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Research Article

PREVALENCE OF MULTI-DRUG RESISTANCE AMONG DIVERSE BACTERIAL COMMUNITIES IN BHAVANI RIVER: AN UNADDRESSED THREAT

SHANMUGA SUNDARI ILANGOVAN[1], KEERTHANA R[2], SUDHARSANA S[1], SHANMATHI S[2], DIVYA S[2], SUGANYA M[2], SHAMPA SEN[1]*, MOHANAPRIYA A[1]

[1]School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India. [2]Department of Biotechnology, Vivekanandha College of Engineering for Women, Tiruchengode, Namakkal, Tamil Nadu, India. Email: shampa.iitg@gmail.com

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ABSTRACT

Objective: Our work focused on accessing the quality and bacterial diversity of rapidly polluted Bhavani river, the mainline water resource of the Kongunadu region.

Methods: Water samples were collected from two sites used by the people for routine use. At the sites of analysis, the water displayed physicochemical values far away from the permissible limits of World Health Organization. Samples were subjected to 16s ribosomal DNA (16s rDNA) sequencing and phylogenetic diversity was inferred from PHYLIP software package. Multi-drug resistance (MDR) of the collected strains were determined by antibiotic susceptibility test.

Results: Phylogenetic diversity prediction by 16s rDNA gene sequencing method disclosed the presence of two major phyla, Firmicutes (71%) and Proteobacteria (21%) affiliated to different genera (*Bacillus*, *Paenibacillus*, *Lysinibacillus, Staphylococcus, Pseudomonas,* and *Acinetobacter*). Of the total population, 35% of the isolates represent novel phenotypes. Antibiotic susceptibility test indicated that >60% of the total bacterial strains showed strong resistance to all the antibiotics. The cumulative resistance percentage ranged from 64 to 82 signifying their adaptive nature to MDR.

Conclusion: The results signify the urge toward the management of river water pollution to prevent the occurrence of MDR bacteria in river Bhavani.

Keywords: Multi-drug resistance, Physiochemical analysis, Phylogenetic diversity.

INTRODUCTION

The majority of the Industries in the Indian subcontinent are located downstream to the rivers. They utilize the river water resources and discharge effluents. Environmental standards of water quality are unconsidered by these industries. 70% of the water quality deterioration is due to the assimilation of municipal, industrial, and agricultural wastes. On a daily basis, water bond problems upshot in the death of more than 14,000 people all around the world. It has been estimated that by 2025, about half of the world population will endure from water-based problems [1], and it will be the foremost root of death and diseases. The same conditions were reflected in the Bhavani river, the most important South Indian perennial river.

The Bhavani river is a main tributary of river Kaveri and the second largest perennial river of Tamil Nadu, South India. The main itinerary of the river is positioned on the entire of North-Western Erode district of Tamil Nadu [2]. Erode and Tirupur districts are well-recognized textile centers of India. The river banks of Bhavani are populated by countless industrial setups such as textile mills, tanneries, and paper mills. According to Pollution Control Board, approximately, 77 dyeing units are functioning across the river apart from tannery and paper industries. The Bhavani, the Noyyal, and the Kaveri are the three major sources of water for these industries. However, tremendous untreated effluents were discharged by those industries into the same rivers. The Tamil Nadu Government banned the entire water usage from Noyyal river as approximately, 75000 m^3 of effluents were discharged into the Noyyal river and into other sewage systems [3].

The deterioration of water quality due to multiple anthropogenic inputs in the river has major effects on human health [4]. The polluted river hampers light penetration, smash up the quality of the receiving streams, turn out to be the lethal toward the aquatic life and endanger more than six million people in the districts. It is well-known that health risks from water born pathogenic organisms are increasing in developing and developed countries [5]. Different aids of organic matter, toxins, and other xenobiotics promote resistance in the bacterial community against antibiotics giving rise to multi-drug resistant revolution. This local adaptation of the microbial community contributes to both disease pathogenesis and transmission [6]. Residents near the river bank face such a massive challenge against microbial associated infections. Microorganisms evolve multi-drug resistance (MDR) as they employ toxic effluents and other contaminants as their substrate [7]. Hence, studies on the antibiotic resistance evolved during the remediation process should be extensively considered.

