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Metal tolerant and antibiotic resistant bacteria from the rhizosphere of water hyacinth: a study from a wetland receiving non point source of contamination

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Abstract

Heavy metals and antibiotics are considered as the emerging environmental pollutants causing both long-term and short-term modifications in the natural microbial communities due to their toxicity. The present study focus on the metal tolerance and antibiotic resistance pattern of bacteria isolated from the rhizosphere of water hyacinth growing in a tropical wetland ecosystem. The water quality analysis, profiling of bacteria from the water hyacinth rhizosphere, bacterial load of water and sediment, and metal tolerance of rhizosphere bacteria against Cu, Zn, Pb and Cd were tested. The antibiotic resistance experiments were also carried out on the rhizosphere bacteria samples with 13 different antibiotics. Results showed that microbial load in rhizosphere were $67x10^3$ CFU/ml and the same of associated water and sediment system were $2.21x10^2$ CFU/ml and $2.56x10^3$ CFU/ml respectively. Five different bacterial genera were identified from the rhizosphere of water hyacinth which belongs to Chromobacterium, Bacillus, Listeria, Pseudomonas and Vibrio. The pattern of heavy metal tolerance observed by isolated strains as follows: Chromobacterium-Pb>Zn≥Cu>Cd, Vibrio - Pb>Zn>Cu>Cd,b Bacillus - Pb>Cu>Zn>Cd, Listeria - Pb>Cu>Zn>Cd, and Pseudomonas - Pb>Zn>Cu>Cd. The antibiotic resistance pattern showed that all the bacterial species were sensitive towards Chloramphenicol, Amikacin, Gentamicin and resistant towards Penicillin and Ampicillin. The percentage resistance of antibiotics showed that Penicillin and Ampicillin have maximum resistance (100%) and Nalidixic acid (20%) showed the minimum. The most frequent resistant pattern observed was P Nv As Va L T (Penicillin-Novobiocin- Ampicillin – Vancomycin- Lincomycin- Tetracycline) which was exhibited by Bacillus and Listeria. All the isolates have a Multiple Antibiotic Resistance (MAR) index value higher than 0.2 confirms the high-risk source of contamination. Pseudomonas and Vibrio were identified as pathogenic strains. The study highlight the occurrence of metal and antibiotic resistant strains in wetlands because of the unregulated use of antibiotics in farming and agriculture which leads to the dominance of newly emerging resistant bacteria.

Keywords: Multiple antibiotic resistance, rhizosphere, metal tolerance, water hyacinth, Kuttanad.

Introduction

Wetlands have the ability to store, adapt and convert the contaminants lost from the land, hence they are known as the 'kidneys of the landscape'. Tropical wetlands are productive ecosystems on earth, harbouring aquatic and terrestrial communities with high biodiversity[1]. Tropical wetlands protect inland areas from erosion, floods, tsunami and safeguard marine ecosystems from terrestrial sedimentation and pollutants[2]. The primary pollutants causing the deterioration of wetland are fertilizers, human sewage, animal wastes, pesticides, heavy metals which originate from many sources including runoff from urban, agricultural, silvicultural and mining areas[3,4]. Among these pollutants, heavy metals are the most dangerous contaminants in aquatic environment. Aquatic organisms are vulnerable to the effects of toxic heavy metal contamination because they are in close association with the soluble metals[5,6].

Any metallic element that has an atomic density greater than 4 g/cm^3 and is toxic even at negligible concentration is referred as 'heavy metals'[7]. Pollution of aquatic system with heavy metals is one of the major threats to the environment as they are toxic, persistent, non degradable and it directly affects flora, fauna and human health[8,9]. Metals have a major role in metabolic processes, development and growth of microorganisms such as bacteria. However, metal concentration beyond tolerance levels forced the microorganisms to acquire various biological mechanisms in order to survive those conditions[10]. Microorganisms that are tolerant to heavy metals can grow in higher metal concentrations and have great potential in bioremediation[11].

