Study on Phenotypic Detection of Carbapenemase Producing Enterobacteriaceae in MBS Hospital, Kota

Pooja Jain[1], Naveen Saxena[2]

[1, 2] Department of Microbiology, Government Medical College, Kota, Rajasthan, India.

ABSTRACT

BACKGROUND

The Carbapenemase Resistant Enterobacteriaceae (CRE) are associated with high rates of morbidity and mortality particularly amongst critically ill patients. Hence rapid laboratory detection of CRE hospitalized patients is highly desirable. The vast majority of carbapenemases belong to three of the four known classes of beta lactamases namely Ambler class A, Ambler class B metallobetalactamases (MBL) and Ambler class Doxacillinases (OXAs). The purpose of this study was to determine the prevalence of carbapenemases producing Enterobacteriaceae in clinical isolates in MBS hospital, Kota.

METHODS

This study was conducted in the Department of Microbiology at MBS Hospital, Kota from June 2020 to December 2020. 68 non repeat isolates (MDR) that were resistant to imipenem (10 mg) according to CLSI breakpoint were included in the present study.

RESULTS

Out of 68 imipenem resistant Enterobacteriaceae, 52 were carbapenemase producing as detected by Modified Hodge Test. As per our study, the prevalence of carbapenemase producing Enterobacteriaceae was 20.8%. Most commonly seen in *K. pneumoniae* isolated from urine and swab of critically ill and debilitated patients of surgical ward.

CONCLUSIONS

Curbing irrational usage of antimicrobials in India is urgently required. Thus, aggressive infection control efforts have been effective at decreasing rates of infections with KPC-producing bacteria in intensive care units and long-term acute care hospitals. Bundled interventions including enhanced environmental cleaning, active surveillance culturing and contact precautions, as well as antimicrobial stewardship are important in controlling KPC-producing bacteria.

KEY WORDS

Multi Drug Resistance Enterobacteriaceae (MDRE), Klebsiella Producing Carbapenemase (KPC), Carbapenem Resistant Enterobacteriaceae (CRE), Metallo Beta Lactamase (MBL), Modified Hodge Test (MHT)

Corresponding Author:
Dr. Pooja Jain,
622, Main Road,
Dadabari, Kota – 324009,
Rajasthan, India.
E-mail: Poojajain3985@gmail.com

DOI: 10.14260/jemds/2021/446

How to Cite This Article:
Jain P, Saxena N. Study on phenotypic detection of carbapenemase producing Enterobacteriaceae in MBS hospital, Kota. J Evolution Med Dent Sci 2021;10(29):2181-2185, DOI: 10.14260/jemds/2021/446

Submission 10-01-2021,
Peer Review 18-05-2021,
Acceptance 25-05-2021,
Published 19-07-2021.

BACKGROUND

Enterobacteriaceae are a major group of pathogens causing hospital acquired infections. Broad spectrum cephalosporins are the empirical agents of choice for treating infections caused by these enteric bacilli. However, the emergence and dissemination of extended spectrum beta lactamases (ESBL) has compromised the use of these agents in certain geographical areas. As a result, the use of carbapenems have increased significantly in some hospitals and carbapenem resistant gram-negative bacilli (GNB) have started emerging. Gram negative organisms can display resistance to carbapenems by overproduction of Amp-C beta lactamases associated with loss of outer membrane porins and or overexpression of efflux pumps. These beta lactamase genes can readily be acquired by gram negative pathogen and usually reside on plasmid or transposons which facilitate the dissemination of these resistant mechanisms.[1] Carbapenem-resistant Enterobacteriaceae have been reported worldwide as a consequence of acquisition of carbapenemase genes.[2] The first carbapenemase producer in Enterobacteriaceae (NmcA) was identified in 1993.[3] since then, a large variety of carbapenemases have been identified in Enterobacteriaceae belonging to 3 classes of β-lactamases: the Ambler class A, B, and D β-lactamases[2]. In addition, rare chromosome- encoded cephalosporinases (Ambler class C) produced by Enterobacteriaceae may possess slight extended activity toward carbapenems, but their clinical role remains unknown.[2,]

