EXECUTIVE SUMMARY

UGC-MAJOR RESEARCH PROJECT

41-1172/2012(SR)

Identification and characterization of Integron-mediated antibiotic resistance in *Escherichia coli* isolated from Yamuna River water

Submitted by

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Summary of the UGC-Major Research Project entitled "Identification and characterization of Integron-mediated antibiotic resistance in *Escherichia coli* isolated from Yamuna River water" sanctioned to Dr. Pooja Gulati, Assistant Professor, Department of Microbiology, Maharshi Dayanand University, Rohtak by University Grant Commission, New Delhi. [F. No. 41-1172/2012(SR)]

SUMMARY

In the present study, *E. coli* isolated from Yamuna River water were characterized for the presence of multiple antibiotic resistance, class 1 & class 2 integrons and their associated gene cassettes. A total of 199 *E. coli* were isolated from eight different sampling sites along the Yamuna River stretch in New Delhi. Biochemical characterization of *E. coli* isolates was performed where one hundred forty one isolates were confirmed as *E. coli*. These isolates were analyzed for antimicrobial susceptibility against a panel of nineteen antibiotics belonging to twelve different antimicrobial classes. High prevalence of antibiotic resistance was observed with resistance frequency of 98.58% and multiple resistance rate of 60% among *E. coli* isolates. One hundred twenty three (86.6%) isolates were designated as multi-drug resistant (MDR). The *E. coli* isolates showed high resistance to β-lactams (Cefazolin and Cefotaxime) and Vancomycin while least resistant to Polymyxin-B and Azithromycin. Multiple Antibiotic Resistance (MAR) index of each sampling site was also evaluated. All the sampling sites had a MAR Index above 0.25 which showed high risk of contamination at these sites. Eighty-one MDR isolates were further screened by PCR amplification for the presence of class 1 and class 2 integrons. Fifteen (15/81; 18.5%) isolates harbored class 1 integron while only one possessed class 2 integron. PCR amplification was carried out for the characterization of variable region of class 1 and class 2 integrons. Eighteen isolates were found to possess a class 1 integron variable region. Three different gene cassettes were reported to be prevalent in *E. coli* isolates of Yamuna River *viz.* '*dfrA17-aadA5*', '*dfrA12*-orfF-*aadA2*' and a putative phage tail tape measure protein. Majority of the isolates possessed *dfrA17-aadA5* as a gene cassette in the variable region of class 1 integron. Class 2 integron variable region showed amplification in only one isolate which harbored '*dfrA1-sat2-aadA2*' gene cassette. Southern blot analysis was done with *intI1* gene acting as a probe in determining the location of integron. Hybridization results depicted that plasmid and not chromosome was responsible for high prevalence and wide dissemination of

integrons in *E. coli* isolates of Yamuna River. Genotyping of integron-positive isolates using repetitive elements like BOX and $(GTG)_5$ was performed. $(GTG)_5$ typing clustered isolates into eighteen different types while BOX-typing clustered *E. coli* isolates into fifteen different types. Most of the isolates possesing '*dfrA17-aadA5*' cassette grouped into a single BOX-PCR type (B_8) suggesting a clonal relatedness among the isolates. However $(GTG)_5$ typing method has a higher discriminating power than BOX type and thus can serve as a potential tool in epidemiological typing of *E. coli*.

SIGNIFICANCE OF WORK

The Yamuna River had been serving as a major source of water, either directly or indirectly, for both human and animal consumption in Delhi and adjoining areas. The present study elucidated antibiotic resistance, class 1 and class 2 integrons in waterborne *E. coli* within Yamuna River highlighting a serious risk it poses to human and animal health through the spread of antibiotic-resistant bacteria. Further, the presence of antibiotic resistant genes carrying integrons in *E. coli* isolates of Yamuna River emphasizes that the city is under a high risk of antibiotic resistance development especially to newer β-lactams. Hence, proper mitigation and control strategies need to be developed to tackle the alleviated resistance among water bodies.