Microbial tolerance to heavy metal is ecologically critical especially if they are antibiotic resistant. Antibiotics are bioactive substances which are used for treatment of disease and growth promotion in human and animal[12]. Antibiotics are one of the emerging environmental contaminants, causing both long term and short term modifications in natural microbial communities. However, they can encounter the microbial population, they are considered as an important pollutants as well. Residues from human environment and agro farms contain antibiotics and antibiotic resistance genes that can contaminate the natural environment through surface run off. The main consequence of antibiotic release in natural environment is the selection and emergence of resistant bacteria[13]. Bacteria have a significant genetic ability to survive in the environmental pressures including the occurrence of antibiotics that may threaten their existence. Antibiotic resistance is a genetic strategy of bacteria to prevent the antibiotic attack and the antibiotic resistant genes can be present inside the bacteria or outside the bacterial cell[14,15]. Studies prove that there is a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the probability that the resistance genes to both (antibiotics and heavy metals) are situated closely together on the same plasmid in bacteria[16,17]. Due to the occurrence of pathogenic bacteria with antibiotic resistance, infectious diseases turn out to be more challenging and more costly to cure. Hence, it is necessary to cautious of the misuse of antibiotics in the society and the disposal of heavy metals into the surroundings[18].

Aquatic weeds are the plants that propagate and complete their life cycle in water and cause damage to aquatic environment. But many aquatic plants are desirable since they play a beneficial role in remediating agricultural, domestic and industrial pollution[19]. Water hyacinth, *Eichhornia crassipes* (Mart.) Solms.-Laubach, is a perennial floating weed considered as one of the world's 100 worst weed invading lakes, ponds, canals, rivers etc. which can freely floated on the surface of water or anchored in mud[20,21]. These nuisant weeds can interfere the water transport, water flow, cause mechanical damage to hydroelectric systems and serves as an agent for the spread of serious diseases in tropical countries[22]. Water hyacinth has a strong capacity to accumulate nutrients, heavy metals and organic pollutants and is an excellent candidate for the water pollution control[23].

In phytoremediation technique, the root zone has a special attention. The pollutants are absorbed by the root, then stored and break down by the plant or the degradation of pollutants by enzymes released from their roots[24]. The term rhizosphere is defined as ''the zone near to roots" have high bacterial activity and is considered as the hot spot for diverse microbial colonization[25]. There are several research has been conducted on the rhizosphere of terrestrial plants, while comparatively little has been done on the rhizosphere of aquatic plants. Studies prove that the bacteria inhabiting in the rhizosphere of water hyacinth has distinct role in the metal accumulation[26-29].

The floating mats of water hyacinth acts as a medium for the attachment of pathogenic bacteria[30]. The rhizosphere microbes of few wild and cultivated plant species have been reported as opportunistic human pathogens that cause skin, wound and urinary tract infections[31-34]. Factors such as protection from UV radiation and high nutritional content influences the prevalence of human pathogenic bacteria in the rhizosphere[33,35].

Kuttanad wetland ecosystem, part of Vembanad-Kol Ramsar site, supports more than 1.6 million people of three districts in the state of Kerala. Five river systems drains to the kuttanad and local people mainly built their households over the bund or at the raised land between the paddy fields and canals/river channels. As a livelihood source, local people are rearing duck, chicken, fish, cattle, goat etc. and the waste materials including excreta from these farms/ households reaches into the nearby water bodies without any treatment. Monsoon flood also brings the upland waste into this wetland system at a large extent. The chocking of water bodies with water hyacinth and other aquatic weeds also poses serious ecological threat to Kuttanad wetland ecosystem.

Hence the present study envisages to highlight the occurence of metal tolerant and multiple antibiotic resistant bacteria isolated from the rhizosphere of water hyacinth which is growing in a wetland with high human interference.

Materials and methods

Study area: Kuttanad has a peculiar agricultural system where farming is carried out below sea level and is known as the '*rice bowl of Kerala*'[36]. The total geographical area of Kuttanad is 1100 sq.km. The remnants of agrochemicals including the residues of fertilizers and pesticides are accumulating in the water, sediment and later to the different biotic components. Kuttanad has good diversity of macrophytes and water hyacinth, the dominant weed causing both ecological and economical threat to the wetland ecosystem. According to the study of Sylas[37] water hyacinth is one of the abundant and highly gregarious aquatic macrophyte of Kuttanad wetland ecosystem growing in rivers, channels, canals, paddy fields and other water logged areas.

Since Kuttanad having a vast geographical area, the present study conducted in a representative geographical region, Kainday, a village situated in the central part of Kuttanad (Figure-1). The entire study was conducted during February to September, 2014 covering two seasons viz., pre-monsoon and monsoon.

