

Molecular Characterisation of Carbapenem-Resistant *Enterobacteriaceae* from Intensive Care Units

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ABSTRACT

Objective: To determine the molecular characterisation of carbapenem-resistant *enterobacteriaceae* (CRE) from patients admitted in intensive care units (ICUs) of a tertiary care hospital.

Study Design: Descriptive study.

Place and Duration of Study: Department of Pathology and Microbiology, Pakistan Institute of Medical Sciences (PIMS), SZABMU, Islamabad, Pakistan, from July 2015 to March 2016.

Methodology: Fifty isolates of CRE from intensive care units samples were processed, collected through samples coming from ICUs. Specimens were cultured on blood and Mac Conkey agar, and organisms were identified. Resistant organisms to imipenem and meropenem were preserved in glycerol broth at -80°C. Their molecular characterisation was done using the conventional PCR targeting NDM, KPC, VIM and IMP genes.

Results: Out of 50 samples, 13 (26%) were from urine, 13 (26%) from endotracheal tube tip, 7 (14%) from blood, 7 (14%) from pus, 7 (14%) from tracheal secretions, 2 (4%) from fluids, and 1 (2%) from catheter tip. NDM genes was isolated in 56% isolates. VIM, KPC, and IMP genes were not found in any isolates. NDM positive isolates included 86% *Klebsiella pneumoniae*, 10% *Escherichia coli*, and 4% *Klebsiella oxytoca*.

Conclusion: NDM gene was present in 28 (56%) samples. Whereas, VIM gene, KPC gene, and IMP genes were not detected in the CRE samples.

Key Words: *Enterobacteriaceae*, Carbapenem resistance, PCR, Molecular characterisation, NDM gene.

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INTRODUCTION

Enterobacteriaceae infections are major cause of morbidity and mortality in world nowadays. These organisms are surviving in the hospital environment because of their acquired resistance to several commonly used antibiotics and, perhaps more important, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons. Prevalence of carbapenem resistant *Enterobacteriaceae* is emerging worldwide, *i.e.* *Acinetobacter* 88%, *Klebsiella* 67%, *E. coli* 49%; other organisms are also showing similar resistance patterns.¹

Enterobacteriaceae are prevalent causes of both health-care and community infections, raising the possibility of metastasise CRE into the community. There are limited therapeutic options available to treat patients infected with these pan resistant organisms. CRE has gained epidemiologic importance nationally and internationally.²

The referral of the patients is the potential source of transmitting the infection to a hospital. Though infection control practices are optimally observed, but the huge patient burden undermines the desired standards. In these conditions, the treating physicians are handicapped due to limited options to treat these multidrug resistant organisms. Molecular characterisation helps in formulating a better empiric therapy and discourage use of ineffective and needless antibiotics. Further, this study will provide a benchmark for more advanced studies to discover the resistance mechanisms other than the studied.

The aim of this study was to determine the molecular characterisation of carbapenem resistant *Enterobacteriaceae* (CRE) from patients admitted in intensive care units (ICUs) of a tertiary care hospital.

METHODOLOGY

This descriptive, cross-sectional study was conducted from July 2015 to March 2016 in Department of Pathology and Microbiology, Pakistan Institute of Medical Sciences (PIMS), Islamabad. A proforma was used as a tool for data collection. Fifty isolates of carbapenem resistant *Enterobacteriaceae* (CRE) from intensive care units samples were analysed with standard laboratory techniques.

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Table I: Technical details about genes, primers & PCR conditions.

Primer	Sequence (5'-3')	PCR product size (bp)	PCR conditions	References
IMP-A IMP-B	GAAGGYGTTTATGTTTCATAC (Y = C or T) GTAMGTTTCAAGAGTGATGC (M = A or C)	587	10 min at 94°C; 36 cycles of amplification consisting of 30 s at 94°C, 40 s at 52°C, and 50 s at 72°C; and 5 min at 72°C for the final extension.	6
VIM2004A VIM2004A	GTTTGGTCGCATATCGCAAC AATGCGCAGCACCAGGATAG	382	Initial denaturation step at 94°C for 5 min, and 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 54°C for 1 min and primer extension at 72°C for 1.5 min.	7
NDM-F NDM-R	GGTTTGGCGATCTGGTTTTTC CGGAATGGCTCATCAGCATC	621	94°C (5min) [94°C (30 s) 55°C (30 s)] × 30 cycles 72°C (50 s) 72°C (5 min)	8
KPC-F KPC-R	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTGTAGGCG	798	94°C 3 min + 30 cycles of (95°C 1 min + 55°C 31 s +73°C 1 min) + 72°C 5 min.	9

