Asian Journal of Environment & Ecology

12(4): 22-32, 2020; Article no.AJEE.57159
ISSN: 2456-690X

Environmental Antimicrobial Resistance (En-Amr) in Surface Water of Thiruvananthapuram City, Kerala

C. R. Sreelakshmi[1], Sheela A. Moses[2] and Salom Gnana Thanga Vincent[1*]

[1]Department of Environmental Sciences, University of Kerala, Kariavattom Campus, Thiruvananthapuram, Kerala, India.
[2]Kerala State Pollution Control Board, Thiruvananthapuram, Kerala, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SGTV designed the study. Author CRS performed the sampling and laboratory analysis. Authors CRS, SAM and SGTV contributed to writing the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEE/2020/v12i430165
Editor(s):
(1) Prof. Daniele De Wrachien, State University of Milan, Italy.
Reviewers:
(1) Zakaria Fouad Fawzy Hassan, National Research Centre, Egypt.
(2) Shaymaa Ismail Shedeed, National Research Centre, Egypt.
(3) Khairul Anuar Mat Amin, Universiti Malaysia Terengganu, Malaysia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57159

Original Research Article

Received 12 March 2020
Accepted 18 May 2020
Published 27 May 2020

ABSTRACT

Aim: The study was done to understand the microbial contamination and antibiotic resistance pattern in surface water environment.
Study Area and Sampling: Water samples collected from selected water bodies in the main urban area of Thiruvananthapuram were analysed for the presence of coliforms and the pattern of antimicrobial resistance in bacterial cultures isolated from the water samples.
Methodology: The total coliform count and faecal coliform count was determined using the multiple tube fermentation technique and the total heterotrophic bacterial count was performed using nutrient agar media. The bacterial cultures were identified using biochemical characterization and Antibiotic susceptibility patterns for the various bacterial isolates were determined using commercial antibiotic disks (Hi Media, Mumbai) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disc diffusion method. The antibiotics used were Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Tetracycline and Meropenem. Multiple Antibiotic resistances (MAR) index was determined for those isolates which showed resistance to more than three antibiotics.

**Corresponding author: E-mail: salom@keralauniversity.ac.in;*

Results: The total heterotrophic bacteria, total coliforms and fecal coliforms were significantly high in all the sites, indicating that the water bodies are sewage contaminated. The biochemical identification of bacterial strains isolated from water sample showed the presence *of E. coli, Bacillus sp, Staphylococcus sp, Klebsiella sp, Clostridium sp, Neisseria sp, Enterobacter sp, Enterococcus sp* and *Streptococcus sp* in varying frequencies in different sites. Among these 58 isolates, 26 strains were found to be resistant against 3 or more antibiotics and hence, designated as multi drug resistant. The isolates were highly resistant to Ampicillin (98%), Chloramphenicol (53%) and Gentamycin (44%); and highly susceptible to Meropenem (86%), Ciprofloxacin (69%) and Tetracyclin (58%). *E. coli* showed maximum resistance to all the antibiotics. One- way ANOVA of the obtained data revealed that there is no significance difference in spatial distribution of antibiotic resistance.

Keywords: Antibiotics; antimicrobial resistance; bacterial strains; surface water; multiple antibiotic resistance.

1. INTRODUCTION

An antibiotic is a chemical substance, which has the ability to decrease the growth of and also can eliminate the micro-organisms. The action of an antibiotic against micro-organisms is choosy in nature, some organisms may affected and others stay as unaffected may be to a particular degree. Antibiotics show variations in their physical, chemical properties as well as in their toxicity towards animals [1]. The potential for the misuse and abuse of antibiotics were known shortly after their introduction [2]. More usage of antibiotics leaves their residue in the environment. Hence, more bacteria develop resistance to them, which makes treating infections that much more challenging. A microbial organism where there is antibiotic presence, it may lead to mutational changes in usually sensitive bacteria, this allows the bacteria to survive and then it get formed as antibiotic resistant bacteria (ARB) that carry antibiotic resistant genes (ARGs) [3]. More number of pathogens has become antibiotic resistant, and some have become resistant to many antibiotics and chemotherapeutic agents, this is known as multidrug resistance. Significant adverse effects such as increase of morbidity and mortality, drug toxicity, long hospitalization period, increase of costs, resistant microorganisms and associated infections are caused by the excessive and inappropriate use of antibiotics [4].

Consequently, diverse environments are being investigated as reservoirs or hubs for the spread of ARGs. In particular, aquatic environments are inhabited by highly diverse microorganisms that represent a vast reservoir of ARGs and by allochtonous microbes originating from various sources, including potential pathogens that are already resistant to antibiotics [5]. Bacteria have developed several mechanisms to make the antibiotics ineffective, which are used against them. The defence mechanisms encoding genes are located on the bacterial chromosome or on extra chromosomal plasmids, and are transmitted to the next generation (vertical gene transfer). Genetic elements, such as plasmids, can be exchanged among bacteria of different taxonomic association (horizontal gene transfer) [6].