Rhizosphere sample collection: Rhizosphere samples were collected by washing the root region in to a sterilized polypropylene bottles using 100 ml sterilized water. The samples of rhizosphere were stored at 4ºC until analysis.

Enumeration of rhizosphere, water and sediment associated bacteria: The samples collected for rhizosphere, water and sediment bacteria were first diluted and then analyzed for total heterotrophic plate count by the spread plate method using Nutrient agar. 200µl of the diluted water samples were aseptically spread in Nutrient agar plates and incubated at 37^0C for 24-48 hours. After incubation, plates with 25-250 colonies were chosen for counting using colony counter and the Total Plate Count (TPC) of bacteria were expressed as number of colony forming units (CFU) /ml.

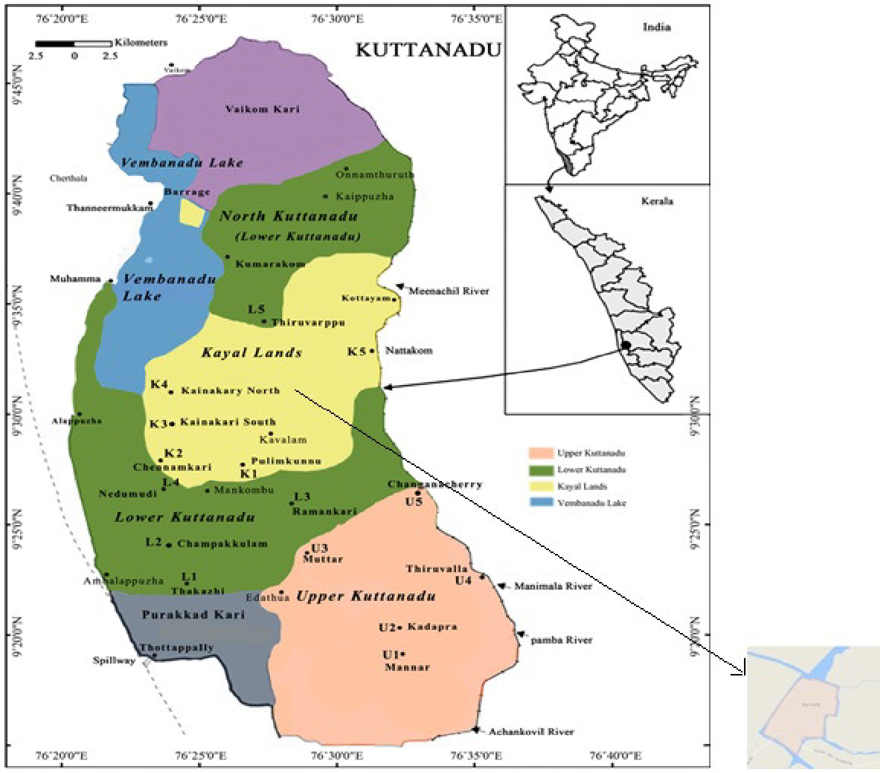

Figure-1: Study area and map.

Isolation and identification of rhizosphere associated bacteria: After enumerating the total bacterial load of rhizosphere associated bacteria, morphologically different colonies were picked up and aseptically streaked on to the surface of sterile nutrient agar slants for identification. The isolates were identified up to genera using the identification scheme provided in Bergey's manual of determinative bacteriology based on various staining and biochemical tests such as Gram-staining, Spore staining, Motility, Kovacs Oxidase, Catalase, IMViC, H_2S production, Lactose fermentation and Sucrose fermentation tests[38].

Heavy metal tolerance analysis: Agar dilution method were used to study the metal tolerance of isolated bacteria[39]. The heavy metals used are Lead (Pb), Cadmium (Cd), Copper (Cu) and Zinc (Zn). Young culture of isolated bacteria were aseptically streaked on the nutrient agar plates and different concentrations of the selected heavy metals were added individually into it (0.1µg/mL to 100 µg/mL). The plates were incubated at 35°C for 24 hours and surface colonies were counted. The lowest concentration of heavy metals which prevents visible growth of a bacterium was considered as the Minimal Inhibitory Concentration (MIC). Selected metal solutions were added to the medium after sterilization and cooling to 45-50°C from filter-sterilized stock solutions. Lead nitrate ($Pb(NO_3)_2$), Zinc sulphate ($ZnSO_4.6H_2O$), Copper sulphate ($CuSO_4.5H_2O$) and Cadmium nitrate ($Cd (NO_3)_2 4H_2O$) were the metal salts used for the study.

