Original Research Paper

Volume - 12 | Issue - 08 | August - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Clinical Microbiology

DETECTION OF CARBAPENEMASE PRODUCING ENTEROBACTERALES AMONG THE CLINICAL ISOLATES OF DIARRHOEAGENIC ESCHERICHIA COLI CAUSING ACUTE GASTROENTERITIS IN CHILDREN BELOW 2 YEARS IN TERTIARY CARE HOSPITAL OF NORTH EAST INDIA.

Preeta Mairembam Ph.D Scholar, Department of Microbiology, RIMS, Imphal

Shan Damrolien* Assistant Professor Department of Microbiology, RIMS, Imphal *Corresponding Author

Ksh. Mamta Devi Professor, Department of Microbiology, RIMS, Imphal

Kh. Sulochana Devi Professor, Department of Microbiology, RIMS, Imphal

ABSTRACT **Introduction:** Enterobacterales that test resistant to at least one of the carbapenem antibiotics (ertapenem, meropenem, doripenem, or imipenem) are called Carbapenem resistant Enterobacterales (CRE) and if they produce a carbapenemase (an enzyme that can make them resistant to carbapenem antibiotics) they are called Carpenemase producing Enterobacterales (CPE). Children with CRE strains in fecal samples are considered as a high risk group by World Health Organization (WHO), which can spread CRE by intimate contact and travel. **Methods:** This cross-sectional study was conducted in the Department of Microbiology, RIMS, Imphal, Manipur, India from Jan 2020 to Feb 2022. A total of 157 children under 2 years of age whose stool culture was positive for diarrhoeagenic Escherichia coli were included in the study. The modified carbapenem inactivation method (mCIM) has been done for detection of carbapenemase producers and the addition of EDTA in eCIM to further differentiate between serine and metallo-β-lactamase producers. **Result and Discussion:** Out of 157 Diarrhoegenic E.coli (DEC) ,Carbapenem resistance was seen in 9 isolates i.e 5.7 %. Out of these 9 isolates, 3 were MBL producers tested by the phenotypic test mCIM and eCIM. All the three MBL producers carried bla NDM-1 gene. mCIM/eCIM assay is designed to simultaneously detect and distinguish the different types of carbapenemases. Carbapenemase genes are often located on plasmids that can be exchanged between Enterobacteriaceae and other Gram-negative bacteria. Carbapenem-resistant K. pneumoniae are currently more frequent and more likely to cause healthcareassociated outbreaks, carbapenem-resistant E. coli pose a greater risk for spread in the community. **Conclusion:** Screening for carbapenemase producer using mCIM and eCIM essay is important along with infection control measure such as active surveillance through rectal screening for CRE carriage on hospital admission, contact precautions, hand hygiene, patient isolation, environmental sanitation, case notification/flagging, antibiotic restriction.

KEYWORDS : mCIM and eCIM, CRE, CPE, NDM-1

INTRODUCTION

Enterobacterales that test resistant to at least one of the carbapenem antibiotics (ertapenem, meropenem, doripenem, or imipenem) or produce a carbapenemase (an enzyme that can make them resistant to carbapenem antibiotics) are called CRE. Carbapenemase-producing CRE make enzymes called carbapenemases that inactivate carbapenems and other β-lactam antibiotics, including penicillins and cephalosporins. The Klebsiella pneumoniae carbapenemase (KPC), was first identified in the United States in 20011 since then it spread all over the [world] and in 2010 India reported its first cases of KPC producing Klebsiella pneumoniae in India [2].

In addition to KPC, there are a number of other carbapenemases associated with mobile genetic elements, includingNew Delhi Metallo-beta-lactamase (NDM), Verona Integron-Encoded Metallo-beta-lactamase (VIM), Imipenemase (IMP), Oxacillinase-48 (OXA-48) etc. This mobile genetic elements may be involved in the horizontal transmission. Thus, the prevalence of carbapenem-resistant Enterobacterales (CRE) has emerged in hospital setting as well as in community and become public health issue around the world[3].

Several studies have been conducted to investigate the fecal carriage characteristics of CRE strains among inpatients[4]. Children with CRE strains in fecal samples are considered as a high risk group by World Health Organization (WHO), which can spread CRE by intimate contact and travel[5]. In children, as acute diarrhoea mostly subsides spontaneously and are generally self-limiting, rehydration and adequate nutrition forms the basis of treatment but in developing country like India,

one third of total paediatric admissions in hospitals are due to diarrhoeal diseases and 17 percent of all indoor paediatric patients deaths are diarrhoea related[6]. Diarrhoeagenic Escherichia coli (DEC) strains are most frequently associated with diarrhoea in children in developing countries[7].