Among aquatic environments, wastewater effluents from humans, livestock, industries and hospitals together with the rivers that receive these effluents have been widely examined for the presence of antibiotic compounds, ARB and ARGs [7] and also they act as meeting and exchange places for human and environmental bacteria, either pathogenic or non-pathogenic [8,9]. This is now becoming a major issue in our country, improved AMR stewardship and the development of new antimicrobials may be the best course to preventing the spread of AMR in the environment [10]. The present study was done to recognize the nature of increasing antimicrobial resistance in bacteria in public water bodies flowing to the Karamana River. The objective of the present investigation is to assess the total and fecal coliform count and the susceptibility of isolates from different water bodies to various antibiotics and also their antimicrobial resistance index.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling

The samples were collected in December, 2018 randomly from the 14 stations in water bodies in the Karamana river basin, passing through Thiruvananthapuram City. The location of

sampling stations is given in Figs. 1 and 2 and in Table 1. Maps showing land use and location of health care stations are given in Figs. 3 and 4. The study was done to evaluate the microbial contamination and antimicrobial resistance property of surface water bacterial isolates. Samples for microbiological tests were collected in sterile grass bottles. The samples were then place on ice in a cooler box and were transported to the laboratory in a cooler box for further analysis.

2.2 Analysis of Coliform Bacteria, Total Heterotrophic Bacteria (THB) and Antibiotic Resistance

The total coliform count and faecal coliform count was determined using the multiple tube fermentation technique [11]. Total heterotrophic bacterial count was done using pour plat method in nutrient agar media. Identification of the bacterial isolates are done using biochemical characterization methods [12]. Antibiotic susceptibility patterns for the various bacterial isolates were determined using commercial antibiotic disks (Hi Media, Mumbai) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disc diffusion method [13]. A total of 6 antibiotics, viz., Ampicillin (AMP, 10 µg), Chloramphenicol (C 30 µg), Ciprofloxacin (CIP 5 µg), Gentamicin (GEN 10 µg), Tetracycline (TE 10 µg), Meropenem (MRP 10 µg) were used in the present study. Multiple Antibiotic resistances (MAR) index was determined for those isolates which showed resistance to more than three antibiotics [14].

MAR index = $\frac{a}{b}$

Where,

'a' is the number of antibiotics to which the isolate shows resistance.

'b' is the number of antibiotics to which the isolate was exposed.

Sampled parameters where subjected to one-way ANOVA to check the statistical differences at p=0.05.