Our efforts have focused on the Bhavani river as it is the suitable place for understanding the potential role of contaminants to amend the bacterial community's resistance to antibiotics. Understanding the factors and cause for the infectious diseases across the river basin relies on the study of physicochemical parameters and antibiotic susceptibility tests. To our knowledge, this is the first report on the phylogenetic diversity of microbial communities in the Bhavani river tributary.

METHODS

Site description and sample collection

Samples were collected from subsurface water level (10-20 m depth) from two polluted sites, site 1 (S1) (11° 26' 44.4546" N 77° 41' 14.7366" E) and site 2 (S2) (11° 20' 58.8156" N 77° 45' 11.289" E) of Bhavani river as shown in Fig. 1. S1 is located near the place Bhavani, harboring many small dyeing units which drains the majority of their effluents into the river without proper effluent treatment. S2 has direct mixing of effluents from textile and paper industries besides domestic wastages. The river water at both the sites was used directly for drinking. Besides, the water resource was used for domestic and fishing purposes. Water samples from both the sites were collected in

sterile glass bottles, brought to the laboratory, processed within 1-3 hrs, and stored at −20°C for further analysis. Collected water samples were analyzed for physicochemical parameters.

Physicochemical analysis

Color, odor, and temperature were measured directly at the sites 1 and 2. The pH was recorded using a pH meter (ELKO–L1613, India). Turbidity, total dissolved solids (TDS), total suspended solids (TSS), biological oxygen demand (BOD), and chemical oxygen demand (COD) were determined and calculated in the laboratory according to standard methods of the American Public Health Association, 1995 [8] and Environmental Protection Agency [9]. The recorded physicochemical parameters were compared to the standard values of World Health Organization (WHO) and tabulated in Table 1.

Isolation and cultivation of bacterial communities

Bacterial colonies were isolated from the water samples by culture dependent method [10]. Briefly, 1 ml of water sample each from S1 and S2 was added to 9 ml of sterile distilled water serially diluted and 100 µl of samples was plated onto nutrient agar medium by spread plate method. Plates were incubated at 37°C for 24-48 hrs. Based on their morphological behavior, such as pigmentation, fast and slow growing conditions, colony morphology, and discrete bacterial colonies, were selected, subcultured, and purified. The isolates were designated and used for further experimental purposes.

Molecular characterization

Bacterial isolates were grown in nutrient broth (Hi media, India) for 24 hrs. Phenol - chloroform extraction procedure was implemented to extract the genomic DNA [11]. 16s ribosomal DNA (16s rDNA) gene was amplified using universal primers 27 F 5'-AGA GTT TGA TCC TGG CTC AG-3' and 1492 R 5'-GGT TAC CTT GTT ACG ACT T-3'[12]. The reaction mixture of volume 50 µl consists of 25 ng of 1 µl template DNA with 1X reaction buffer containing 10 mM Tris (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$, 200 µM of each dNTP, 10 pM of each primer, and 0.05 U of Taq DNA polymerase in thermal cycler at specified conditions as reported earlier [13,14]. Amplified polymerase chain reaction (PCR) products were examined in agarose gel electrophoresis (1% agarose), stained with ethidium bromide, and visualized under UV transilluminator.

