EMERGENCE OF HIGH-RISK COLISTIN RESISTANCE MCR-1 GENE IN CARBAPENEM-RESISTANT ENVIRONMENTAL BACTERIAL ISOLATES FROM DELHI, INDIA

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INTRODUCTION

Antibiotic resistance is a significant global health concern. In addition to fundamental applications in clinical settings, antibiotics are extensively used in agriculture, the food industry and aquaculture. The presence of antibiotics in the ecosystem is a potent stimulus to elicit a bacterial adaptation response to develop antibiotic resistance. The increased use of colistin, a last resort drug, due to failure of Carbapenems, has possibly contributed to the development and spread of resistance to colistin among Gram negative bacteria. Colistin belongs to the family of Polymyxins, cationic lipopeptides, with broad-spectrum activity against Gram negative bacteria.

METHODOLOGY

In this study, we obtained non-duplicate bacterial isolates from sewage water and the river Yamuna in Delhi and phenotypically screened them for colistin resistance. The colistin resistance gene mcr-1 was detected among the screened bacterial isolates by PCR based amplification with specific primers. Similarly, chromosome-based genes phoPQ, pmrAB and mgrB were amplified from resistant isolates of Klebsiella pneumoniae; moreover, carbapenem-resistant genes blaNDM, blaVIM and OXA were amplified in positive bacterial isolates. ESBL determinants blaCTX-M, blaSHV and blaTEM were further amplified in colistin resistant bacteria. Furthermore, WGS were performed in mcr-1 positive isolates with carbapenemase to study the genetic environment of colistin resistant bacteria. Minimum inhibitory concentrations (MICs) of 9 different classes of antibiotics were determined against all mcr-1-positive isolates through the broth micro-dilution method as per the CLSI and EUCAST guidelines. The in vitro conjugation approach was applied for the transfer of mcr-1, Carbapenemase and other ESBL resistant determinants.

RESULTS

The PCR amplification of 10 isolates was positive for the mcr-1 gene. Bacterial identification was carried out using the 16S rRNA approach which revealed the bacteria to be E. coli, Aeromonas veronii, Providencia rettgeri, Aeromonas dhakensis and K. pneumonia. Following a literature survey, we believe that this is the first report of the mcr-1 gene in Aeromonas Spp. worldwide, and the first report in Providencia rettgeri from India. Moreover, mcr-1 has not been reported from sewage water in India. The modulation of chromosomal genes in 5

colistin-resistant Klebsiella pneumonia isolates was screened. Sequencing confirmed one isolate with missense mutation in the mgrB gene and phoPQ two component system. ESBL determinants like blaCTX-M, blaTEM and blaSHV were detected in all mcr-1 positive isolates. Furthermore, a group-specific analysis of CTX-M revealed the presence of blaCTX-M-1 and blaCTX-M-25. The WGS approach results confirmed the existence of integrons, insertions and metallic and non ESBL genes in colistin-resistant bacterial isolates. Carbapenemase genes, OXA, blaVIM and blaNDM, were identified in K. pnumoniae and E.coli. Conjugation studies showed the successful transfer of mcr-1, carbapenemase and other ESBL resistant determinants.

DISCUSSION

The presence of colistin-resistant bacteria in natural water sources is a serious threat to public health. The discharge of pharmaceutical waste into natural water sources creates a selection pressure for the spread of antibiotic resistance. The overuse of antibiotics among livestock animals also favours the spread of antibiotic resistance in the aquatic environment. In this study, we have reported the first case of colistinresistance mcr-1 found in Aeromonas Species and E. coli, and the existence of the blaNDM and OXA genes in water samples taken from Delhi, India. The mcr-1 and blaNDM genes are mostly located on bacteria plasmids and can be easily transferred to sensitive strains through the horizontal transfer of genes. The presence of such colistin-resistant bacteria in the environment is of serious concern for the general public, agriculture and human health. In this study, we have reported that most of the colistin-resistant isolates and NDM-1 and mcr-1 positive isolates were found in samples taken from sewage and the river Yamuna. The results of our conjugation studies further highlight the risk that the mcr-1 gene and other resistant determinates will spread to other bacteria including clinically important pathogens.