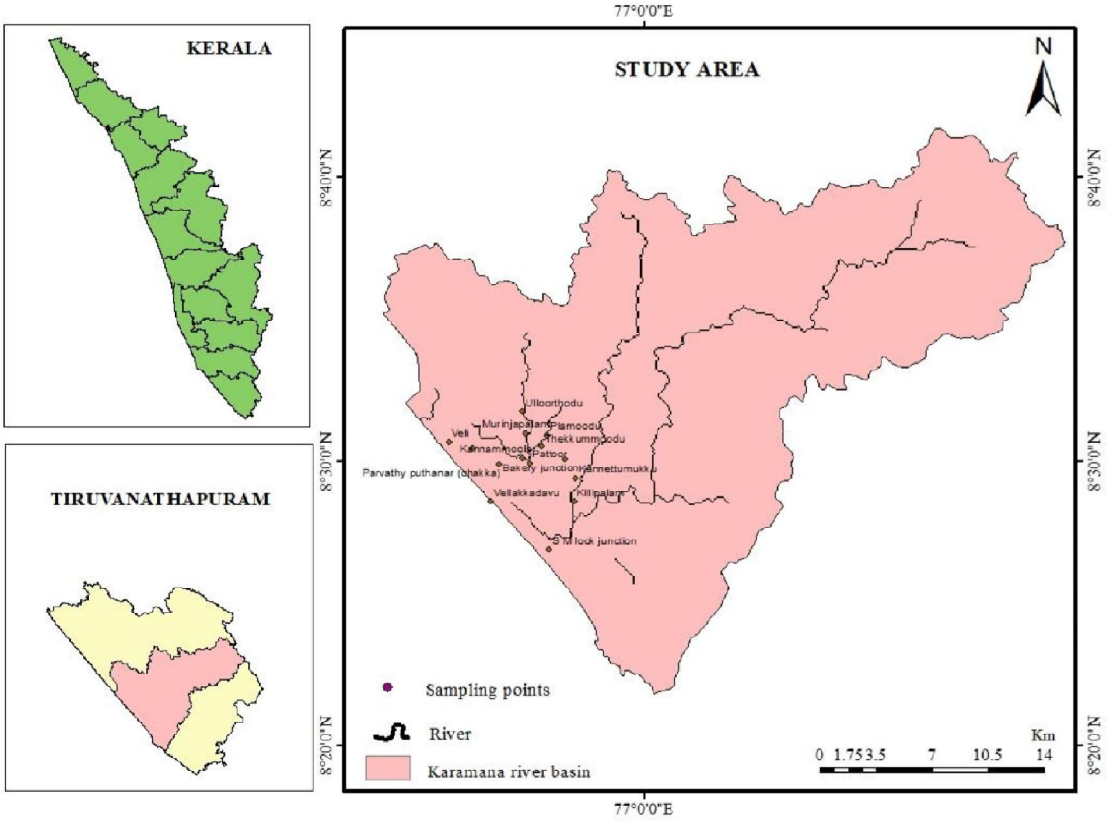

Fig. 1. Map of the study area

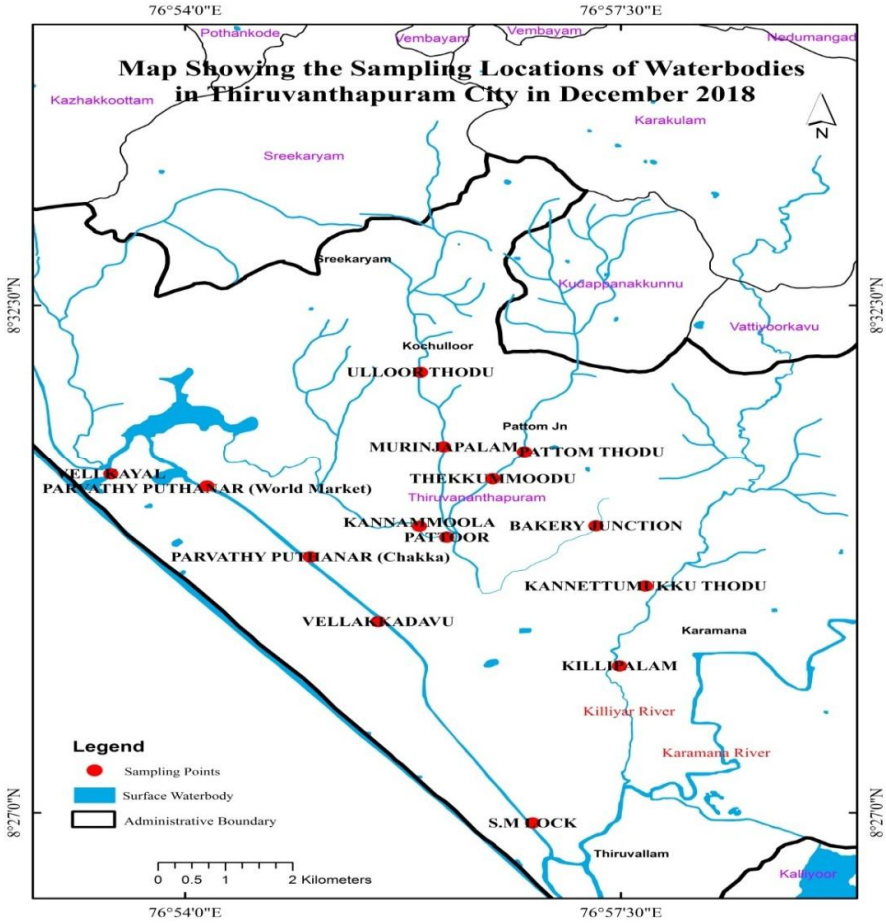

Fig. 2. Map showing location of sampling stations

3. RESULTS AND DISCUSSION

3.1 Total Coliform, Fecal Coliform and Total Heterotrophic Bacterial Count

The maximum permissible value of total coliform in water is 1 per 100 ml [15] and 10 per 100 ml [16]. The presumptive test showed presence of coliform bacteria in all the water samples. Table 1 showed that the total coliform count was significantly high in the downstream of Kannamoola drain (S1) and Amayizhanchan stream (S2), northern part of Parvathiputhanar (S3 and S4) and in Veli lake (S5) with 2400 MPN/100 ml and lower at the upstream of Amayizhanchan stream (S10) with 23 MPN/100 ml. The faecal coliform count was high at the same locations (S1 – S5) and in Parvathiputhanar at Vellakadavu (S7) with 2400 MPN/100 ml. Faecal coliform presence was also assessed in all the samples. In the study area, polluted drains lie in the urban area (Fig. 3) and there are three health care institutions having more than 500 beds and ten having bed strength between 200 and 500 beds (Fig. 4). The presence of coliform organism in water is considered as the indication of faecal contamination as their source in the intestinal tract of human and other warm blooded animals. This shows that the bacterial contamination in the water bodies is mainly caused by the human excreta and domestic sewage, which creates unhygienic and unhealthy condition and makes the water unfit for any use [17].

In the present study, presence of bacterial isolates in the water samples in all the locations indicated undesirable contaminations of samples. The maximum total viable bacterial load of 1.3×10^{13} found in the downstream of Amayizhanchan stream (S2) and of 1.28×10^{13} in the Pattom stream (S11). In the previous study on the water quality of Karamana river basin, bacteriological analysis of the samples clearly indicated

microbial contamination in the river. Almost all the stations showed higher index for total coliforms and faecal coliform. In all seasons *E. coli* was present in all analysed samples [18].