The KPC β lactamases are mostly plasmid-encoded enzymes from *K. pneumoniae.* Their current spread worldwide makes them a potential threat to currently available antibiotic-based treatments. The emergence and global spread of carbapenemase producing Enterobacteriaceae is of great concern to health services worldwide. These bacteria are often resistant to all beta lactam antibiotics and frequently co-resistant to most other antibiotics.[4] The Carbapenamase Resistant Enterobacteriaceae (CRE) are associated with high rates of morbidity and mortality particularly amongst critically ill patients. Hence rapid laboratory detection of CRE hospitalized patients is highly desirable. The vast majority of carbapenemases belong to three of the four known classes of beta lactamases namely Ambler class A, Ambler class B metallo beta lactamases (MBL) and Ambler class Doxacillinases (OXAs).[5] Genes coding for beta lactam enzymes mutate continuously in response to heavy pressure of antibiotic use leading to development of newer beta lactamases with a broad spectrum activity. Among these carbapenemases especially transferable metallo beta lactamases are most important because of their ability to hydrolyse virtually all drugs in that class including carbapenems.[6] There are several mechanisms for carbapenem resistance such as the lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux mechanisms.[6] But the most important mechanism of resistance to carbapenems is carbapenemase production. [7]

Objectives

1. to determine the prevalence of carbapenemases producing Enterobacteriaceae in clinical isolates in MBS hospital, Kota. It includes:
2. to detect Enterobacteriaceae like *E. coli*, klebsiella, enterobacter, citrobacter, serratia etc. in various clinical samples and study their antibiotic sensitivity pattern to isolate imipenem resistant Enterobacteriaceae amongst them to follow further.
3. to detect phenotypic carbapenem resistant amongst Enterobacteriaceae isolated by Double Disc Synergy Test (DDST), Combined Disc Synergy Test (CDST) and MHT.

METHODS

The retrospective study was conducted in the Department of Microbiology at MBS Hospital, Kota from June 2020 to December 2020. All samples received for culture in the bacteriology laboratory from patients visiting and or admitted to the hospital were included in the study. The study was carried out for all samples including Swab, Sputum, Urine, Stool, Blood, Pus, CSF and other body fluids. From these specimens 4735, continuous non repeat isolates of Family Enterobacteriaceae isolated were screened. Among these bacterial strains, 250 multidrug resistance Enterobacteriaceae (MDRE) were further tested for imipenem sensitivity. 63 non repeat isolates that were resistant to imipenem (10 mgm) according to CLSI breakpoint were included in present study. These isolates were multi drug resistant (MDR). ATCC *E. coli* 25922 was used as control strain.

All the clinical isolates other than Enterobacteriaceae were excluded from the study. Antimicrobial susceptibility of all the isolates was performed by the disc-diffusion (Modified-Kirby Bauer disc diffusion method) according to CLSIs guidelines. The following antibiotics were tested by disc diffusion method, ampicillin (20ug), co-trimoxazole (25ug), cefotaxime (30ug), ceftriaxone (30ug), cefaclor (30ug), cefepime (30ug), tetracycline (30ug), piperacillin-tazobactam (100/10 ug), imipenem (10 ug), amikacin (30 ug), gentamicin (10 ug), levofloxacin (5 ug). The strains which were resistant to all the above drugs i.e. multi drug resistant (MDR) strains were further tested for imipenem sensitivity. All isolates showing intermediate susceptibility or resistant to imipenem by the disc diffusion method will be further tested for carbapenemase production using modified Hodge test (MHT) using ertapenem. Strains which produced carbapenemase enzyme formed an inward indentation of the growth (clover leaf shape) along the streaking line of the test strain.

Statistical Analysis

The statistical package for social science {SPSS} version 20 was used for data analysis. Mean, median, and SD were used to describe quantitative data. Qualitative data were summarized using frequency and percentage.

RESULTS

Total 250 MDR Enterobacteriaceae were isolated in 7 months study. Amongst them 125 were *E. coli,* 103 klebsiella, 1 citrobacter, 7 providentia spp., 7 proteus spp. (4 *P. vulgaris* and 3 *P. mirabilis*), 1 morganella. If seen ward wise then 116

isolates from surgical ward, 33 from Medicine, 35 from Paediatrics, 9 from Obs-Gynae, 32 from ortho, 10 from TB, 2 from ENT. If seen sample wise 78 from urine, 21 from blood, 98 from swab, 4 from sputum, 35 from pus and 10 from stool.

All strains taken in study were MDR strains i.e. resistant to ampicillin-sulbactum, gentamicin, piperacillin / tazobactam, amikacin, co-trimoxazole, tetracycline, cefotaxime, ceftriaxone, cefaclor, cefepime, imipenem. Many but not all carbapenemase producers were resistant to levofloxacin.

Imipenem resistant strains were sensitive to aztreonam, tigecycline and polymyxin- B except proteus spp. which is inherently resistant to polymyxin - B.