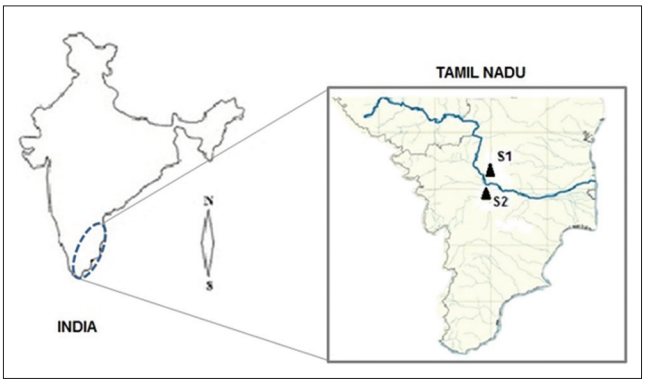


Fig. 1: Samples collected at two highly contaminated sites S1 and S2 in the Bhavani river tributary, India (Source: http://en.wikipedia.org/wiki/Kaveri)

Table 1: Physicochemical analysis of Bhavani river at sampling sites, S1 and S2

Physical characters	Sampling site 1 (S1)	Sampling site 2 (S2)	Standard values of WHO
Color	Brownish	Brownish black	-
Odor	Pungent smell	Pungent smell	-
pH	8.8	9.6	7-8.5
Temperature (°C)	25	29	40
Turbidity (N.T.U)	18	23	5
TDS (mg/L)	1050	1410	1000
TSS (mg/L)	660	740	150
BOD (mg/L)	254	480	50
COD (mg/L)	185	392	1000

TDS: Total dissolved solids, TSS: Total suspended solids, BOD: Biological oxygen demand, COD: Chemical oxygen demand, WHO: World Health Organization

Nucleotide accession numbers

All the 16s rDNA PCR products were sequenced (Macrogen, South Korea), aligned, and checked for chimeric sequences [15]. The sequences were submitted to Gene Bank database, and the accession numbers GenBank: KF751671-KF751682 and GenBank: KJ748624-KJ748629 were obtained.

Phylogenetic analysis

Phylogenetic affiliations of the 16s rDNA partial sequence for all the bacterial isolates to its nearest type strains were determined with BLAST (NCBI) [16] and taxonomically arranged using SEQMATCH (RDP) [17] algorithms. Each 16s rDNA partial sequence was aligned to its nearest bacterial strains by multiple sequence algorithm CLUSTAL X [18] with default parameters and converted to PHYLIP format with BIOEDIT (version 7.1). A phylogenetic tree was inferred with PHYLIP (version 3.695) software package. A neighbor-joining tree was constructed, and the robustness of the topology was inferred by bootstrap analysis with 1000 replications.

Antibiotic susceptibility test

The obtained bacterial strains were tested for their resistance to different classes of antibiotics. Pure bacterial isolates were assessed for susceptibility profile by disc diffusion method according to manufacturer's instruction (Hi media, India) which obeys Clinical Laboratory Standards Institute guidelines [19,20]. Briefly, all the isolates were inoculated in Tryptic soy broth and incubated for 2-8 hrs at 37°C. After attaining moderate turbidity, isolates were swabbed in Muller–Hinton agar plates with a sterile cotton swab. Antibiotic-impregnated discs containing tetracycline (30 µg), gentamycin (10 µg), erythromycin (10 µg), kanamycin (10 µg), and ampicillin (10 µg) were placed on petri plates using automatic disk dispenser (Hi media, India) and incubated for 24 hrs at 37°C. *Escherichia coli* strain (ATCC® 25922™) was used as a control. Zone of inhibition in millimeter (mm) was measured manually, and the results were tabulated in Table 2. Cumulative resistance percentage was calculated and tabulated (Table 2) using the formula:

$$\text{Cumulative resistance percentage} = \frac{\text{Number of isolates resistant to each antibiotic}}{\text{Total number of isolates}}$$

RESULTS

Physicochemical analysis of Bhavani river

The obtained physicochemical parameters for sites S1 and S2 were compared with standards of WHO and tabulated as shown in Table 1. The water color was brownish black in S1 and blackish blue in S2 with pungent smell at both the sites. The pH was recorded as 8.8 and 9.6 in S1and S2, respectively. Turbidity values were recorded as 18 N.T.U and 23 N.T.U which are far away from the permissible limits of WHO (5 N.T.U). TDS at S1 and S2 was found to be 1050 mg/l and 1410 mg/l and were considerably higher than the standards of WHO (1000 mg/l), indicating the presence of organic and other pollutants in the water.TSS of S2 (740 mg/l) was significantly higher than S1 (660 mg/l), respectively. A high load of BOD was observed in both the sites S1 (254 mg/l) and S2 (480 mg/l) compared to the value of WHO (50 mg/l). However, COD of S1 and S2 was found to be within the WHO limits (1000 mg/l).