The samples included urine, pus, blood, catheter tip, tracheal secretions, endotracheal tube and fluid. Culturing of bacterial isolates was done on blood agar and Mac Conkey's agar media. Urine samples were cultured on cystine lactose electrolyte deficient (CLED) agar. Primary identification was carried out by gram staining, growth characteristics, on Mac Conkey agar followed by standard biochemical methods *i.e.* API 20E (Biomérieux, France).

Antibiotic susceptibility testing was carried out using Kirby-Bauer disk diffusion method.³ The results were interpreted according to Clinical Laboratory Standard Institute 2014 Guidelines.⁴

Escherichia coli ATCC 25922 was used as control in susceptibility testing; negative control regarding ESBL phenomenon and absence of genes whereas *K. pneumoniae* ATCC 700603 was used as positive control regarding ESBL phenomenon. Positive controls regarding IMP, VIM, KPC, and NDM gene were acquired from Quaid-i-Azam University.⁵ Conventional PCR was done to find out NDM, KPC, IPM, VIM gene. Table I describes the method of PCR.⁶⁻⁹

RESULTS

Carbapenem-resistant *Enterobacteriaceae* isolated from samples from 10 Intensive care units of the hospital, were included. Minimum age of patient was second day of life while maximum was 80 years.

Majority of samples were received from medical intensive care unit 24 (48%), followed by surgical intensive care unit 14 (28%), pediatric intensive care unit 4 (8%), and neonatal intensive care unit 8 (16%). Out of 50 samples, 13 (26%) were from urine, 13 (26%) from endotracheal tube tip, 7 (14%) from blood, 7 (14%) from pus, 7 (14%) from tracheal secretions, 2 (4%) from fluids and 1 (2%) were from catheter tip.

KPC gene was not detected in all 50 samples using forward and reverse primers mix, with standard PCR conditions and reagents. Expected band size of KPC

PCR product was 798bp. VIM gene was not detected in all 50 samples using forward and reverse primers mix, with standard PCR conditions and reagents. Expected band size of VIM PCR product was 382bp. IMP gene was not detected in all 50 samples using forward and reverse primers mix, with standard PCR conditions and reagents. Expected band size of IMP PCR product was 587bp. Expected PCR product size for NDM gene was 621bp. NDM gene was amplified in 28 samples out of 50 (56%).

NDM gene was isolated in 24 (86%) isolates of *Klebsiella pneumoniae*, followed by *E. coli* 3 (10%), and *Klebsiella oxytoca* 1 (4%).

DISCUSSION

During the past decade, the increase in antibiotic resistance in *Enterobacteriaceae* has become a major concern worldwide. Beta-lactams have been widely used as the mainstay of treatment for severe infections due to these bacteria, with carbapenems often representing last-resource drugs. Carbapenem resistance due to acquired carbapenemases has emerged and spread worldwide since the early 2000s and spread rapidly among the bacteria and also became common source of hospital-acquired infections. Carbapenemases have been studied in depth, and is characterised on the basis of enzymes production, now classified as class B (metallo-beta-lactamases, MBLs), class A and class D (serine carbapenemases).¹⁰ The most widespread carbapenemases so far in *Enterobacteriaceae* is the KPC-type class-A carbapenemase, which has been found in *Klebsiella pneumoniae*, in the United States, Asia, the United Kingdom, Israel, and southern Europe.¹¹ In the Indian subcontinent, NDM is the most prevalent carbapenemase. Interestingly, acquired carbapenemases have mainly been restricted to geographical areas and to specific bacterial species, and outbreaks as well as spread in other countries has often been associated with imported cases from those countries where the bacteria are endemic. Population

mobility is known to be a main factor in globalisation and spread of antimicrobial drug-resistance among organisms.¹² For example, the emergence of KPC-producing *Enterobacteriaceae* in the United States in 2001,¹¹ could be later associated with the emergence of travel-related outbreaks in other countries.^{10,11,13,14}