Therefore, this study was conducted to find out diarrhoeagenic Escherichia coli causing acute gastroenteritis in children below 2 years and to detect carbapenem-resistant enterobacterales (CRE).

Materials &Methods:

This cross-sectional study was conducted in the Department of Microbiology, RIMS, Imphal, Manipur, India from Jan 2020 to Feb 2022. A total of 157 children under 2 years of age who attended Paediatric Department with acute gastroenteritis and whose stool culture was positive for diarrhoeagenic Escherichia coli were included in the study.

Culture:Fresh stool specimens were collected in a sterile wide mouthed, dry, leak proof screw capped container and processed as soon as possible. The stool samples were inoculated on MacConkey agar, XLD (Xylose Lysine Deoxycholate) agar and incubated overnight in 35 degree celsius. The colony morphology, gram staining, motility testing and further biochemical tests for the identification was done as per standard procedure. Serotyping of the Escherichia coli isolates for enteropathogenic (EPEC) were done using E. coli OK O pool antisera pool 1 (O26, O103, O111, O145, O157), pool 2 (O55, O119, O125, O127, O128) and pool 3 (O86, O114, O121, O126, O142) procured from SSI Diagnostica.

Antibiotic susceptibility testing: Antimicrobial sensitivity testing (AST) was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion method for the following antibiotics- ampicillin (10µg), amoxicillin clavulanate (30µg), amikacin (30µg), gentamicin (10µg), cotrimoxazole (25µg), ciprofloxacin (5µg), ofloxacin (5µg), ceftriaxone (30µg), piperacillintazobactum (100/10µg), imipenem (10µg). For Colistin, minimum inhibitory concentration (MIC) was determined by microbroth dilution method as per CLSI guidelines[8].

Phenotypic detection Carbapenemase producing enterobacterales (CPE): Modified carbapenem inactivation method - mCIM and eCIM. The modified carbapenem inactivation method (mCIM) has been done for detection of carbapenemase producers by mCIM and the addition of EDTA in eCIM to further differentiate between serine and metallo-β-lactamase producers as per CLSI M100-S28 supplement in 2018 to specifically identify metallo-carbapenemases[8].

An isolate is positive for metallo-carbapenemase production when the eCIM zone size increases by ≥5 mm compared to the zone size

observed for the mCIM and is considered negative for a metallo-carbapenemase if the increase in zone size is < 4 mm.

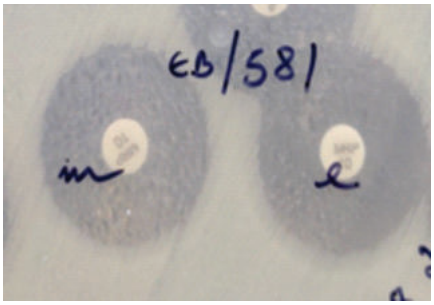

Figure 1 mCIM and eCIM test - Negative

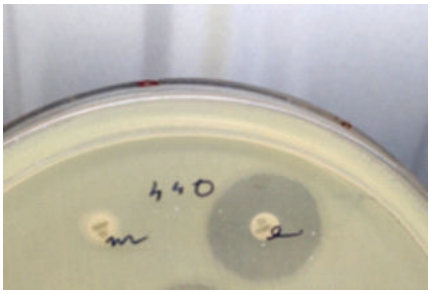

Figure 2 mCIM and eCIM - MBL producer

Genotypic detection of CPE:

Detection of NDM-1 by PCR using primers targeting blaNDM-1 using control strains. DNA was isolated by using QiagenDNA extraction kit – (QIAamp DNA mini extraction kit Lot no: 157056144). PCR product were electrophoresed on 2% agarose gel along with a 100 bp DNA ladder (as a molecular wt marker) and visualized using a UV transilluminator. For NDM-1 primer used was FP (5'-ACC GCC TGG ACC GAT GAC CA-3'), RP (5'-GCC AAA GTT GGG CGC GGT TG-3'). Primers were procured from Eurofins Scientific India Pvt Ltd.