Publications

1. **Megha Kaushik**, Sanjay Kumar, Rajeev Kumar Kapoor, Jugsharan Singh Virdi and **Pooja Gulati** (2018). 'Integrons in *Enterobacteriaceae*: Diversity, distribution and epidemiology'. International Journal of Antimicrobial Agents.**51**:167-76.
2. **Megha Kaushik**, Neha Khare, Sanjay Kumar, **Pooja Gulati** (2018). 'High prevalence of antibiotic resistance and integrons in *E. coli* isolated from an urban river water, India'. Microbial Drug resistance **(MDR 2018-0194 : accepted)**.
3. **Megha Kaushik**, Rajeev Kumar Kapoor, Sanjay Kumar and **Pooja Gulati** (2018). Integrons as vehicles in the dissemination of antibiotic resistance genes in aquatic environment: Threat Detection and Risk Assessment. Science of the Total Environment. **(STOTEN-D-18-0359.under review).**

GenBank submissions

1. *Escherichia coli* strain IO-9 class 1 integron dihydrofolate reductase (*dfrA17*) and aminoglycoside adenylyltransferase (*aadA5*) genes, complete cds - **KX573886**
2. *Escherichia coli* strain IT-26 class 1 integron, partial sequence - **KX573885**
3. *Escherichia coli* strain WZB-11 class 2 integron, partial sequence - **KX500024**
4. *Escherichia coli* strain IT-7 , class 1 integron, truncated phage tail tape measure protein, partial CDS – **MH230085**
5. *Escherichia coli* strain IO-1, class 1 integron, dihydrofolate reductase (*dfrA12*), an open reading frame and aminoglycoside adenyltransferase (*aadA2*), partial CDS- **MH249046**
6. *Escherichia coli* strain WZB-11 class 2 integron, dihydrofolate reductase (*dfrA1*), streptothricin (*sat2*) and aminoglycoside adenyltransferase (*aadA2*), partial CDS- **MH370610**

Presentations

1. **Megha Kaushik**, Sanjay Kumar, Pooja Gulati (2017). Comparative analysis of antibiotic resistance in various *E. coli* from Yamuna River through its flow in National Capital territory of Delhi. Microbes for Health and Wealth, MaharshiDayanand University, Rohtak, Nov. 14, 2017 **(Poster presentation)**.
2. **Megha Kaushik**, Sanjay Kumar, Pooja Gulati (2016). Dissemination of antibiotic-resistant genes in *E. coli* from Yamuna River, New Delhi. India International Science Festival (IISF) – Young Scientists' Conclave (YSC), National Physical Laboratory, New Delhi, Dec 8^{th}-11^{th}, 2016. **(Oral presentation)**
3. **Megha Kaushik**, Neha Khare, Sanjay Kumar, Pooja Gulati (2016). Isolation, characterization and phylogrouping of *Escherichia coli* from Yamuna River, New Delhi. **103^{rd} Indian Science Congress**, University of Mysore, Manasagangotri, Mysuru, Jan 3^{rd} – 7^{th} 2016. **(Poster presentation)**
4. **Megha Kaushik**, Sanjay Kumar and **Pooja Gulati** (2015). Detection of integron amongst different phylogroups of *Escherichia coli* isolated from Yamuna River, New Delhi. **56^{th} Annual Conference of Association of Microbiologists of India** (AMI 2015), Jawaharlal Nehru University, New Delhi, Dec 7^{th} -10^{th} 2015. **(Poster presentation)**
5. **Megha Kaushik**, Neha Khare, Sanjay Kumar, **Pooja Gulati**. (2013) Isolation, identification and antibiotic profiling of strains of *E. coli* isolated from Yamuna river water. 54^{th} Annual Conference of Association of Microbiologist of India (AMI), Maharshi Dayanand University, Rohtak, Haryana, Nov. 17-20, 2013. **(Poster presentation) BEST POSTER AWARD**