Isolation of bacterial strains

A total of 18 bacterial strains were isolated from two sites (S1 and S2). Based on their discrete morphological behavior observed after 3 subcultures, the bacterial isolates were selected and designated as SSKSD01-SSKSD18.

16s rDNA gene amplification

Genomic DNA was isolated for all the 18 isolates, and PCR amplification for 16s rDNA gene was carried out with eubacterial Universal Primers 27 F and 1492 R to obtain a single amplicon of ~1500 bp. All the PCR products of 16s rDNA were sequenced. SSKSD13 was found to be a repetitive sequence of SSKSD04 after sequence analysis and was discarded. Hence, the forthcoming results will be discussed with 17 bacterial isolates.

Phylogenetic analysis

The obtained nucleotide sequences were analyzed in BLASTn (NCBI) and taxonomically arranged with SEQ-MATCH (RDP) to obtain the phylogenetic affiliations of the nearest bacterial type strains as given in Table 3. Phylogenetic diversity analysis shows distinct variation in species level from both the sites. Considering, 97.5% threshold value [12], 35% of the total population belongs to new phenotype. This includes SSKSD10 from S1 and SSKSD04, SSKSD11, SSKSD14, and SSKSD15 from S2. Diversity analysis of the 17 bacterial isolates from S1 and S2 reveals that they belong to two major class Bacilli (71%) and Gammaproteobacteria (21%). The class Bacilli dominates by 71% representing different genera of *Bacillus*, *Paenibacillus*, *Lysinibacillus,* and *Staphylococcus*. The class Gammaproteobacteria represents 21% of total population of S1 and S2 containing *Pseudomonas* and *Acinetobacter* species.

Bacterial diversity at S1

A total of eight bacterial species were obtained from S1, and a phylogenetic tree was constructed as shown in Fig. 2 *Bacillus* was the predominant genus contributing to 38% of total population of S1. Isolates SSKSD01, SSKSD05, and SSKSD08 belonged to *Bacillus* species. Isolates SSKSD03 and SSKSD07 were found to be *Paenibacillus* species contributing to 25% of total population next to genus *Bacillus.* Isolate SSKSD06 was identified as *Pseudomonas* species showing 99% sequence similarity to *Pseudomonas aeruginosa* (GenBank: HE978271) while isolate SSKSD12 showed 99% sequence similarity to *Lysinibacillus fusiformis* (GenBank: AJ310083), each isolate contributing to 12% of total population of S1. Isolate SSKSD10 was found to be a *Fictibacillus* species showing 93% sequence similarity to *Fictibacillus nanhaiensis* (GenBank: GU477780) probability of being a new phenotype.

Bacterial diversity at S2

Phylogenetic diversity as shown in Fig. 3 represents a total of nine bacterial isolates corresponding to different genera. *Pseudomonas* and *Bacillus* are the two major genera each sharing 33% of total population of

Table 2: Antibiotic susceptibility profile of bacterial isolates[1]