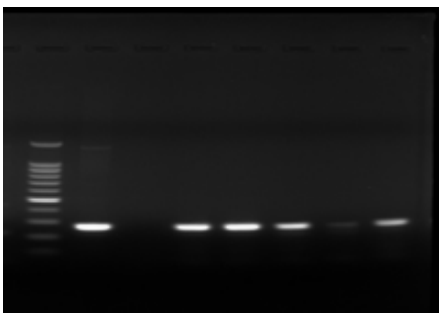

Figure 3 : Agarose gel electrophoresis of PCR-amplified products of NDM-1 gene (264bp). Lane 1: 100bp DNA marker, Lane 2: Positive control, Lane 3: Negative control, Lane 4,5,6 & 8 Positive sample, Lane 7: Negative sample

Control strains: Escherichia coli ATCC 25922, Escherichia coli ATCC BAA-2469 (NDM-1), Klebsiella pneumoniae ATCC BAA-1705 (KPC), Klebsiella pneumoniae ATCC BAA-1706 were the control strains used for antibiotic susceptibility, phenotypic test and PCR.

Result:

Out of 157 Diarrhoegenic E.coli (DEC) isolated from January 2020 to February 2022, Carbapenem resistance was seen in 9 isolates i.e 5.7 %. Out of these 9 isolates, 3 were MBL producers tested by the phenotypic test mCIM and eCIM. All these three carried bla NDM-1 gene.

Table 1. Sensitivity pattern Diarrhoegenic E.coli N= 157

Antibiotics	Sensitivity in %
Ampicillin	0
Cefixime	13.4
Nalidixic acid	11.5
Cotrimoxazole	35
Tetracycline	40.8
Imipenem	94.3
Meropenem	94.3
Gentamicin	96.2
Amikacin	98.7
Chloramphenicol	90
Ciprofloxacin	15.6

Table 2: Susceptibility pattern CRE: (n=9)

Antibiotics	Sensitivity in %
Ampicillin	0
Cefixime	0
Nalidixic acid	0
Cotrimoxazole	11.1
Tetracycline	22.2
Chloramphenicol	77.7
Ciprofloxacin	0
Gentamicin	88.9
Amikacin	100

DISCUSSION:

E.coli is one of the most common pathogen causing acute gastroenteritis[9] in this region, among this isolates of E.coli, CRE was 5.7 % and from the isolated CRE strains, the NDM-1 type carbapenemase gene (blaNDM-1) was 33.3%. The major resistance mechanism of CRE is the production of carbapenemases such as NDM and NDM-1 type carbapenemase gene (blaNDM-1) was the most common. This finding is consistent with the finding of Fen Pan et al study on fecal carriage and molecular epidemiology of CRE[12]. NDM-1 and its minor variants, a class B carbapenemase first clinically isolated from a patient at a hospital in New Delhi, India, has been identified all over the world and not only detected in E.coli and K.pneumoniae[11].

The first CLSI recommended growth-based carbapenemase detection test was the modified Hodge Test (MHT) in 2009, however MHT results are often difficult to interpret, and false-positive results are observed for isolates producing ESBL or AmpC βlactamase with porin loss12. mCIM and eCIM assay has been used to detect carbapenemase producing enterobacterales. mCIM/eCIM assay is designed to simultaneously detect and distinguish the different types of carbapenemases13. In this study, we used PCR targeting carbapenemase genes i.e blaNDM-1 as the gold standard to evaluate the performance of phenotypic tests of mCIM/eCIM assay and our study correctly detect carbapenemase producers and further differentiate between serine and metallo-β-lactamase producer and this finding is consistent with the study conducted by Tsai et al[14].

In our study, there was no significant difference in the antimicrobial resistance pattern observed among carbapenemase producer and non-carbapenemase producer DEC for antibiotics like Ampicillin, Trimethoprim-sulfamethoxazole, Nalidixic acid, Ciprofloxacin and both shows high sensitive to Amikacin, Gentamicin and chloramphenicol. Our findings were in concordance with a study performed by Prasad et al15 which concluded that most of the DEC isolates from children with diarrhea were resistant to Ampicillin, Trimethoprim-sulfamethoxazole, Nalidixic acid. In this study DEC isolates shows 94.7 % sensitive to Imipenem and Meropenem and similar pattern of sensitivity is also report by Prasad et al which shows 94.7 % sensitive to Imipenem, Meropenem and Ertapenem in their study conducted in tertiary hospital of North-East India.