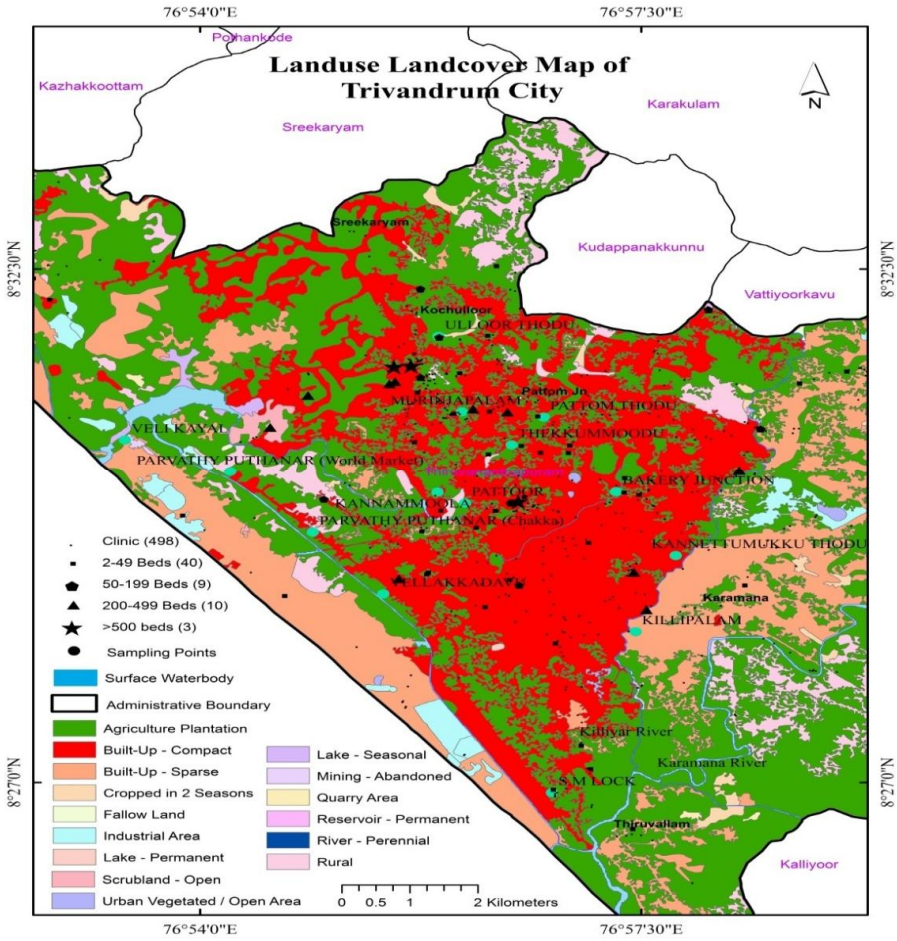

Fig. 3. Land use map of the study area

Table 1. Total coliform count, fecal coliform count and total heterotrophic bacterial (THB) count of the water samples

Samples	Location	TC per 100 ml	FC per 100 ml	THB (CFU / ml)
S1	Kannamoola stream	2400	2400	7.6×10^{12}
S2	Amayizhanchan stream- Pattoor	2400	2400	1.3×10^{13}
S3	Parvathiputhanar-Chakka	2400	2400	8.1×10^{12}
S4	Parvathiputhanar (World Market)	2400	2400	1.02×10^{13}
S5	Veli Lake	2400	2400	3×10^{12}
S6	Parvathiputhanar-S.M. Lock	240	240	9.2×10^{12}
S7	Parvathiputhanar-Vellaikadavu	2400	2400	5.2×10^{12}
S8	Killiyar-Killippalam	43	9	9.4×10^{12}
S9	Kannettumukku stream	240	7	6.5×10^{12}
S10	Amayizhanchan stream-Bakery junction	23	15	1.1×10^{13}
S11	Pattom stream-Pattom	460	9	1.28×10^{13}
S12	Ulloor stream	43	9	5.7×10^{12}
S13	Ulloor stream-Murnijapalam	240	35	3.9×10^{12}
S14	Pattom stream-Thekkummoodu	240	93	7.2×10^{12}