Strain designation	Nearest type strain	Antibiotics (zone of inhibition in mm)				
		TET[a]	GNT[b]	ERY[c]	KNM[d]	AMP[e]
SSKSD01	*Bacillus horneckiae*	26(S)	15(S)	28(S)	26(S)	22(S)
SSKSD02	*Pseudomonas aeruginosa*	12(R)	11(R)	12(R)	13(R)	11(R)
SSKSD03	*Paenibacillus brasilensis*	15(I)	13(I)	10(R)	12(R)	15(I)
SSKSD04	*Acinetobacter soli*	14(R)	10(R)	12(R)	11(R)	12(R)
SSKSD05	*Bacillus safensis*	19(S)	08(R)	24(S)	24(S)	10(R)
SSKSD06	*Pseudomonas aeruginosa*	13(R)	12(R)	10(R)	12(R)	12(R)
SSKSD07	*Paenibacillus lautus*	12(R)	15(I)	11(R)	17(I)	12(R)
SSKSD08	*Bacillus subtilis*	18(I)	11(R)	10(R)	13(R)	13(R)
SSKSD09	*Pseudomonas alcaligenes*	11(R)	09(R)	13(R)	10(R)	12(R)
SSKSD10	*Fictibacillus nanhaiensis*	13(R)	11(R)	12(R)	13(R)	09(R)
SSKSD11	*Bacillus subtilis*	13(R)	11(R)	10(R)	12(R)	10(R)
SSKSD12	*Lysinibacillus fusiformis*	10(R)	12(R)	14(I)	11(R)	13(R)
SSKSD14	*Bacillus anthracis*	17(I)	14(I)	11(R)	18(S)	11(R)
SSKSD15	*Staphylococcus pasteuri*	10(R)	10(R)	13(R)	12(R)	13(R)
SSKSD16	*Bacillus horneckiae*	26(S)	20(S)	29(S)	30(S)	22(S)
SSKSD17	*Staphylococcus pasteuri*	12(R)	08(R)	11(R)	10(R)	14(R)
SSKSD18	*Pseudomonas alcaligenes*	11(R)	10(R)	13(R)	12(R)	12(R)
% resistance		64.7	70.6	76.4	70.5	82.3
Resistance		14 R	12 R	13 R	13 R	13 R
Sensitivity		19 S	15 S	23 S	18 S	17 S

[1]The control strain *E. coli* (ATCC® 25922™) behaved same as given in interpretation chart in the disc assays. [a]TET - Tetracycline (30 µg), [b]GNT - Gentamycin (10 µg), [c]ERY - Erythromycin (10 µg), [d]KNM- Kanamycin (10 µg), eAMP - Ampicillin (10 µg). R, I, and S indicate the Resistance, Intermediate, and Sensitivity levels of Antibiotic discs

Table 3: Phylogenetic affiliation of the 17 bacterial isolates analyzed in the study

Strain designation	Accession no	Nearest type strain (accession num)	Putative phylum	% identity
SSKSD01	KF751671	*Bacillus horneckiae* (EU861362)	Firmicutes	99
SSKSD02	KJ748625	*Pseudomonas aeruginosa* (X06684)	Proteobacteria	99
SSKSD03	KF751672	*Paenibacillus brasilensis* (AF273740)	Firmicutes	99
SSKSD04	KJ748629	*Acinetobacter soli* (EU290155)	Proteobacteria	94
SSKSD05	KF751673	*Bacillus safensis* (AB681259)	Firmicutes	99
SSKSD06	KF751674	*Pseudomonas aeruginosa* (HE978271)	Proteobacteria	99
SSKSD07	KJ748627	*Paenibacillus lautus* (AB073188)	Firmicutes	97
SSKSD08	KF751675	*Bacillus subtilis* (AJ276351)	Firmicutes	99
SSKSD09	KF751676	*Pseudomonas alcaligenes* (D84006)	Proteobacteria	99
SSKSD10	KJ748624	*Fictibacillus nanhaiensis* (GU477780)	Firmicutes	93
SSKSD11	KJ748626	*Bacillus subtilis* (AJ276351)	Firmicutes	97
SSKSD12	KF751677	*Lysinibacillus fusiformis* (AJ310083)	Firmicutes	99
SSKSD14	KJ748628	*Bacillus anthracis* (AB190217)	Firmicutes	97
SSKSD15	KF751679	*Staphylococcus pasteuri* (AB009944)	Firmicutes	99
SSKSD16	KF751680	*Bacillus horneckiae* (EU861362)	Firmicutes	97
SSKSD17	KF751681	*Staphylococcus pasteuri* (AB009944)	Firmicutes	99
SSKSD18	KF751682	*Pseudomonas alcaligenes* (D84006)	Proteobacteria	99