Conclusion: This study highlight the importance DEC as an etiological agents of diarrhea in children population of the North East region of India and also reveal the presence of CRE among the isolates of DEC. Carbapenemase genes are often located on plasmids that can be exchanged between Enterobacteriaceae and other Gram-negative bacteria16. Carbapenem-resistant K. pneumoniae are currently more frequent and more likely to cause healthcareassociated outbreaks, carbapenem-resistant E. coli pose a greater risk for spread in the community[16]. CRE infections has limited treatment option so, it is important to developed a strategies to control the spread CRE. Implementation of enhanced CRE control measures in healthcare settings requires reliable identification of CRE by the microbiology laboratory, therefore, screening for carbapenemase producer using mCIM and eCIM essay is equally important along with infection control measure such as active surveillance through rectal screening for CRE carriage on hospital admission, contact precautions, hand

hygiene, patient isolation, environmental cleaning, case notification/ flagging, antibiotic restriction.

REFERENCES

1. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae [J]. Antimicrob Agents Chemother. 2001;45:1151–61.
2. Parveen RM, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of Klebsiella pneumoniae in South India. Int J Pharma Biosci. 2010;1(2):1–11.
3. Satlin MJ, Chen L, Patel G, Gomez-Simmonds A, Weston G, Kim AC, et al. Multicenter clinical and molecular epidemiological analysis of bacteremia due to Carbapenem-resistant Enterobacteriaceae (CRE) in the CRE epicenter of the United States [J]. Antimicrob Agents Chemother. 2017;61. https://doi. org/10.1128/AAC.02349-16
4. Vaishnavi C. Translocation of gut flora and its role in sepsis Indian J Med Microbiol. 2013;31:334–42.
5. Schwartz KL, Morris SK. Travel and the spread of drug-resistant Bacteria [J]. Curr Infect Dis Rep. 2018;20:29.
6. Park K. Acute diarrhoeal diseases. In: Park K, editor. Parks textbook of preventive and social medicine, 15th edition. Jabalpur: Banarsidas Bhanot. 1998:171-4.
7. Nataro JP, Kaper JP. Diarrhoeagenic Escherichia coli. Clin Microbiol Rev. 1998;11(1):142-201.
8. Clinical & Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 30th ed; 2020. CLSI supplement M100-S30
9. Sarda Angom, Shan Damrolien, Tsering Wangmu, Ksh. Mamta Devi, Kh. Sulochana Devi, C. Syamsundar Singh."Bacterial enteropathogen causing acute diarrhea in children in the tertiary care hospital, Imphal" IJCMPH April 2021 Volume 8, Issue 4 2007.
10. Fen Pan , Dongxing Tian , Bingjie Wang, Wantong Zhao, Huihong Qin, Tiandong Zhang and Hong Zhang. Fecal carriage and molecular epidemiology of carbapenem-resistant Enterobacteriaceae from outpatient children in Shanghai. BMC Infectious Diseases (2019) 19:678
11. Moellering RC Jr. NDM-1--a cause for worldwide concern [J]. N Engl J Med. 2010;363:2377–9
12. Pasteran F, Mendez T, Rapoport M, Guerriero L, Corso A. Controlling falsepositive results obtained with the Hodge and Masuda assays for detection of class a carbapenemase in species of enterobacteriaceae by incorporating boronic acid. J Clin Microbiol. 2010;48(4):1323–32.
13. Sfeir MM, Hayden JA, Fauntleroy KA, Mazur C, Johnson JK, Simner PJ, et al. EDTA-Modified Carbapenem Inactivation Method: a Phenotypic Method for Detecting Metallo-beta-Lactamase-Producing Enterobacteriaceae. J Clin Microbiol. 2019;57(5):e01757–18
14. Ya-Min Tsai , Shining Wang , Hui-Chuan Chiu, Cheng-Yen Kao and Li-Li Wen Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiology (2020) 20:315.
15. Abhijit Kumar Prasad, Wihiwot Valarie Lyngdoh, Thigujam Surbala Devi, Elantamilan Durairaj. Presence of Resistant DEC Strains in a Tertiary Healthcare Center in North East India in Children under 18 Years. Journal of Laboratory Physicians 2022.
16. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011 Oct;17(10):1791-8.