In this study of 50 samples, 13 (26%) were isolated from urine and endotracheal tube tip each, 7 (14%) from blood, 7 (14%) from pus, and tracheal secretions each, 2 (4%) from fluids, and 1 (2%) from catheter tip. Barran *et al.* from Turkey in 2016 isolated majority of CRE strains from internal medicine intensive care unit (ICU) (27.07%). This was followed by post-anesthesia care unit (PACU) (9.94%), surgical ICU (5.52%).¹⁵

The unique nature of the intensive care unit (ICU) environment makes this part of the hospital a focus for the emergence and spread of many MDR pathogens. There are plenty of opportunities for the cross-transmission of resistant bacteria from patient to patient, as patients are commonly exposed to broad-spectrum antimicrobial agents and any breach in infection control practices will spread the MDR bacteria more rapidly. Increased resistance to multiple drugs is associated with hospital-acquired infections among ICU patients, and the resistance rates are almost universally higher among ICU patients than non-ICU patients. Longer time hospitalisation (>7 days) increases the risk of about two- to three-times to be infected with a pathogen possessing an antimicrobial-resistance.

In this study, *bla* NDM gene is present in 28 (56%) samples. Whereas, *bla* VIM gene, *bla* KPC gene, *bla* IMP gene are not detected at all in the CRE samples. NDM gene positive isolates, 24 (86%) were *Klebsiella pneumoniae*, 3 (10%) were *Escherichia coli* and 1 (4%) was *Klebsiella oxytoca*.

Naheed *et al.* conducted a study in Pakistan in 2013. They collected clinical samples from two hospitals of Lahore and Islamabad, Pakistan. On PCR amplification 23.6% isolates were harbouring *bla* NDM-1 gene, 25.1% isolates having *bla* VIM gene, and 1.5% isolates display *bla* IMP gene.¹⁶ *Bla* NDM-1 is located on remarkably plastic plasmids - genetic structures that can move with ease from one bacterium to another - and it is now very clear that *bla* NDM-1 plasmids are highly promiscuous. It can easily spread among various species and transmit the resistance genes.

Resistance to commonly prescribed antibiotics is increasing day-by-day in our country. Surveillance for antimicrobial resistance among bacterial pathogens in hospitals, particularly in ICU settings with a preexisting higher resistance burden, is mandatory in establishing and/or modifying guidelines for empirical treatment of severe infections in ICU patients caused by these antimicrobial-resistant pathogens.

In 2014, Rahman *et al.* conducted a study in India and noted that all carbapenem-resistant isolates in their study were *bla* NDM-positive by PCR.¹⁴

In 2012, Hu *et al.* reported 75.3% *bla* KPC gene detected in their isolates, *bla* VIM gene in 2.6% isolates, and *bla* IMP gene in 4% isolates. They screened 77 isolates of *Enterobacteriaceae* for the *bla* NDM-1 gene; however, none was found to be NDM-1-producing.¹⁷

The massive use of antibacterial drugs in the medical and veterinary practice, food industries, agriculture and commercial ethanol production and increased production of these drugs globally has also contributed to the environmental antibiotic pollution leading to destruction of useful bacteria and favouring the selection of resistant bacteria.

However, there are many opportunities to prevent the emergence and spread of these resistant pathogens through improved use of established infection control measures like patient isolation, hand washing, glove use, and appropriate gown use and judicious use of antibiotics.

there were certain limitations of the study. Clinical antibiotic history and outcome of the patients were not correlated. The sample size was also very small.

CONCLUSION

In this study, NDM gene was present in 56% samples. VIM gene, KPC gene, and IMP gene were not detected in none of the CRE samples. NDM positive isolates included 86% *Klebsiella pneumoniae*, 10% *Escherichia coli*, and 4% *Klebsiella oxytoca*. In 44% isolates, no gene was detected, which implies that other genes encoding carbapenemases enzymes may be present in this part of the world.

CONFLICT OF INTEREST:

Authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

HR: Sample collection and initial processing.

AF: PCR and final analysis and writing the manuscript.

MS: Statistics and result analysis.

RK: Final proof reading and carrying out corrections.

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