



ORIGINAL RESEARCH PAPER

Microbiology

SURVEILLANCE OF NDM-1 PRODUCING ENTEROBACTERIACEAE FROM WATER SAMPLES IN AND AROUND PUDUCHERRY.

KEY WORDS: Carbapenemases; Enterobacteriaceae; NDM (New Delhi Metallo beta lactamases)

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ABSTRACT **Aim:** Presence of NDM-1 - lactamase in environmental samples around New Delhi was reported in 2011. However, there are no such reports from this region. In this study, an attempt has been made to isolate, identify, Enterobacteriaceae which carry NDM-1 gene. **Methodology:** Fifteen water samples were collected from lakes, ponds and taps in and around Puducherry, South India. 500ml of water was collected in a sterile bottle and filtered through sterile membrane filters (0.2 µM, Millipore) and the membrane filter was cultured. **Results:** It was observed that the Enterobacteriaceae species isolated from lakes (n=4) and ponds(n=2) were all susceptible to Meropenem, Amikacin, Ciprofloxacin, Ceftazidime, Ceftriaxone and Gentamicin by disc diffusion method. Our study brings out the fact that the carbapenem resistant Enterobacteriaceae are restricted only to the hospital environment in Puducherry rather than the natural environment as has been found in Delhi study.

INTRODUCTION

Carbapenems are potent beta lactam antibiotics used in the hospitals for the treatment of a number of infections caused by multidrug resistant organisms. However, with the emergence of carbapenemases, the treatment of these multidrug resistant organism infections poses a serious threat to the public health systems. The prevalence of carbapenemase producing enzymes continues to rise of which the most important one is Class B enzymes which has the ability to hydrolyse all beta lactams but not monobactams. Presence of these carbapenem hydrolysing enzymes have not only been detected in clinical isolates but also in good number of environmental isolates. A variety of environmental species of Proteobacteria, Firmicutes pose intrinsic carbapenem resistance. Acquired resistance often implies acquisition of transferrable genetic elements causing horizontal spread of these resistant genes. NDM-1 gene have been demonstrated in isolates from environment water and tap water samples in New Delhi among unrelated Gram-negative species.

Enterobacteriaceae, the largest microbiome colonizing gastrointestinal tracts of humans and animals are widely distributed in nature. They are the most important pathogens causing variety of infections and extended spectrum beta lactams, carbapenems and fluoroquinolones are often used for the treatment of these infections—. They are not only present in the gastrointestinal tract but also represent a large part of bacterial communities colonizing hospital effluents and other sewage treatment plants. Presence of NDM-1 - lactamase in environmental samples around New Delhi was reported in 2011. However, there are no such reports from this region. The understanding of antibiotic resistance, mechanism of resistance, the resistance pattern of Enterobacteriaceae isolates from environmental samples in a geographical area will help in formation of an applicable antibiotic policy in the hospital which can assist the clinicians in reducing the dissemination of multidrug resistance and to identify the mechanism of carbapenem resistance in isolates belonging to the family Enterobacteriaceae. Since most of the studies focus on clinical isolates, we planned to perform a microbiological study of carbapenem resistant pathogens belonging to the family Enterobacteriaceae isolated from environmental water samples in and around Puducherry, India.

MATERIALS AND METHODS

Sample collection

The presence of carbapenem resistance were studied from environmental samples in the surrounding areas of Puducherry. A total of Fifteen water samples were collected from different locations in and around Puducherry. For each sample, five hundred millilitres of target water samples were collected from lakes (n=4), ponds (n=2) and drinking water taps (n=9) in screw capped wide mouthed sterile bottle. Each sample was collected approximately 30 cm below the water surface and bottles were opened only under the water. The collected water samples were transported immediately to the laboratory for immediate processing.

Microbial isolation

For microbial isolation, membrane filtration technique was used. 500 millilitres of collected water samples were filtered through sterile membrane filters (0.2 µM, Millipore, India). The membrane was then placed upside down on the surface of 5% sheep blood and MacConkey agar plates and allowed for few minutes before being taken off. Then, the surface of MacConkey agar and sheep blood agar were streaked for isolated colonies and incubated at 37°C. for 24 hours. Further, the membrane was introduced into McCartney bottles containing brain heart infusion (BHI) broth using sterile forceps and the broth was sub-cultured on MacConkey agar and blood agar after overnight incubation at 37°C.

Biochemical and antimicrobial resistance profile

From the initial bacterial growth, colonies were characterized based on the morphology, Gram stain, Standard biochemical methods and only those isolates belonging to the family Enterobacteriaceae were selected for further antimicrobial susceptibility testing by Kirby Bauer disc diffusion method as per CLSI guidelines 2011. Antimicrobial susceptibility testing for detecting multidrug resistant (MDR) organism was done using a panel of antibiotics viz. Meropenem 30µg (Hi Media-Mumbai) and in-house prepared discs Ciprofloxacin 5µg, Amikacin 30µg, Gentamicin 10µg, Ceftazidime 30µg and Ceftriaxone 30µg. Only those strains belonging to the Enterobacteriaceae family resistant to Meropenem by routine disc diffusion method was planned to analyse further for the presence of carbapenem resistant genes using Polymerase Chain Reaction (PCR).