Antibiotic resistance testing: The antibiotic resistance were tested according to the method of Bauer et al. (1966). The young cultures of isolated bacteria were swabbed uniformly on Mueller-Hinton agar plates and antibiotic discs (Hi-media) were put on the agar surface. These plates were incubated at 35°C for 24 h in an incubator. The inhibition zones around the antibiotic disc were measured in millimetre and average values were taken. Lack of bacterial growth around the antibiotic-impregnated disc were considered as inhibition zone. Antibiotic resistance was determined by comparing the diameter of clear zone around each antibiotic disc with zone size interpretive chart (Table-1) of Hi-media laboratories, Bombay.

Multiple Antibiotic Resistance (MAR) indexing of the isolates: The MAR index provide the information about the source of contamination and is calculated as the ratio of number of antibiotics to which organism is resistant to total number of antibiotics to which organism is exposed. If an isolate having MAR index value more than 0.2 is considered to have the contamination from human, commercial poultry farms, swine and dairy cattle where antibiotics are regularly used[41].

Beta hemolysis: Beta hemolysis is considered to be the indication of bacterial pathogenicity. It represents the complete disruption of hemoglobin in the red blood cells of a bacterial colony. The isolated bacteria were streaked over the surface of blood agar plates which was supplemented with 5% of sheep blood[42]. The total lysis of the red blood cells were shown by a colourless zone or lightened yellow surrounding the colonies.

Results and discussion

Heterotrophic plate count of rhizosphere, water and sediment associated bacteria: The viable bacteria from the rhizosphere of water hyacinth, water and sediment samples were enumerated. The microbial load in rhizosphere was $67x10^3$CFU/ml and the same in water and sediment system was $2.21x10^2$CFU/ml and $2.56x10^3$CFU/ml (Figure-2). Pratap[34] reported the heterotrophic plate count of root zone bacteria of water hyacinth in which the observed bacterial load were ranged from $61x10^4$ CFU/ml to $77x10^4$ CFU/ml. Similar reports were made by Raisa et al.[43] and they reported the bacterial load in the rhizosphere of water hyacinth were ranged from log 5.3 CFU/ml to log 7.6 CFU/ml and the associated water system was ranged from log 4.3 CFU/ml to log 4.9 CFU/ml. Microbial load in rhizosphere was higher than that of growing water body and sediment in the study area and similar results were reported by Zhan et al.[44] and Raisa et al.[43]. The heavy bacterial count in the rhizosphere region was a clear indication of chemotaxis of microbes towards rhizosphere region of water hyacinth. This was attributed to the presence of different amino acids found in the root exudates secreted by water hyacinth[34].

Table-1: Zone interpretative chart

Antimicrobial agent	Symbol	Disc content	Resistant (mm or less)	Intermediate	Sensitive
Amikacin	AK	30 mcg	14	15-16	17
Ampicillin	AMP	10 mcg	11	12-14	15
Chloramphenicol	C	30 mcg	12	13-17	18
Gentamicin	GEN	10 mcg	12	13-14	15
Kanamycin	K	30 mcg	13	14-17	18
Lincomycin	L	2 mcg	9	10-14	15
Nalidixic acid	NA	30 mcg	13	14-18	19
Novobiocin	NV	30 mcg	17	18-21	22
Penicillin G	P	1 mcg	14	15-18	19
Streptomycin	S	10 mcg	11	12-14	15
Tetracycline	TE	30 mcg	14	15-18	19
Vancomycin	VA	30 mcg	14	15-16	17

