BRAZILIAN JOURNAL OF MICROBIOLOGY xxx (2018) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

Study of pandrug and heavy metal resistance among *E. coli* from anthropogenically influenced Delhi stretch of river Yamuna

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ARTICLE INFO

Article history:
Received 7 October 2016
Accepted 21 November 2017
Available online xxx

Associate Editor: Elizabeth Marques

Keywords:
Antibiotic resistance
ESBLs
Heavy metal resistance
Polluted environment
Resistance genes

ABSTRACT

Escalating burden of antibiotic resistance that has reached new heights present a grave concern to mankind. As the problem is no longer confined to clinics, we hereby report identification of a pandrug resistant *Escherichia coli* isolate from heavily polluted Delhi stretch of river Yamuna, India. *E. coli* MRC11 was found sensitive only to tobramycin against 21 antibiotics tested, with minimum inhibitory concentration values >256 μg/mL for amoxicillin, carbenicillin, aztreonam, ceftazidime and cefotaxime. Addition of certain heavy metals at higher concentrations were ineffective in increasing susceptibility of *E. coli* MRC11 to antibiotics. Withstanding sub-optimal concentration of cefotaxime (10 μg/mL) and mercuric chloride (2 μg/mL), and also resistance to their combinatorial use, indicates better adaptability in heavily polluted environment through clustering and expression of resistance genes. Interestingly, *E. coli* MRC11 harbours two different variants of *bla*TEM (*bla*TEM-116 and *bla*TEM-1 with and without extended-spectrum activity, respectively), in addition to *mer* operon (*mer*B, *mer*P and *mer*T) genes. Studies employing conjugation, confirmed localization of *bla*TEM-116, *mer*P and *mer*T genes on the conjugative plasmid. Understanding potentialities of such isolates will help in determining risk factors attributing pandrug resistance and strengthening strategic development of new and effective antimicrobial agents.

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https://doi.org/10.1016/j.bjm.2017.11.001

Please cite this article in press as: Azam M, et al. Study of pandrug and heavy metal resistance among *E. coli* from anthropogenically influenced Delhi stretch of river Yamuna. *Braz J Microbiol.* (2018), https://doi.org/10.1016/j.bjm.2017.11.001

Introduction

Pollution of the aquatic environment through anthropogenic activities, such as discharge of municipal sewage and untreated or partially treated industrial waste, creates means for selection, proliferation and dissemination of resistant traits among bacteria. Dissemination of resistance genes that diminishes the treatment options of infectious diseases has compromised human and animal health against multidrug resistant bacteria.[1] Pollution of aquatic environments is common in developing countries like India, one such case occurs with the largest tributary of river Ganga – Yamuna, having its origin from Yamunotri Glacier of Uttar Kashi in Uttarakhand.[2] It enters the capital territory Delhi at Palla village 15 km upstream of Wazirabad barrage, that acts as a reservoir accounting for more than 70% of Delhi's water supply. Moreover, it is mainly the Delhi stretch of river Yamuna that receives high amount of discharge in terms of domestic sewage and industrial waste from urban centres and there are also various industrial settings established in and around the capital city.[3] Providing a better niche for their establishment, pollution of aquatic environment selects bacteria by causing exchange of genetic determinants as well as acquisition of resistance traits crucial for it to thrive under the increasing pressure of pollutants. There are several reports pertaining to the dissemination of antimicrobial resistance genes, and it is known that their related genetic factors originate and are later transferred, from one bacteria to other bacteria present at a distant place.[4–6]

Development of resistance in bacteria makes it potent to compete with the sensitive ones, particularly in selective environments. Co-resistance to antibiotics and metals share common structural and functional strategies, conferred by chromosomal or mobile genetic elements.[7,8] There is an increasing concern regarding potential of metal contaminated environment, acting as a pool, for sequestering antibiotic resistance genes, both in environmental and clinical isolates.[9,10] Studies reporting assembly of resistance determinants on the same genetic element, insinuates towards the need to elucidate the role of polluted environment in acquisition of resistance in both pathogenic and non-pathogenic isolates of aquatic habitats.[11–14] Exploring the resistance profiles among microbes inhabiting the aquatic ecosystem in urban areas, is of significant importance, as they directly or indirectly influence the sanitation outlook of the area. The presence of mercury and other heavy metals in water samples collected from river Yamuna has been reported in the studies of Sehgal et al.[15] and Malik et al.[16] Tolerance of mercury at different concentrations, suggests operation of different modes of detoxification encoded by genes located on *mer* operon among bacterial inhabitants of the polluted environment. Additionally, *mer* operon genes have been frequently observed to be genetically linked to antibiotic resistance genes.[12,17] Taken together, investigation of *mer* operon genes as a representation of resistance to mercury, becomes pre-requisite to determine its role in selection and survival of bacterial isolates in the polluted environments. Thus, in the present study, cefotaxime resistant *Escherichia coli* isolates from Delhi stretch of river Yamuna were assessed for its susceptibility towards wide range of antibiotics and heavy metals. Genetic determinants imparting ESBL positive phenotype, conjugation frequency and biofilm formation in different media composition were studied for the isolate. Additionally, growth kinetics and susceptibility pattern to antibiotic and heavy metal either singly or in combination were also performed.

Methodology

Isolation and characterization of bacteria

Upon collection of the water samples from thirteen diverse locations – spread across Delhi stretch during March–April and September–October, 2012 and 2013 – bacterial screening was done by spreading 100 μL of serially diluted samples on MacConkey agar plates supplemented with cefotaxime (4 μg/mL). Non-duplicate bacterial colonies with distinct colony morphology were selected and subjected to characterization by IMViC test and 16S rRNA gene sequence analysis.

Antimicrobial susceptibility test

The antibiotic susceptibility test was done by disc diffusion method following Clinical and Laboratory Standards Institute[18] guidelines. All isolates were subjected to drug susceptibility test against 21 antibiotics as described previously.[19] Representing 13 different categories that includes cephalosporins, carbapenems, aminoglycosides and fluoroquinolones. Multiple Antibiotic Resistance (MAR) index was calculated, as described by Krumperman.[20] Categorization of bacterial isolates into, multidrug resistant, extensively drug resistant and pandrug resistant, was strictly done on the basis of their resistance profiles following guidelines of Magiorakos et al.[21] Minimum inhibitory concentration (MIC) of antibiotics was determined by broth micro-dilution method using Luria Bertani (LB) broth and E-test following CLSI guidelines with minor modifications in the culture medium. Similarly, resistance tests against heavy metals were determined by broth micro-dilution method by using different concentrations of heavy metal salts of cadmium, copper, chromium, mercury, lead and zinc. The cultures (96-well culture plates) were incubated at 37 °C for 14–18 h and Optical Density (OD) was measured at 600 nm. The minimum concentration of each antibiotic and heavy metal salt inhibiting the growth of isolate was considered as its MIC. An isolate designated in this study as MRC11, showing broader resistance to different class of antibiotics and heavy metals, was selected for further studies.

Phenotypic screening for ESBL production

Following susceptibility test to 3rd generation cephalosporins [ceftazidime (CAZ), cefotaxime (CTX) and ceftriaxone (CTR)], selected bacterial isolates were screened for the production of extended spectrum β-lactamase enzyme(s) by Phenotypic Disc Confirmatory Test (PDCT) on Mueller-Hinton Agar plates as described earlier.[19] *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls, respectively.

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