MDR strains were mainly isolated from surgical wards. Commonly isolated strains were *E. coli* and *K. puemoniae* from urine and swab samples. (Figure 1, 2, 3)

Study	Prevalence of CRE
Morocco (2012)	14.2 %
Ontario (2008 – 2011)	31 %
MBS Hospital, Kota	20.8 %

Table 1. Comparison of Our Study with Other Studies

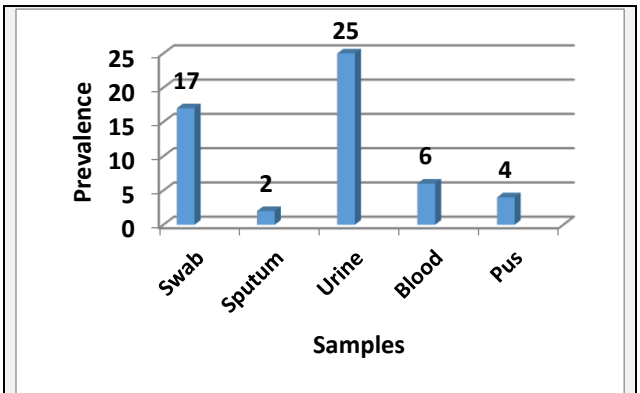

Figure 1. Sample Wise Distribution of Carbapenemase Producing Organisms

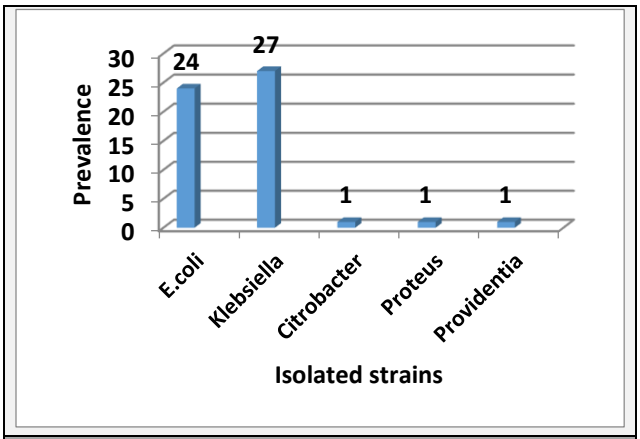

Figure 2. Organisms Producing Carbapenemase Enzyme

Amongst 250 MDR Enterobacteriaceae, 68 were imipenem resistant Enterobacteriaceae isolates, 21 isolates were from swab, 2 isolates were from sputum, 31 isolates were from urine, 9 isolates were from blood, 5 isolates were from pus. These included the following isolates *E. coli* spp. (30), klebsiella spp. (33), citrobacter spp.(1), proteus spp.(1), providentia spp (3). If seen ward wise, isolates from Surgical ward (28), Medicine ward (8), Paediatric ward (14), Obs-Gynae (5), Orthopaedic ward (10), ENT (1), TB (2).

Out of 68 imipenem resistant Enterobacteriaceae, 52 were carbapenemase producing as detected by Modified Hodge Test. Amongst them 24 were *E. coli*, 27 were klebsiella, 1 was citrobacter spp., 1 was providentia spp., 1 was *Proteus vulgaris*. If seen ward wise, 23 isolates from Surgical ward, 5 from Medicine, 10 from Paediatrics, 4 from Obs-Gynae, 8 from Ortho, 2 from TB, 1 from ENT. Sample wise, 25 from urine, 6 from blood, 17 from swab, 2 from sputum, 4 from pus.

MDR strains were tested for imipenem sensitivity. Imipenem resistance was seen mainly from surgical wards. Commonly isolated strains were *E. coli* and *K. pneumoniae* mainly from urine and swab samples (Figure 4,5,6). Imipenem resistant strains were tested for carbapenemase production by MHT. *K. pneumoniae* was the most common carbapenemase producing organism, most commonly isolated from urine samples of patients admitted in surgical ward (Figure 7,8,9).

DISCUSSION

Antimicrobial resistance is a threat to public health and patient safety. Infections with resistant organisms are associated with increased morbidity and mortality, extended stays in hospitals, reduced treatment options, untreatable infections, increased healthcare costs, significantly limits treatment options for life-threatening infection. Resistance mechanisms (carbapenemases) are mobile (spread readily via plasmids). Co-resistance to other agents is common.