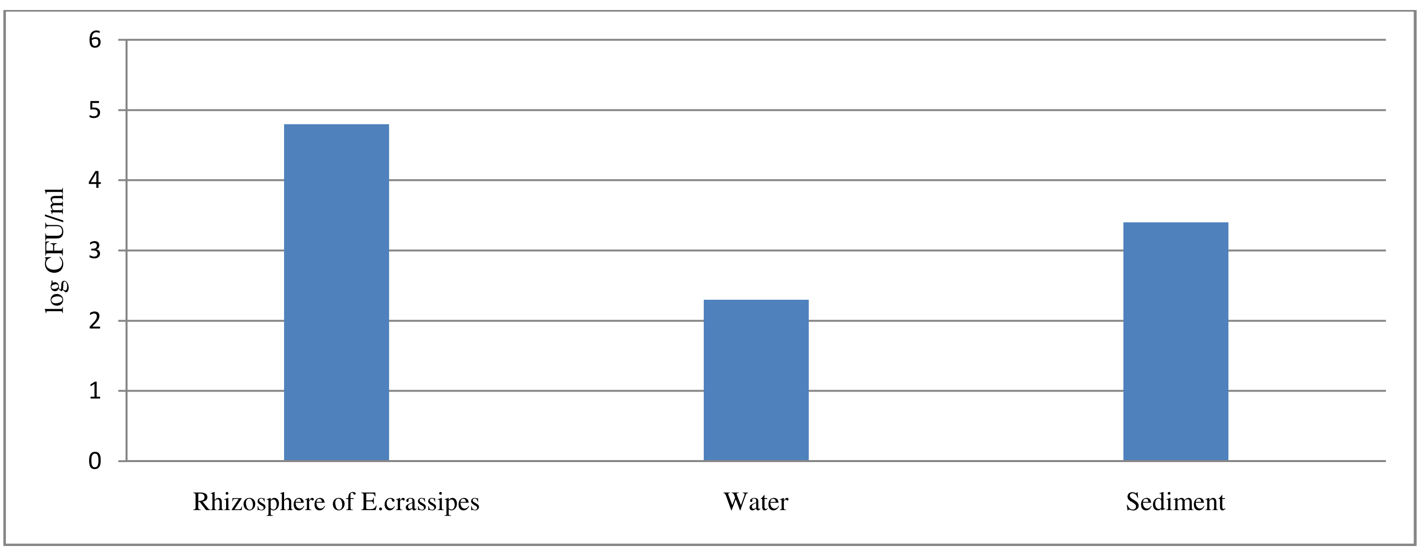

Figure-2: Heterotrophic bacterial load of rhizosphere associated bacteria, water and sediment of water hyacinth.

Characterization of rhizosphere associated bacteria: In the present study, a total of five different bacterial genera were identified from the rhizosphere of water hyacinth which belongs to *Chromobacterium, Bacillus, Listeria, Pseudomonas,* and *Vibrio*. Zhan et al.[44] and Raisa et al.[43] reported most of these genera from the root zone of water hyacinth. Similar studies were done by Abou-Shanab[27] in which total of 85 chromate-resistant bacteria were isolated from the rhizosphere of water hyacinth grown in Mariout Lake, Egypt which belongs to *Pseudomonas diminuta, Brevundimonas diminuta, Nitrobacteria irancium, Ochrobactrum anthropi* and *Bacillus cereus*. Rhizosphere bacterial population can improve the biomass production and can enhance heavy metal tolerance of plants in polluted environments[45,46].

Metal tolerance of isolates from the rhizosphere of Water hyacinth: The results of the heavy metal tolerance experiment showed that the all the isolates have comparatively less tolerance to Cd than other tested heavy metals, which showed Cd toxicity even at low concentration (0.5µg/ml) (Table-2). *Chromobacterium* and *Listeria* could survive only up to 5 µg/ml of Zn but *Vibrio* and *Pseudomonas* could survive up to 50 µg/ml of Zn (Table-2). All the isolates could survive up to 100 µg/ml of Pb and showed 100% mortality in further concentrations (Table-2). Maximum resistance to Cu (50 µg/ml) exhibited by the isolate *Bacillus* (Table-2). The pattern of heavy metal resistance by isolates as follows: *Chromobacterium* - Pb>Zn≥Cu> Cd, *Vibrio*-Pb>Zn>Cu>Cd, *Bacillus* -Pb>Cu>Zn> Cd, *Listeria* - Pb> Cu>Zn>Cd and *Pseudomonas* - Pb>Zn>Cu> Cd. Bacterial species like *Listeria, Kurthia, Acinetobacter, Alcaligenes* and *Bacillus* isolated from the rhizosphere of *E. crassipes* showed high metal resistance i.e. 100-500 µg/ml, which was reported by both Kabeer et al.[43] and Raisa et al.[47].

