenems.<sup>16</sup> However, Leavitt and colleagues have shown that *K. pneumoniae* strains resistant to ertapenem but sensitive to meropenem and/or imipenem do not produce carbapenemase. Those strains show changes in permeability coupled with the presence of ESBL genes.<sup>17</sup> Our findings also indicate that several isolates are susceptible to meropenem and/or imipenem, but not to ertapenem. This emphasizes the need for molecular analysis to confirm the presence/absence of carbapenemase genes in the isolates.

There are four main phenotypic tests, MHT, mCIM, CarbaNP, and CarbaNPt direct tests recommended by CLSI which can be used for identification of carbapenemase production. Based on a study conducted to compare these four phenotypic tests, MHT was shown to have a poor sensitivity and specificity compared to mCIM. Specifically, MHT lacks sensitivity for the identification of MBL and NDM which may have given false negative results in the present study.<sup>18,19</sup> MHT can also give false positive results with AmpC hyper-producing strains and with CTX-M production combined with porin loss. OXA-type carbapenemases have considerably lower MIC values and may have been missed during screening.<sup>20,21</sup>

## Conclusion

This study revealed the presence of CPE as a significant concern within two tertiary healthcare facilities in Sri Lanka. Infection prevention and control strategies should be implemented to prevent the spread of CPE. Due to the low sample size, the study is underpowered, which limits the detection of true effects in bivariate analysis. Further studies with larger sample sizes and the use of more reliable tests are needed to obtain data on the prevalence of CPE in other areas of Sri Lanka.

## Declarations

**Acknowledgement:** None

**Conflicts of Interest:** The authors declare that there are no conflicts of interest.

**Funding:** The study was self-funded.

**Ethics statement:**

Ethical approval was obtained from the Ethics Review Committees of the Faculty of Medical Sciences, University of Sri Jayewardenepura (MLS 1717), Colombo South Teaching Hospital (Application Number 617), and Sri Jayewardenepura General Hospital.

**Authors' contributions:**

SHJ and AHJ participated in specimen collection, data collection, laboratory procedures, data analysis and drafting of the manuscript.

Both SHJ and AHJ equally contributed to the paper. JK contributed to the conceptualization of the research, laboratory procedures, data analysis and drafting of the manuscript. NSC and KJ contributed to the specimen collection and data collection.

All authors have made a significant contribution to the paper. Each author approves the final version of the article being uploaded.

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