As per our study, the prevalence of carbapenemase producing Enterobacteriaceae was 20.8 %. Most commonly seen in *K. pneumoniae* isolated from urine and swab of critically ill and debilitated patients of surgical ward. This is comparable to the study of Morocco (April 2012), where the prevalence was found to be 14.2 %.[8] As per the study done in Ontario from January 2008 to December 2011, the prevalence of KPC was 31 %. The incidence of KPC was continuously increasing due to clonal spread of bla-KPC gene. They were mainly isolated from urine of patients.[9]

Spread of resistance by mobile genetic element in health care settings has been well known. Selection pressure due to frequent use of carbapenemase has caused an increase in carbapenem resistant strains. Treatment options include aztreonam, colistin, polymyxin B, and tigecycline except proteus spp. which is inherently resistance to polymyxin-B. Tigecycline is not recommended for treatment of infections of the blood or urine as it has low concentrations.

The rate of surgical site infections (SSI) for the types of interventions is very high. Most common type of contamination is during surgical procedure itself. Surgical infections can best be minimized by meticulous observation of the fundamental principles of antisepsis and theatre disciplines.

All postoperative patients and majority of medical patients were receiving IV antibiotic therapy. It appears that continued prophylaxis was the reason in majority although in a proportion it was therapeutic for treating established SSI. These antimicrobials were causing harm rather than benefit by selecting out multi resistant Enterobacteriaceae, both ESBL and MBL producers.

In medical wards, incidence of infection or phlebitis and bacterial colonization of the catheter increases when catheters

are left in place for > 72hrs. Being associated with an increased risk of infection. Peripheral catheter sites are normally rotated at 72 to 96 hrs interval, to reduce both risk of infection and patients discomfort associated with phlebitis. While isolates producing ESBL remain sensitive to carbapenems, carbapenemase producing isolates are resistant to all antibiotics except few. Few are sensitive to levofloxacin also. Thus heralding an era of untreatable infection.

Selection pressure exerted by 3rd generation cephalosporins and even unrelated classes are sufficient to select carbapenem resistance due to co-selection associated linkage as a single plasmid often carries resistance genes to multiple classes which makes control of antimicrobials prescribing urgent. The bla -CTX-M-15 gene borne on plasmid conferring acquired resistance to 3rd generation cephalosporins in Enterobacteriaceae first emerged in India in mid 1990s, which is now the globally dominant ESBL isolates. So the problem is not restricted to hospital only but also seen in community settings. Emergence of carbapenemase producers in Indian hospitals and potentially in the community should serve as the last straw. The Indian government and regulatory agencies should impose strict statutory guidelines implementing interventions for limiting in appropriate usage of antimicrobials, particularly for the post-surgical prophylaxis.

Enterobacteriaceae are part of the human gut flora. Dissemination of resistant clones carried as part of the normal human gut flora occurs unsuspected which is amplified many folds by the selection pressure exerted by the antimicrobials and gives rise to infection in vulnerable patients, that could be life threatening. Defensive practise, ignorance of rational antibiotic prescribing principles, lack of awareness of the problem of the alarming rise in multi resistance and pharmaceutical promotions are possible contributing factors leading to unnecessary antimicrobial usage. Inadequate infection control is further compounding the problem.

Research is needed to establish the prescribing habit of doctors in Indian hospitals and the influencing factors including behavioural so that an appropriate intervention strategy can be formulated to curb unnecessary prescribing. Most nosocomial infections are preventable by simple means even when resources are scarce, but would require an institutional approach. Curbing irrational usage of antimicrobials in India is urgently required.

CONCLUSIONS

KPC - producing bacteria have increasingly been isolated worldwide. The spread of KPC-producing *K. pneumoniae* is worrying. Hospital - acquired infections in severely ill patients. accumulate and transfer resistance determinants. MDR KPC-producing bacteria may be the source of therapeutic dead-ends. Careful and conservative use of antibiotics combined with good control practices is required. Strict infection control measures have to be implemented to prevent further spread of KPC-producing bacteria.

The epidemic of carbapenemase producers cannot stop spontaneously. Such community-based outbreaks will be difficult to control. The factors that enhance the spread of carbapenemase producers in the community are multiple and associated with lack of hygiene, overuse and over-the-counter use of antibacterial drugs, and increased worldwide travel. In addition, many carbapenemase producers carry unrelated drug-resistance determinants. Therefore, selection pressure with structurally unrelated antibacterial drugs (not only β - lactams) may contribute to their spread. We cannot predict either the speed of diffusion of those carbapenemase producers in the community or their prevalence at a steady state (5 % – 50 %). The actual prevalence of carbapenemase producers is still unknown because many countries that are likely to be their main reservoirs have not established any search protocol for their detection. The prevalence may substantially differ, depending on the country. The prevalence is estimated to be 3 % – 5 % in France and > 80 % in India.[10,11] The epidemic will likely be caused mainly by nosocomial carbapenemase producers in *K. pneumoniae* of all types (KPC, IMP, VIM, NDM, and OXA-48). It is likely that in certain countries high rates of different types of carbapenemase producers may already exist, for example, in Greece (VIM and KPC) and in the Indian subcontinent (NDM, KPC, OXA-181).