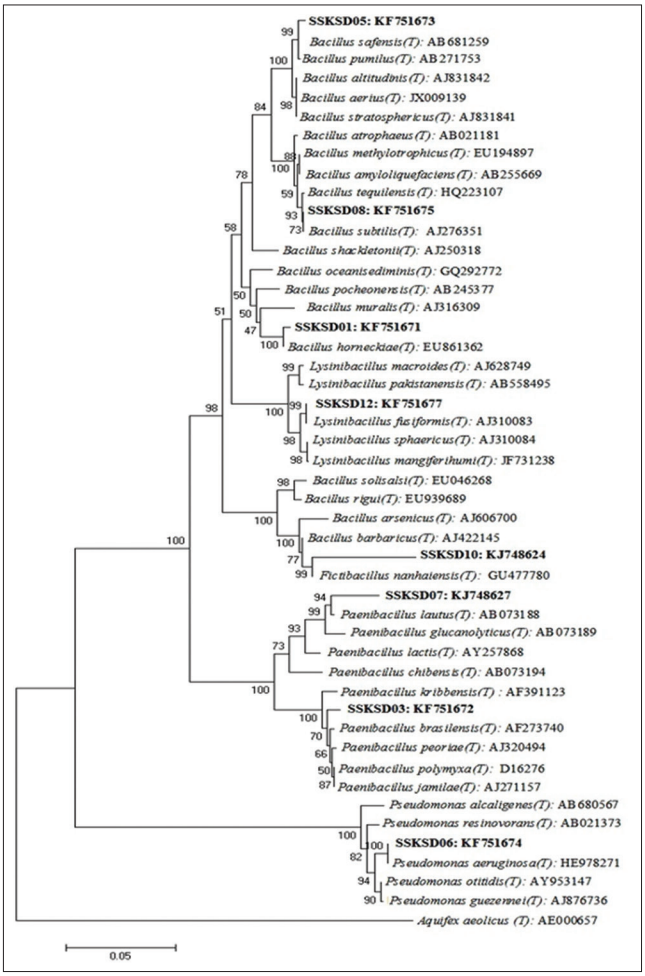


Fig. 2: Neighbor-joining phylogenetic tree for 16s ribosomal DNA gene sequences of bacterial isolates of S1. Distance was calculated by Jukes-Cantor method. Boot strap values are expressed as percentage of 1000 replications. *Aquifex aeolicus*^{T} (Accession no: AE000657) was used as an out group

