Screening of Environment Water for the Presence of blaNDM-1 Gene Containing Microorganisms

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ABSTRACT

The acquisition of blaNDM-1 gene by Gram negative bacteria has emerged as critical threat to human health as these organisms display high resistance to available antibiotics. The presence of this gene has been observed in both clinical as well as environmental settings. The present study was carried out to evaluate the prevalence of blaNDM-1 containing microorganisms in water sources. A total of 241 water samples were collected from different sites in Islamabad, Rawalpindi and areas of Khyber Pakhtunkhwa. Meropenem resistant organisms were isolated and PCR was used for the detection of the blaNDM-1 gene among the resistant isolates of all the water samples, 43 meropenem resistant bacteria were isolated among which 12 isolates were found to carry blaNDM-1 gene. The blaNDM-1 isolates were resistant to most of the antibiotics but sensitive to colistin and polymyxin B. The presence of blaNDM-1 gene harboring organisms in aquatic environment is concerning and poses public health threat.

Key Words: Water, blaNDM-1, Pakistan, Gram negative bacteria, Meropenem resistant bacteria, Colistin, Polymyxin B.
hospital sites, seepage) of Islamabad, Rawalpindi and the province of Khyber Pakhtunkhwa, Pakistan. Fifteen ml of water samples were collected in conical tubes and brought to laboratory within 24 hours of collection. Each water sample was inoculated on MacConkey’s agar media supplemented with meropenem (0.5 g/L) to isolate meropenem resistant Gram negative bacteria. The resistant organisms were further identified using Gram staining and biochemical tests. Combined disks diffusion method was used for the detection of metallo-β-lactamase production using 10 µg disk of meropenem and 0.5M EDTA as an inhibitor.3 After 24 hours incubation, the plates were observed for an increase in the zone of inhibition as compared to the meropenem disk only. Antimicrobial susceptibility testing was performed by disc diffusion method using Mueller-Hinton agar according to CLSI guidelines.7 Escherichia (E.) coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853 strains were used for quality control. All data were analyzed using Microsoft Excel (2010) where the frequencies and percentages for the qualitative variables were calculated.

Out of 83 water samples obtained from rivers, lakes and seepage, a total of 30 meropenem resistant strains were isolated. Among the 70 water samples from hospitals and hotels, 13 isolates were found to be meropenem resistant. Of these 13 isolates, 4 were from hospitals and 9 were from hotels water supply. So a total of 43 meropenem resistant isolates were isolated from all water samples, of which, 16/43 (37.2%) were Pseudomonas spp, 3/43 (6.9%) were Salmonella spp, 9/43 (20.9%) were Shigella spp, 2/43 (4.6%) were E. coli, 4/43 (9.3%) were Enterococcus, 8/43 (18.6%) were K. pneumoniae and 1/43 (2.3%) was Citrobacter freundii. All samples from filtration plants, supply plants and home well water sources were negative for any meropenem resistant organism.

All meropenem resistant strains were positive for MBL production by phenotypic assay. These were further screened for blaNDM-1 gene by PCR using forward primer; 5’-CAGCGGAGCTTGTGCG-3’ and reverse primer; 5’-TCGCCAAGCTGAGCA-3’.8 Amplified products were separated on agarose gel and visualized under UV light. Among the 43 isolates, 12 were positive for blaNDM-1 (Figure 1). Out of these 12 isolates, 3 were Pseudomonas spp, 2 were Enterococcus spp, 3 were K. pneumoniae, 2 were Shigella spp, one each was E. coli and Citrobacter freundii. These positive isolates were primarily from open water sources such as rivers, lake and seepage followed by tap water (Table I). This is a matter of concern as people living close to these open sources use this water for daily use such as washing, bathing and also for all domestic needs of the animals. Furthermore, the lakes are recreational spots and people visiting these areas usually play in the water and do activities such as boating and fishing. The presence of resistant organisms in tap water from hospitals and hotels indicates the mixing of water supply with such sources which contain resistant organisms. The presence of antibiotic resistant organisms in water from other areas of Pakistan has been reported9,10 but this study reports the presence of blaNDM-1 positive organisms in water sources.

All 43 meropenem resistant isolates showed 100% resistance against imipenem, cefotaxime, ceftazidime, aztreonam and 50% resistance to amikacin while 100% susceptibility against polymyxin B and colistin was observed. All NDM-1 positive strains in this study were found to be resistant to the effects of meropenem, imipenem, amoxicillin/calvulanic acid, ceftazidime, ceftriaxone, cefotaxime and aztreonam while 100% susceptibility was observed for colistin and polymyxin B.

No metallo-β-lactamase producing bacteria were isolated from the filtration plant, supply cooler plant and home well water, suggesting that these sources were free of meropenem resistant organisms. As the aim was to isolate only bacteria presenting resistance to meropenem, the authors were unable to conclude whether these sources were free from any other resistant organisms.

The importance of environment in dissemination of resistant organisms has been documented and requires more attention. The contamination of environment is increasing through many ways as bacteria often colonize the oral or gut of the patient and then can transmit through food, hands and water in our community. The screening of NDM-1 in environment sources is needed to evaluate the burden of resistance genes in the environment. The presence of highly resistant organisms in water samples calls for an immediate response and priority.
sources should be carried out and standard hygienic measures should be taken.

REFERENCES