Prevalence of Multiple Antibiotic-Resistant Coliform Bacteria in the Water of River Ganga

Karabi Biswas, Dipak Paul, Sankar Narayan Sinha[*]

Environmental Microbiology Research Laboratory, Department of Botany, University of Kalyani, Kalyani, West Bengal, India

Email address:

biswaskarabi@yahoo.in (K. Biswas), dipak23paul@yahoo.com (D. Paul), sinhasn62@yahoo.co.in (S. N. Sinha)

Abstract: The current investigation was done to assess the bacterial load and detect the presence of indicator bacteria that acquire the spread of feacal contamination of water of river Ganga at Serampore, West Bengal, India. Total of 110 bacterial isolates, 25 feacal coliform, 50 *Escherichia coli* and *Enterobacter* sp were isolated from river water. The objective of this study also showed the antibiotic resistivity of the different isolates. This result showed the notable presence of indicator coliform bacteria in the water and need for conventional treatment of water.

Keywords: Antibiotic-Resistant, Coliform Bacteria, River Ganga

1. Introduction

The presence of antibiotic resistant bacteria associated with river water has been a crucial public health concern [1]. The occurrence of antibiotic resistant bacteria is increasing in aquatic environments in last few decades [2]. Several investigations have been performed to study the prevalence of antibiotic resistant bacteria in different water bodies [3,4]. Indiscriminate use of antibiotics in human and animals for treatment of different diseases leads to release of antibiotics into the environment [5,6]. Various mechanisms are responsible for the capability of microorganisms to tolerate antibiotics, and the occurrence of resistance to these antibiotics within bacterial species has risen notably since the commercial use of antibiotics [7]. Different indicators microorganisms have been used worldwide as a tool to indicate the contamination of water by human wastes [8]. The anthropogenic effects on coastal areas through inflow of domestic effluents can be assessed by determining feacal bacteria present in water bodies and the occurrence of bacterial resistance to antimicrobial substances [9].

In India, the River Ganga is the longest and most important river that passes along 29 class I cities, 23 class II cities and approximately 50 towns because of which wastes of different types such as industrial, sewage etc are discharged into this mighty river eco-system [10-12]. The aim of the present study is to investigate the prevalence of multiple antibiotic-resistant coliform group of bacteria isolated from the water of the River Ganga.

2. Materials and Methods

2.1. Study Area

The River Ganga represents a highly polluted aquatic environment which receives direct discharges of domestic, commercial, industrial and agricultural wastewaters. The river water samples were collected from Serampore (22º45′30.65″N 88º20′31.51″E), West Bengal, India. The main activity at this area includes passenger vessel jetties and human interference.

2.2. Collection of Sample

Water samples for microbiological analysis were collected from surface and bottom of study site. Aseptic plastic bottles were used for sample collection and were transported to the laboratory immediately in an ice box for analysis.

2.3. Enumeration of Bacterial Population

Counting of bacterial population was carried out by using standard plate count method. Water samples were aseptically transferred to the laboratory and serially diluted water samples were plated on Petridishes for enumeration of total viable count, feacal pollution indicator such as total and feacal coliform, *Escherichia coli*. Total coliform were counted on MacConkey agar, which was incubated for 24 h at 37°C. All colonies showing pink-red colouration were counted as total coliform. Feacal coliforms were counted on

EMB agar and incubated at 44.5°C for 24 h. *Escherichia coli* were counted as dark coloured colonies with metallic green sheen on EMB agar.

2.4. Identification of Bacterial Isolates

Different bacterial isolates were characterized by cultural, morphological and further identified by biochemical examination in accordance with Bergey's Manual of Determinative Bacteriology [13].

2.5. Antibiotic Susceptibility Test

Antibiotic resistance of bacteria of Enterobacteriaceae family was determined by the disc diffusion method [14]. The isolates were screened against five antibiotics such as ampicillin, chloramphenicol, tetracycline, norfloxacin and co-trimoxazole. A bacterial suspension of overnight grown cultures was prepared and turbidity was adjusted to a 0.5 McFarland standard. A sterile cotton swab was used to inoculate the bacterial suspension on the surface of a Mueller Hinton Agar plate. The bacterial isolates were scored as susceptible, intermediate or resistant to a given antibiotic by the inhibition zone diameter around the antibiotic disc.