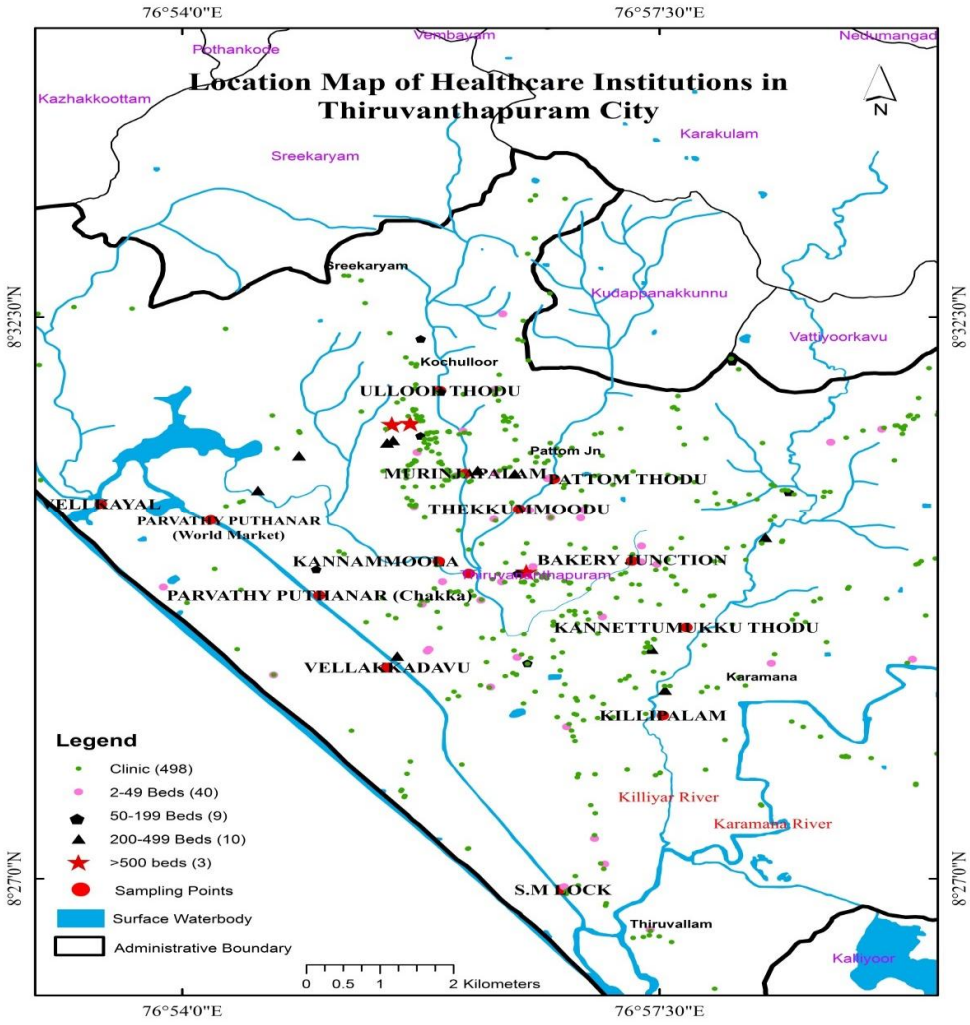

Fig. 4. Map showing location of health care stations in Thiruvananthapuram city

Table 2. Identification of bacterial isolates

Strain	Gram staining reaction	IMVIC test				Inference
		Indole	Methyl - Red	Voges - Proskauer	Citrate	
S1 A	–	+	+	–	–	*E. coli sp.*
S1 B	+	–	–	+	+	*Bacillus sp.*
S1 C	+	–	+	+	–	*Staphylococcus sp.*
S1 D	–	–	+	+	+	*Klebsiella sp.*
S2 A	–	–	–	–	–	*Neisseria sp.*
S2 B	–	+	+	–	–	*E. coli*
S3 A	–	–	–	+	+	*Enterobacter sp.*
S3 B	+	–	+	+	–	*Staphylococcus sp.*
S3 C	–	+	+	–	–	*E. coli*
S4 A	+	–	–	–	+	*Bacillus sp.*
S4 B	+	–	–	–	–	*Clostridium sp.*
S4 C	+	–	–	+	+	*Bacillus sp.*
S4 D	–	+	+	–	–	*E. coli*
S4 E	+	–	+	+	–	*Staphylococcus sp.*

Strain	Gram staining reaction	IMVIC test				Inference
		Indole	Methyl - Red	Voges - Proskauer	Citrate	
S5 A	_	_	+	+	+	*Klebsiella sp.*
S5 B	+	_	_	_	_	*Clostridium sp.*
S5 C	_	+	+	_	_	*E. coli*
S5 D	+	_	_	+	+	*Bacillus sp.*
S5 E	+	_	_	_	_	*Clostridium sp.*
S6 A	_	_	+	+	+	*Klebsiella sp.*
S6 B	_	+	+	_	_	*E. coli*
S6 C	+	_	+	+	_	*Staphylococcus sp.*
S6 D	+	_	_	+	+	*Bacillus sp.*
S6 E	+	_	_	+		*Streptococcus sp.*
S6 F	+	+	+	_	_	*Enterococcus sp.*
S7 A	+	_	+	+	_	*Staphylococcus sp.*
S7 B	_	+	+	_	_	*E. coli*
S8 A	_	_			_	*E. coli*
S8 B	+	_	_	_	_	*Clostridium sp.*
S8 C	+	_	_	_	_	*Clostridium sp*
S8 D	+	_	_	+	+	*Bacillus sp.*
S8 E	+	_	_	+	+	*Bacillus sp.*
S9 A	+	_	+	+	_	*Staphylococcus sp.*
S9 B	_	_	_	+	+	*Enterobacter sp.*
S9 C	_	_	+	+	+	*Klebsiella sp.*
S9 D	+	_	_	+		*Streptococcus sp.*
S10 A	+	_	_	+	+	*Bacillus sp.*
S10 B	_	_	+	+	+	*Klebsiella sp.*
S10 C	+	_	+	+	_	*Staphylococcus sp.*
S10 D	_	_			_	*Neisseria sp.*
S10 E	_	+	+	_	_	*E. coli*
S11 A	_	_			_	*Neisseria sp.*
S11 B	+	_	_	+	+	*Bacillus sp.*
S11 C	+	+	_	_	_	*Enterococcus sp.*
S11 D	_	+			_	*E. coli*
S12 A	+	_	+	+	_	*Staphylococcus sp.*
S12 B	+	+	_	_	_	*Enterococcus sp.*
S12 C	_	_	+	+	+	*Klebsiella sp.*
S13 A	+	_	_	+	+	*Bacillus sp.*
S13 B	_	_	_	+	+	*Enterobacter sp.*
S13 C	_	+	+	_	_	*E. coli*
S14 A	_	+	+	_	_	*E. coli*
S14 B	+	_	_	+	+	*Bacillus sp.*
S14 C	_	_	+	+	+	*Klebsiella sp.*
S14 D	_	_	_	+	+	*Enterobacter sp.*
S14 E	+	+			_	*Clostridium sp*
S14 F	+	_	+	+	_	*Staphylococcus sp.*
S14 G	_	_			_	*Neisseria sp.*

