GENOMIC DIVERSITY OF MCR-1 CARRYING ESCHERICHIA COLI ISOLATES RECOVERED FROM SURFACE WATER AND SEDIMENT IN ECUADOR

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INTRODUCTION

This study aimed to characterise the genetic structure of colistin resistance (mcr) genes among Escherichia coli isolates recovered from surface water and sediment in Ecuador. From 459 isolates, four Escherichia coli showed multidrug-resistant phenotypes, which harboured the mcr-1 gene and β-lactamases, such as the blaTEM, blaCTX-M-15, blaCTX-M-55 or blaCTX-M-65 genes. Three E. coli isolates (U20, U30 and U144) shared a similar genetic environment surrounding the mcr-1 gene, which was located on plasmids. Only one E. coli isolate (U175) showed the mcr-1 gene to be chromosomally located. Moreover, the core genome multilocus sequence typing (cgMLST) analysis revealed that these isolates belong to different lineages. This study represents the first detection of the mcr-1 gene in multidrug-resistant E. coli isolates from environmental samples in Ecuador.

METHODOLOGY

The sampling points were selected according to spatial suitability criteria, such as population density, agricultural areas, river network, irrigation channels, proximity to hospitals, logistic feasibility and upstream and downstream areas from the discharge point. Fifty-nine water (1.0 L) and sediment (100 g, depth 1 cm) samples were collected from January to October 2018 from rivers and irrigation channels in the provinces of Pichincha (n=6), Cotopaxi (n=12), Tungurahua (n=15), Manabí (n=13) and Guayas (n=13). The isolates were obtained from isolation in both Chromocult and MacConkey agar. All isolates were screened via PCR to detect genes conferring resistance to colistin (mcr-1 to 5). The whole genome of all the isolates that were positive for mcr genes was sequenced using the Illumina MiSeq platform. The sequences were assembled into contigs using QIAGEN CLC Genomics Workbench version 10.0. A core genome MLST (cgMLST) scheme for E. coli was created using Ridom SeqSphere+ version 7.0. Genome assemblies were also uploaded to the Center for Genomic Epidemiology in order to extract information on ARGs and plasmid replicons for E. coli using ResFinder 4.1 and PlasmidFinder 2.1, respectively.

RESULTS

A total of 459 Enterobacteriaceae-like morphology isolates were recovered from different sites and on different dates. Among them, only 4 isolates carried mcr genes as demonstrated by PCR amplification. Moreover, MALDI-TOF analysis revealed that the mcr-positive isolates were Escherichia coli. Multidrug-resistant phenotypes were detected, showing an extended-spectrum β-lactamases (ESBL) phenotype in all mcr-positive E. coli isolates. All mcr-positive E. coli isolates were classified as belonging to the non-wild type (NWT) according to their colistin MIC determination (> 4 mg/L). Additionally, all the isolates showed an ESBL phenotype and resistance to clinically-relevant antibiotics such as cefotaxime, ceftazidime, cefepime and aztreonam. All mcr-positive E. coli isolates co-harboured different β-lactamases, including blaTEM-1B, blaCTX-M-15 (U175), blaCTX-M-55 (U20) and blaCTX-M-65 (U30 and U144). The cgMLST analysis showed that the E. coli isolates belong to different lineages.

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.

There have been no previous reports of E. coli co-harbouring mcr-1 and blaCTX-M-15 genes in Ecuador. The genetic environment surrounding mcr-1 showed that the nikB gene was present in the upstream region (although ISApI1 was found to be absent), whereas the pap2 and ydfA genes were present in the downstream region. Interestingly, the mcr-1 gene was chromosomally located in one isolate (U175), and the genetic environment, in which two copies of ISApI1 flanked mcr-1, was similar to those exclusively described in relation to some E. coli chromosomes from Japan and Vietnam (GenBank accession numbers: LC424789, LC424782 and AP021890). These results support our previous observations because transconjugation assays failed in E. coli U175. The cgMLST analysis showed that the E. coli isolates belong to different lineages, which include the ST10 (isolate U175) that has been detected in mcr-positive E. coli of animal origin from Spain (Migura-Garcia et al., 2020) and in clinical samples from Colombia (Saavedra et al., 2017) and China (Huang et al., 2020). Likewise, all the isolates harboured a wide diversity of aminoglycoside, β-lactam, phenicol and tetracycline resistance genes.