RESULTS & DISCUSSION

The prevalence study of carbapenem resistance in water samples in Puducherry was carried out. A total of eleven bacterial isolates belonging to Enterobacteriaceae family was isolated from fifteen different water samples collected from various places in and around Pondicherry. The most common isolates were *Escherichia coli* (n=5), *Klebsiella* sp (n=3), *Enterobacter* sp (n=2), *Proteus mirabilis* (n=1). Enterobacteriaceae species isolated from lakes (n=4) and ponds(n=2) were all susceptible to Meropenem, Amikacin, Ciprofloxacin, Ceftazidime, Ceftriaxone and Gentamicin by disc diffusion method. However, none of the drinking tap water samples (n=9) yielded any Gram negative bacteria.

It was observed that isolates from places like Bahour village, Kuruvintham village, Villianur village, Oulgaret municipality, Ousdu village and Ariyankuppam were susceptible to meropenem through antibiotic susceptibility testing by disc diffusion. Enterobacteriaceae species were not isolated from tap water samples from places such as Muthirapalayam, Mettupalayam, Kundupalayam, JIPMER campus, Lawspet, Thattanchavady and Othiyanchalai. Characterization of various isolates belonging to the Enterobacteriaceae family obtained from various environmental samples is summarized in Table 1.

It is a well-known fact that antimicrobial resistance especially to beta lactam antibiotics among the commonly isolated organism like members of the Enterobacteriaceae family is a matter of public health importance globally. Until recently the presence of these carbapenemases were more commonly associated with non-fermenting Gram Negative bacilli like *Pseudomonas aeruginosa*. It did not draw much attention because of the intrinsic resistance exhibited by these non-fermenters to a variety of antibiotics. However, with the discovery of NDM -1 from India, the scenario has changed and a number of studies were published based on this new MBL enzyme. Studies also have detected the presence of bla NDM gene among the bacteria isolated from sewage samples and public tap waters from New Delhi, India . The isolates include *Vibrio cholerae* which indicates that the gene is being disseminating in environment also. These findings are worrisome since they indicate transfer of these resistant genes out of the clinical settings to the environment.

During water sample analysis, the Enterobacteriaceae species collected in and around the Puducherry did not exhibit carbapenem resistance. Results of this study depicts the absence of meropenem resistance in Enterobacteriaceae isolates isolated from environmental water samples. This study underlined the reliable data for researchers and clinicians working in the area of carbapenem resistant Enterobacteriaceae isolates in environmental samples.

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Table 1.Characterization of Enterobacteriaceae species from environment, Puducherry

S. No	No. of clinical isolates	Place	Sample	Disk diffusion for Meropenem
1	<i>Klebsiella</i> spp <i>Esch. coli</i> ,	Bahour village	Bahour Lake	Susceptible
2	<i>Esch. coli</i> <i>Enterobacter</i> spp <i>Klebsiella</i> spp	Kuruvinatham village	Kuruvinatham Lake	Susceptible
3	<i>Esch. coli</i> <i>Enterobacter</i> spp	Villianur village	Sankarbarani Lake	Susceptible
4	<i>Esch. coli</i> <i>Enterobacter</i> spp <i>Klebsiella</i> spp	Oulgaret municipality	Kanagan Lake	Susceptible
5	<i>Klebsiella</i> spp <i>Enterobacter</i> spp	Ousdu village	Ousteri Lake	Susceptible

6	Culture negative for Enterobacteriaceae	Muthirapalayam	Tap water	-
7	Culture negative for Enterobacteriaceae	Mettupalayam	Tap water	-
8	Culture negative for Enterobacteriaceae	Kundupalayam	Tap water	-
9	Culture negative for Enterobacteriaceae	JIPMER, campus	Tap water	-
10	Culture negative for Enterobacteriaceae	Lawspet	Tap water	-
11	Culture negative for Enterobacteriaceae	Thantanchavady	Tap water	-
12	Culture negative for Enterobacteriaceae	Othiyanchalai	Tap water	-
13	Culture negative for Enterobacteriaceae	Saram	Tap water	-
14	Culture negative for Enterobacteriaceae	Muthialpet	Tap water	-
15	<i>Enterobacter</i> spp <i>Klebsiella</i> spp	Ariyankuppam	Pond	Susceptible

REFERENCES

- Walsh TR. Emerging carbapenemases: a global perspective. *Int J Antimicrob Agents*. 2010 Nov;36 Suppl 3:S8-14.
- Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis*. 2011 May;11(5):355-62.
- Yang F, Mao D, Zhou H, Luo Y. Prevalence and Fate of Carbapenemase Genes in a Wastewater Treatment Plant in Northern China. *PLOS ONE*. 2016 May 26;11(5):e0156383.
- Hrenovic J, Ganjto M, Goic-Barisic I. Carbapenem-resistant bacteria in a secondary wastewater treatment plant. *Water SA*. 2017 Apr;43(2):186-91.
- Woodford N, Wareham DW, Guerra B, Teale C. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? *J Antimicrob Chemother*. 2014 Feb 1;69(2):287-91.
- Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, et al. Carbapenemase-Producing Organisms: A Global Scourge. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2018 Apr 3;66(8):1290-7.
- Mathur P, Singh S. Multidrug Resistance in Bacteria: A Serious Patient Safety Challenge for India. *J Lab Physicians*. 2013;5(1):5-10.
- Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bernes P, Hartemann P, et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS Hyg Infect Control*.
- Biedenbach D, Bouchillon S, Hackel M, Hoban D, Kazmierczak K, Hawser S, et al. Dissemination of NDM Metallo- β -Lactamase Genes among Clinical Isolates of Enterobacteriaceae Collected during the SMART Global Surveillance Study from 2008 to 2012. *Antimicrob Agents Chemother*. 2015 Feb;59(2):826-30.