The 58 isolates taken from the 14 different water samples were identified using biochemical methods (gram staining and IMViC test). Among the isolates *E. coli, Bacillus sp, Staphylococcus sp, Klebsiella sp, Clostridium sp, Neisseria sp, Enterobacter sp, Enterococcus sp* and *Streptococcus sp* were identified. The number of *E. coli* (12) was maximum followed by *Bacillus sp.* (11), *Staphylococcus sp.* (9), *Klebsiella sp.* (7), *Clostridium sp.* (6), *Neisseria sp and Enterobacter sp. (4), Enterococcus (3) and the least number among isolates is Streptococcus* (2).

3.2 Antimicrobial Susceptibility Test

The antibiotic resistance test was examined based on the measurement of zone formation,

given according to the Clinical and Laboratory Standards Institute [19]. The strains with no zone or with size of the zone formation less than 10 mm in diameter were regarded as resistant strain. On the other hand the strains is said to be sensitive when the zone formation is equal to or more than 15 mm in diameter, as given by the CLSI.

Table 3. Antibiotic resistance pattern and multiple antibiotic resistance of isolated cultures

Sample stations	Bacterial isolates	AMP	TET	C	CIP	GEN	MRP	MAR index
S1	*E. coli sp.*	R	S	R	S	R	S	0.5
	Bacillus sp.	R	R	S	S	R	S	0.5
	Staphylococcus sp.	R	S	R	S	R	S	0.5
	Klebsiella sp.	R	R	S	S	S	I	0.33
S2	*Neisseria sp.*	R	R	R	S	S	S	0.5
	E. coli	R	R	R	S	R	R	0.83
S3	*Enterobacter sp.*	R	I	S	S	R	I	0.33
	Staphylococcus sp.	R	R	I	R	R	I	0.66
	E. coli	R	R	S	S	R	R	0.66
S4	*Bacillus sp.*	R	R	R	S	R	S	0.66
	Clostridium sp.	R	R	R	S	R	I	0.66
	Bacillus sp.	R	R	I	S	S	S	0.33
	E. coli	R	R	R	S	R	S	0.66
	Staphylococcus sp.	R	R	I	S	R	S	0.5
S5	*Klebsiella sp.*	R	R	R	S	R	S	0.66
	Clostridium sp.	R	S	S	R	S	S	0.33
	E. coli	R	I	R	I	R	S	0.5
	Bacillus sp.	R	R	R	I	R	S	0.66
	Clostridium sp.	R	R	S	S	S	S	0.33
S6	*Klebsiella sp.*	R	S	S	S	R	S	0.33
	E. coli	R	S	S	S	R	S	0.33
	Staphylococcus sp.	R	S	R	R	R	S	0.66
	Bacillus sp.	R	S	R	I	S	S	0.33
	Streptococcus sp.	R	S	R	I	R	S	0.5
	Enterococcus sp.	R	S	R	R	S	S	0.5
S7	*Staphylococcus sp.*	R	S	I	I	S	S	0.16
	E. coli	R	R	S	S	S	S	0.33
S8	*E. coli*	R	R	R	R	R	S	0.83
	Clostridium sp.	R	S	R	S	R	S	0.5
	Clostridium sp	R	S	I	S	S	S	0.16
	Bacillus sp.	R	S	I	R	R	S	0.5
	Bacillus sp.	R	S	R	I	S	S	0.33
S9	*Staphylococcus sp.*	R	S	R	S	R	S	0.5
	Enterobacter sp.	R	S	R	S	S	S	0.33
	Klebsiella sp.	R	S	S	S	S	S	0.16
	Streptococcus sp.	R	S	I	S	S	S	0.16
S 10	*Bacillus sp.*	R	R	R	R	R	S	0.83
	Klebsiella sp.	R	S	S	S	S	S	0.16
	Staphylococcus sp.	R	S	S	S	S	S	0.16
	Neisseria sp.	R	S	R	R	S	S	0.5
	E. coli	R	R	R	R	R	I	0. 66
S 11	*Neisseria sp.*	R	S	S	S	S	S	0.16
	Bacillus sp.	R	S	I	S	S	S	0.16
	Enterococcus sp.	R	S	R	S	S	S	0.33
	E. coli	R	R	R	R	R	S	0.83
S 12	*Staphylococcus sp.*	R	S	R	S	S	S	0.33
	Enterococcus sp.	R	R	I	S	S	S	0.33
	Klebsiella sp.	R	S	S	S	S	S	0.16