3. Results and Discussion

Table 1. *Morphological, physiological and biochemical tests of the bacterial isolates.*

Tests	Feacal coliform	*Escherichia coli*	*Enterobacter* sp
Shape	Rods	Rods	Rods
Gram reaction	-	-	-
o-nitrophenyl-ß-galactopyranoside test	+	+	+
Ornithine	+	-	+
Urease	-	-	-
Nitrate	+	-	+
H$_2$S production test	-	-	-
Esculin hydrolysis	+	-	+
Indole production test	-	+	-
Methyl Red	-	+	-
Voges Proskauer's	+	-	+
Citrate utilization	+	-	+
Oxidase	+	-	+
Carbon Utilization tests			
Glucose	+	+	+
Lactose	+	-	+
Xylose	+	+	+
Rhamnose	+	-	+
Raffinose	+	-	+
Trehalose	+	+	+
Starch Hydrolysis	-	-	-
Gelatinase test	-	-	+
Casein hydrolysis	-	-	-

+ indicates positive results; – indicates negative results

A total of 110 coliform bacteria were isolated from the studied water samples. Of 110 total bacterial isolates 25 feacal coliform, 50 *Escherichia coli* and 35 *Enterobacter* sp were isolated from river water. The bacterial isolates were characterized with the help of morphological, physiological and biochemical tests. Morphological, physiological, and biochemical characteristics of bacterial isolates were outlined in Table 1.

An antibiotic is a kind of ubiquitous contaminant in the aquatic environment with industrial effluents and sewage discharge. The bacterial isolates were exposed to 5 antibiotics for susceptibility testing, and the zones of inhibition were observed and recorded in millimeters. Table 2 describes resistance of isolates to different antibiotics.

Table 2. *Antibiotic sensitivity test.*

Antibiotics	Feacal coliform	*Escherichia coli*	*Enterobacter* sp
	Zone of Inhibition (mm)		
Ampicillin	13	16	18
Chloramphenicol	14	19	10
Co-trimoxazole	15	18	13
Norfloxacin	8	10	7
Tetracycline	12	18	12

All the isolates were showed significant resistant against all the tested antibiotics. *E. coli* showed highest degree of resistance against all the antibiotics than other isolated bacteria. The overall resistance pattern showed high resistance towards co-trimoxazole, chloramphenicol and low resistance against norfloxacin.

This study revealed the prevalence of multiple antibiotic resistant bacteria in Ganga river, Serampore, West Bengal, India. Presence of antibiotic resistance bacteria in a given environment may be an indication that an area is contaminated with antibiotics. The feacal coliform bacteria are numerous in this riverine water might be due to the presence of anthropogenic wastes particularly sewage. These microorganisms may be repeated from the total coliform group by their capability to grow and proliferate at elevated temperature. This investigation is thus pertinent to the present situation of environmental pollution and climate change. Due to these plenty of reasons, importance has been put on the antibiotic resistance status of such bacterial isolates. It is exhibited that the bacterial isolates showed marked resistance to antibiotics like chloramphenicol, co-trimoxazole, ampicillin, tetracycline and low resistance to norfloxacin. Thus it can be concluded that the bacterial isolates from River Ganga water is resistant to most of the antibiotics that may be attributed to genetic modification of the bacterial strain.

Antibiotic resistance of feacal bacteria in surface water of different water bodies has been studied by various researchers such as rivers [15], estuaries [16], lakes [17] and coastal waters [18]. This is mainly because the natural characteristics of the river ecosystem have faced dreadful changes, as untreated sewage discharges and industrial effluents carry high loads of pathogenic bacteria, especially enteric groups, and pose a potential threat to human health [19]. Several studies have used the antibiotic resistance pattern of feacal indicator bacteria to investigate the source of feacal pollution in the aquatic environment [20,21].

The main reasons causing the marine environmental

contamination were improper and unnecessary use of antimicrobial drugs by human and animals [2]. The present assessment of the microbial population for antibiotic resistance profile to different classes of antibiotics showed a high proportion of strains resistance to β-lactam antibiotics, followed by resistant to macrolides. These results complied with the finding of Foster [22].

4. Conclusion

In the present study, show the prevalence of multiple antibiotic-resistant coliform bacteria, which are known to be indicators of water contamination in the river Ganga, Serampore, West Bengal, India. The majority of microbial populations isolated from this area were resistant to antibiotics. Further studies are needed to establish the role of antibiotics substances in control of bacterial populations in River Ganga and subsequent management of these problems are vital to prevent the emergence of drug-resistant bacteria.

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