K. pneumoniae plays a major role in spreading carbapenemase production in patients with risk factors like receiving broad-spectrum antibiotic therapy, patients in intensive care units, immunocompromised patients, transplant patients, surgical patients etc. Early identification of carbapenemase producers in clinical infections, at the carriage state or both is therefore mandatory to prevent development of those hospital-based outbreaks. The dearth of novel antibacterial drugs in the pipeline means that we must conserve the efficacy of existing antibacterial drugs as much as possible. Carbapenemase producers in Enterobacteriaceae are different from other multidrug-resistant bacteria in that they are susceptible to few antibacterial drugs. No vaccines are readily available for preventing infections with carbapenemase producers. This finding is particularly true for *E. coli*, which is part of the human intestinal flora. Therefore, everything must be done to prevent infections as common as pyelonephritis from becoming life threatening because of the lack of any effective treatment.

Research is needed to establish the prescribing habit of doctors in Indian hospitals and the influencing factors including behavioural so that an appropriate intervention strategy can be formulated to curb unnecessary prescribing. Most nosocomial infections are preventable by simple means even when resources are scarce, but would require an institutional approach. Curbing irrational usage of antimicrobials in India is urgently required. Thus, aggressive infection control efforts have been effective in decreasing the rate of infections with KPC-producing bacteria in intensive care units and long-term acute care hospitals. Bundled interventions including enhanced environmental cleaning, active surveillance culturing and contact precautions, as well as antimicrobial stewardship are important in controlling KPC-producing bacteria

Prevention of spread of carbapenemase producers relies on early detection of carriers.[12,13] Patients who undergo screening should include patients who were hospitalized while abroad and then transferred to another country, and patients at risk (e.g., patients in intensive care units, transplant patients, immunocompromised patients). Screened patients should be kept in strict isolation before obtaining results of the screening (at least 24 - 48 hours). Because the reservoir of carbapenemase producers remains the intestinal flora, faecal

and rectal swab specimens are adequate for performing this screening. Those specimens may be plated directly on screening media.

No vaccines are readily available for preventing infections with carbapenemase producers. This finding is particularly true for *E. coli*, which is part of the human intestinal flora. Therefore, everything must be done to prevent infections as common as pyelonephritis from becoming life threatening because of the lack of any effective treatment. Carbapenemase producers in Enterobacteriaceae are different from other multidrug-resistant bacteria in that they are susceptible to few (if any) antibacterial drugs. The dearth of novel antibacterial drugs in the pipeline means that we must conserve the efficacy of existing antibacterial drugs as much as possible.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jemds.com.

REFERENCES

[1] Benouda A, Touzani O, Khairallah MT, et al. First detection of oxacillinase-mediated resistance to carbapenems in Klebsiellapneumoniae from Morocco. Ann Trop Med Parasitol 2010;104(4):327-30.
[2] Borer A, Saidel-Odes L, Riesenberg K, et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. Infect Control Hosp Epidemiol 2009;30(10):972-6.
[3] Carrër A, Poirel L, Yilmaz M, et al. Spread of OXA-48-encoding plasmid in Turkey and beyond. Antimicrob Agents Chemother 2010;54(3):1369-73.
[4] Pillai DR, Melano R, Rawte P, et al. Klebsiellapneumoniae Carbapenemase, Canada. Emerg Infect Dis 2009;15(5):827-9.
[5] Pitout JDD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 2008;8(3):159-66.
[6] Poirel L, Héritier C, Tolün V, et al. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiellapneumoniae. Antimicrob Agents Chemother 2004;48(1):15-22.
[7] Patel G, Huprikar S, Factor SH, et al. Outcomes of carbapenem-resistant Klebsiellapneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol 2008;29(12):1099-106.
[8] Poirel L, Hombrouk-Alet C, Freneaux C, et al. Global spread of New Delhi metallo-β-lactamase 1. Lancet Infect Dis 2010;10(12):832.
[9] Poirel L, Ros A, Carrër A, et al. Cross-border transmission of OXA-48-producing enterobacter cloacae from Morocco to France. J Antimicrob Chemother 2011;66(5):1181-2.
[10] Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev 2007;20(3):440-58.
[11] Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, et al. Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother 2008;52(3):1028-33.
[12] Walsh TR, Toleman MA, Poirel L, et al. Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev 2005;1892):306-25.
[13] Walsh TR, Weeks J, Livermore DM, et al. Dissemination of NDM-1 positivebacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 2011;11(5):355-62.