Sample stations	Bacterial isolates	AMP	TET	C	CIP	GEN	MRP	MAR index
S 13	*Bacillus sp.*	R	S	I	S	S	S	0.16
	Enterobacter sp.	R	S	R	I	S	S	0.33
	E. coli	R	S	R	S	R	S	0.33
S14	*E. coli*	R	R	R	S	S	S	0.5
	Bacillus sp.	R	R	R	S	S	I	0.5
	Klebsiella pp.	R	S	I	S	S	S	0.16
	Enterobacter sp.	R	S	S	I	S	S	0.16
	Clostridium sp	R	S	R	S	S	S	0.33
	Staphylococcus sp.	R	S	R	S	S	S	0.33
	Neisseria sp.	S	S	S	S	S	S	0

AMP-Ampicillin; TET-Tetracyclin; C-Chloramphenicol; CIP-Ciprofloxacin; GEN-Gentamycin; MRP-Meropenem

Among these total bacterial isolates, 57 isolates were resistant and one of them was susceptible to Ampicillin. In the case of Tetracyclin, 22 isolates shows resistance, 34 were susceptible and 2 were intermediate. A total of 31 isolates were resistant to Chloramphenicol, 16 were susceptible and only one of them is intermediate. About 10 isolates were resistant to Ciprofloxacin, and 40 isolates were susceptible to the antibiotic and 8 were found intermediate. 26 isolates out of 58, 32 were susceptible to Gentamycin. In the case of Meropenem, only two of isolates were resistant, 50 isolates were susceptible and 6 of them were intermediate. In the decreasing order, the isolates were highly resistant to Ampicillin (98%) followed by Chloramphenicol (53%) and Gentamycin (44%); and highly susceptible to Meropenem (86%) followed by Ciprofloxacin (69%) and Tetracyclin (58%). A total of 26 isolates showed resistance to three or more antibiotics belonging to different classes and thus designated as Multi Drug Resistance (MDR) strains. The high values of these antibiotics may be due to the overuse or misuse of the antibiotics. Potential hotspots of ARB, as hospitals and health care facilities, for example a high input of antibiotic load to sewerage in complex urban area such as Milan urban area lead to AMR development, which suggests that the common domestic use of antibiotics play an important role. Moreover the residence time in sewerage also important for the development of AMR [20].

In this study it was found that different isolates showed a varied pattern of resistance to different antibiotics used in the study (Fig. 2). Among the 9 strains, the isolates showed 100% resistance to Ampicillin except *Neisseria sp*. which shows 75% of resistance to Ampicillin. *E. coli* showed 83% resistance to Gentamicin, 75% resistance to Chloramphenicol and 66% resistance to Tetracycline; *Bacillus sp.* showed 54% resistance to Tetracycline and Chloramphenicol, 45% resistance to Gentamicin. *Staphylococcus sp*. showed 55% resistance to both Chloramphenicol and Gentamicin. *Klebsiella sp*, showed 28% resistance to Tetracycline and Gentamicin. *Clostridium sp*, *Neisseria sp* and *Enterobacter sp*. showed 50% resistance to Chloramphenicol, whereas, *Enterococcus sp* showed 66% of resistance to Chloramphenicol.

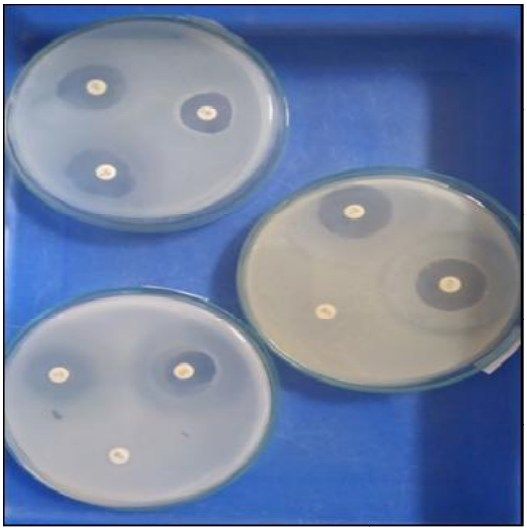

Fig. 5. Representative picture showing antibiotic susceptibility test results

It also has been observed that same bacterial cultures isolated from different stations showed different antibiotic resistance pattern. The isolates showing resistance to three or more antibiotics were designated as Multi Drug Resistance (MDR). Among the identified 9 strains, except *Enterobacter* sp, all the other strains showed multi drug resistant (MDR) character at different stations. MAR index value higher than 0.2 is considered to have originated from high risk sources of contamination. Hence

in the present study, out of the 58 isolates 45 isolates have MAR index value greater than 0.2 indicates that those samples were from highly polluted sources, whereas only 13 isolates were considered to have originated from least contaminated sources as the MAR index value for those isolates were less than 0.2. The highest MAR index 0.83 was found for *E. coli* in the downstream of Amayizhanchan stream (S2) and in Pattom stream (S11) and *Bacillus sp*. in the upstream of Amayizhanchan stream at Bakery Junction ie., these areas are highly contaminated in the middle of the city with establishments, health care establishments, high rise buildings, etc. Similar results were obtained from a study of backwater showing higher value of MAR index means that the microbes were mostly originated from human contamination like hospital discharge and were distributing its resistance potential to other aquatic microbes in the aquatic environment [21]. However, the bacteria isolated from Pattom stream at Thekkummoodu (S14) are highly sensitive to the entire antibiotics used in the study. One-way ANOVA was used to study the spatial differences in AMR, which showed that there was no significance between the different groups.