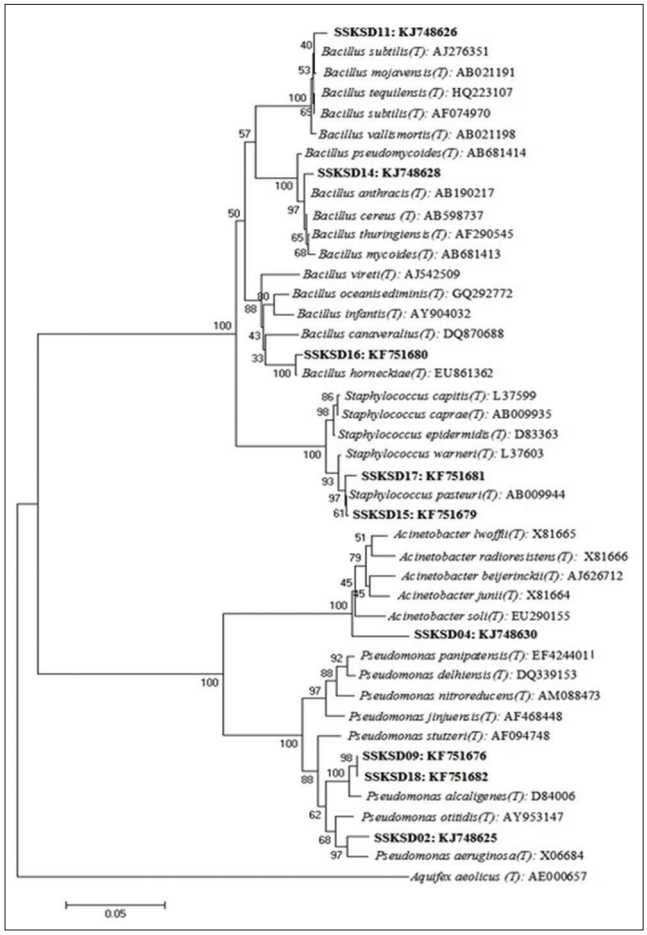


Fig. 3: The phylogenetic tree was constructed by Neighbor-joining method with scale bar indicating Jukes-Cantor distances. Bootstrap values are expressed as a percentage of 1000 replications. *Aquifex aeolicus*^{T} (Accession no: AE000657) was used as an out group

S2. Isolates SSKSD09 and SSKSD18 fell in the same clad of a phylogenetic tree with sequence similarity 99%, respectively, with *Pseudomonas alcaligenes* (GenBank: D84006). Isolate SSKSD02 showed 99% sequence similarity to its type strain *P. aeruginosa* (GenBank: X06684). Isolates SSKSD11 and SSKSD16 share 97% sequence similarity to the type strains *Bacillus subtilis* (GenBank: AJ276351) and *Bacillus horneckiae* (GenBank: EU861362), respectively. SSKSD14 was clustered with the type strain *Bacillus anthracis* (GenBank: AB190217) with 97% sequence similarity exhibiting 11% of total population of S2. Isolates SSKSD15 and SSKSD17 had 99% sequence similarity and fell under the same clade of phylogenetic tree with *Staphylococcus pasteuri* (GenBank: AB009944) contributing to 22% of total population. Strain SSKSD04 was clustered to the type strain *Acinetobacter soli* (GenBank: EU290155) with 94% sequence similarity, representing 11% of total population of S2.

Antibiotic resistance

All the 17 isolates were subjected for their resistance or sensitivity toward different classes of antibiotics. Their percentage resistance to antibiotics was further calculated and tabulated in Table 2.

Maximum isolates showed resistance to all the five classes of antibiotics. Nine isolates showed resistance to all the antibiotics, and two isolates designated SSKSD01 (*Bacillus* sp.) and SSKSD12 (*Lysinibacillus* sp.) showed sensitivity to all the antibiotics. The remaining isolates exhibited resistance to more than one antibiotic. Percentage resistance for isolates to all the antibiotics shown in Table 2 indicates that >60% of isolates shows resistance to antibiotics with a maximum of 82% resistance to Ampicillin. In addition, the cumulative resistance of the isolates toward the antibiotics ranges from 64% to 82%. Cumulative effectiveness of the antibiotics were observed as, ampicillin (82%) > erythromycin (76%) > gentamycin = kanamycin (70%) > tetracycline (64%).

DISCUSSION

Microbial communities are sensitive to environmental changes and are suggested as indicators of drinking water quality [21]. Improperly treated water [22] can lead to viral and bacterial infections [23]. Water color is the reflection of dissolved constituents that may be dyes or pigments or any other dissolved substances. The color of the water was observed as brownish black and blackish blue in sampling sites S1 and S2. This indicates the presence of colored solutes in the river water which may be due to the mixing of industrial effluents located nearby. The pungent smell at both the sites and blackening of the water may be due to the presence of hydrogen sulfide, a common material observed in waste water.