4. CONCLUSION

The total and faecal coliform count were significantly high in the water bodies in the urbanised area especially due to the bypass of sewage from the old sewer lines. A total of 58 bacterial strains were isolated from 14 water samples. From this isolates, the occurrence of *E. coli* (12) was maximum followed by *Bacillus* sp (11), *Staphylococcus* sp, *Klebsiella* sp, *Clostridium* sp, *Neisseria* sp and *Enterobacter* sp, *Enterococcus sp* and the least number among isolates was *Streptococcus* sp. The bacterial isolates were highly resistant to Ampicillin (98%), and highly susceptible to Meropenem (86%). *E. coli* showed maximum resistance to all the antibiotics except Meropenem and Ciprofloxacin. *Bacillus sp*. also shows high resistance to the antibiotics except Meropenem. *Neisseria sp*. isolated from Pattom stream in Thekkummoodu exhibit high sensitivity towards all the antibiotics tested. Among the 58 isolates, 45 isolates showed multiple antibiotic resistances (MAR) which indicates that those samples were from highly polluted sources namely upstream and downstream of Amayizhanchan stream and Ulloor stream. The presence of antibiotic resistant bacteria in a given environment may be an indication that an area is contaminated with antibiotics. This issue can be reduced by creating awareness among the public, pharmaceutical industry, health care stations and policy makers about its risks and about the proper use of antibiotics. Government have to implement very strict rules to check indiscriminate use of antibiotics and ensure proper treatment of wastes before discharge to the environment.

ACKNOWLEDGEMENTS

The authors thank University of Kerala and Kerala State Pollution Control Board for providing facilities for this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Waksman SA. What is an antibiotic or an antibiotic substance? Mycologia. 1947; 39(5):565-569.
2. Ma MY, Rho JP. Considerations in antimicrobial prescribing. The Medical Clinics of North America. 1995;79(3):537-549.
3. Martinez JL. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proceedings of the Royal Society B: Biological Sciences. 2009;276(1667):2521-2530.
4. Craig WA, Uman SJ, Shaw WR, Ramgopal V, Eagan LL, Leopold ET. Hospital use of antimicrobial drugs: Survey of 19 hospitals and results of antimicrobial control programs. Ann Intern Med. 1978;89:793–795.
5. Shao S, Hu Y, Cheng J, Chen Y. Research progress on distribution, migration, transformation of antibiotics and antibiotic resistance genes (ARGs) in aquatic environment. Critical Reviews in Biotechnology. 2018;38(8):1195-1208.
6. Davison J. Genetic exchange between bacteria in the environment. Plasmid. 1999;42(2):73-91.
7. Valitalo P, Kruglova A, Mikola A, Vahala R. Toxicological impacts of antibiotics on aquatic micro-organisms: A mini-review. International Journal of Hygiene and Environmental Health. 2017;220(3):558-569.

8. Amos GC, Ploumakis S, Zhang L, Hawkey PM, Gaze WH, Wellington EM. The widespread dissemination of integrons throughout bacterial communities in a riverine system. The ISME Journal. 2018; 12(3):681.
9. Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: Antibiotic-resistant bacteria in hospital wastewater systems. Journal of Hospital Infection. 2016;93(4):395-402.
10. Williams MR, Stedtfeld RD, Guo X, Hashsham SA. Antimicrobial resistance in the environment. Water Environment Research. 2016;88(10):1951-1967.
11. American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. 21st Ed. American Public Health Association, Washington DC. 2005;1220.
12. Cappuccino JG, Sherman N. Microbiology: A laboratory manual. San Francisco: Pearson/Benjamin Cummings. 2005;507.
13. Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1966;45(4): 493-496.
14. Krumperman PH. Multiple antibiotic resisrtance indexing of *Eschericia coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 1983;46(1):165-170.
15. I.C.M.R. Drinking water standards. New Delhi: ICMR; 1975.
16. World Health Organization. Guidelines for drinking-water quality. World Health Organization; 1993.
17. Usharani K, Umarani K, Ayyasamy PM, Shanthi K, Lakshmanaperumalsamy P. Physico-chemical and bacteriological characteristics of Noyyal River and ground water quality of Perur, India. Journal of Applied Sciences and Environmental Management. 2010;14(2).
18. Harikumar PS. Water quality status of Thiruvananthapuram district, Kerala; 2017. Jayalakshmi V, Lakshmi N, Singara Charya MA. Assessment of physico-chemical parameters of water and waste waters in and around Vijayawada. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011;2(3):1040-1046.
19. CLSI, C. Performance of standards for antimicrobial disk susceptibility tests; approved standards. M2-A10; 2009.
20. Turolla A, Sabatino R, Fontaneto D, Eckert EM, Colinas N, Corno G, Mangiaterra G. Defence strategies and antibiotic resistance gene abundance in enterococci under stress by exposure to low doses of peracetic acid. Chemosphere. 2017;185: 480-488.
21. Pokhrel H, Baishya S, Phukan B, Pillai D, Rather MA. Occurrence and distribution of multiple antibiotic resistance bacteria of public health significance in backwaters and aquaculture farm. Journal Homepage. 2018;7(02). Available:http://www.ijcmas.com

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57159