Physicochemical analysis of the river water reveals the water was highly contaminated. The water displayed alkaline pH conditions with higher levels of TSS, COD, and BOD at both the sites. The river water had alkaline pH at both the sites. Textile effluents usually have alkaline pH as an effect of bleaching process [24-26]. Hence, the pH at both the sites may be due to the mixing of textile effluents. Turbidity is the direct indication of water pollution where the water has more suspended

solids that give a turbid condition. TDS is the presence of unfilterable residues/solids present in the water. At both sites S1 and S2, turbidity and TDS values were observed to be higher than the permissible limits indicating the polluted nature of the river water. TSS is the reflection of filterable solids dissolved in the river water. Increase in TSS causes a serious threat to aquatic life, and it also increases the BOD of the water. TSS values higher that of WHO standard values were observed in both the sites. This could be the possible reason for the increase in the level of BOD at both the sites. Altogether, the physicochemical parameters were higher than the permissible limits of WHO. Our results correlate to the previous work done on Bhavani river [27]. Our observation infers that both the sampling sites were highly polluted. Physicochemical values indicate that the water may not be affordable for domestic usage and fishing as it could cause serious infectious diseases. Moreover, data published by Krishna (2012), on the physicochemical study of the river Kaveri explains the degraded quality of the river due to anthropogenic activities [28]. Though, the Tamil Nadu pollution control board urged the industries to refrain from toxic effluents; the problem persists until date.

Our studies by culture dependent method and 16s rDNA gene sequence analysis of bacterial communities disclose the presence of different bacterial communities with new phenotypes. Taxonomic analysis of bacterial isolates from S1 and S2 revealed the presence of two major classes, Bacilli and Gammaproteobacteria. The majority of the isolates belonged to *Bacillus* and *Pseudomonas* genera similar to previous reports observed in various drinking water systems [29,30]. SSKSD04 and SSKSD10 showed <95% sequence similarity and these two species may belong to new genera which needs further confirmation experiments. SSKSD04 was phylogenetically affiliated to its nearest type strain *A. soli,* a bacterium newly isolated from forest soil [31] and causes blood stream infection [32]. SSKSD12 isolated from S1 had 99% sequence homology to *L. fusiformis*. The same species was earlier reported by Gupta *et al*. [33], isolated from heavy metal-contaminated industrial effluent. SSKSD03 and SSKSD07 were clustered to *Paenibacillus* genus. This is in agreement with the work of [34], where the authors isolated *Paenibacillus* species from textile industry wastewater. From the Figs. 2 and 3, it is clear that most of the species belongs to the family Bacilli indicating the optimal environmental factors favoring the growth of these bacterial communities. Evident reports are available for the higher survival of *Bacillus* species in drinking water [35], in lake existing as multi-resistant bacteria [36], causing food borne illness to anthrax [37].

Prevalence of antibiotic-resistant bacteria were found worldwide in river water [38], drinking water [21], wastewater treatment plants [36], pulp paper mill effluents [39,40], and tannery effluents [41]. The isolates were subjected for their antibiotic susceptibility profile to different classes of antibiotics. The drug ampicillin is a class of aminopenicillin antibiotic which inhibits cell wall synthesis. Drugs, such as tetracycline, kanamycin, and gentamycin, are the inhibitors of protein synthesis with specific mechanism to 30S ribosomal subunit, and the drug erythromycin is specific to 50s ribosomal subunit of 17 isolates, nine isolates exhibited strong antibiotic resistance to all five antibiotics clearly exhibiting MDR, as shown in Table 2. Six isolates showed resistance to more than two antibiotics. Two isolates, SSKSD01 and SSKSD16 were sensitive to all the classes of antibiotics. Previous reports on the antibiotic-resistant bacteria from Bhavani river [42,43] further confirms our observations. Out of nine isolates, four species were *Pseudomonas* genera with sequence similarity to the nearest type strains *P. alcaligenes* and *P. aeruginosa*. Previous reports exist on *P. alcaligenes* causing endocarditis and *P. aeruginosa* causing osteomyelitis, pneumonia, urinary tract infections, gastrointestinal infections, and meningitis [44] and the risk level of infection by these organisms becomes higher when it turn out to be antibiotic resistance [45]. The existence of multi-drug resistant bacteria was previously reported in the river Bhavani [42,43]. We also identified that S2 showed wild MDR bacterial population compared to S1. This clearly indicates the increased risk of people residing at S2 toward multi-drug resistant bacterial infections.

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