So et al.[26] isolated bacterial strains which is resistant to Cu and Zn from the water hyacinth rhizosphere by enrichment with the respective metals at 25 mg /l for 6 days. This study revealed that the inoculation of a metal resistant rhizospheric bacterium into water hyacinth increase the removal capacity of plant roots. A spherical anti-copper strain, namely ACU was isolated from the rhizosphere of water hyacinth by Zhang et al.[28]. The study was proved with substantial copper-removing capability of ACU even at copper concentrations as high as 69 mg/l. When compared the present study with these previous findings, the isolated bacterial strains from the water hyacinth rhizosphere have moderate tolerance towards the heavy metals like Zn, Cd and Cu. The results showed that these bacteria can survive in elevated heavy metal concentrations, might be due to the differences in their cell wall structure or variations in their resistance mechanisms[48].

Antibiotic resistance of bacteria isolated from rhizosphere of water hyacinth: The results of the antibiotic resistance exhibited that all the isolates were sensitive towards Chloramphenicol, Amikacin, Gentamicin and resistant towards Penicillin and Ampicillin (Table-3). All these antibiotics are generally used in agriculture, poultry and health sector. The presence of multidrug resistance pattern in bacteria will resulted in a condition where there are no antibiotics available for the effective treatment against the pathogenic bacteria[49].

Table–2: Metal tolerance of rhizosphere bacteria from Water hyacinth.

Bacterial Isolates	Heavy metal concentration (MIC- µg/ml)			
	Cu	Zn	Pb	Cd
Chromobacterium	05	05	100	0.5
Vibrio	30	50	100	0.5
Bacillus	50	20	100	0.5
Listeria	20	05	100	0.5
Pseudomonas	05	50	100	0.5

The percentage resistance of antibiotics showed that Penicillin and Ampicillin have maximum resistance (100%) followed by Tetracycline (80%),Vancomycin(80%), Novobiocin(60%), Lincomycin(60%) and Nalidixic acid(20%) (Figure-3).

Multiple Antibiotic Resistance (MAR) index and resistance pattern of **bacteria isolated from rhizosphere of water hyacinth:** MAR index and resistance pattern showed that four resistance patterns were shown by the isolates from the rhizosphere (Table-4). All isolates acquired Multiple Antibiotic Resistance (MAR) and the most repetitive resistant pattern was P Nv As Va L T (Penicillin-Novobiocin- Ampicillin – Vancomycin - Lincomycin- Tetracycline) which was exhibited by *Bacillus* and *Listeria.* The occurrence of MAR bacteria in rhizosphere may be due to the use of antibiotics in the nearby farming villages, which reached the aquatic environment through land run-off[50-52]. All the isolates have a MAR index value > 0.2 (Table-4) which indicate high-risk source of pollution.

Pathogenicity of isolated bacteria from the rhizosphere of water hyacinth: *Pseudomonas* and *Vibrio* were identified as pathogenic, both exhibited positive results in beta hemolysis test, where as *Chromobacterium, Bacillus* and *Listeria* were identified as non pathogenic strains. Pratap[34] found that the roots of the water hyacinth supports the growth of pathogenic microorganisms such as *E.coli, S.typhi, V.cholera*, *V.parahaemolyticus* and the amino acids like hydroxyl proline, glutamic acid, threonine and leucine were found in the root region of water hyacinth attracts the pathogenic microbes. Abhirosh et al.[53] studied the prevalence of pathogenic bacteria like *V. cholera* and *V. parahaemolyticus* from Kumarakom lake reported that *Vibrios* varied from 32 to 45%. Table-5 illustrates the examples for the occurrence of some potentially human pathogenic species of bacteria in the rhizosphere of different plants.

Table–3: Antibiotic susceptibility of rhizosphere bacteria from Water hyacinth.

Antibiotics Used	Isolates (Zone of inhibition in mm)				
	Chromobacterium	*Vibrio*	*Bacillus*	*Listeria*	*Pseudomonas*
C	S^{25}	S^{22}	S^{20}	S^{21}	S^{20}
NA	S^{20}	R^{12}	S^{20}	S^{22}	S^{20}
P	R^{0}	R^{0}	R^{0}	R^{0}	R^{0}
AK	S^{23}	S^{22}	S^{19}	S^{15}	S^{22}
S	S^{18}	I^{12}	S^{16}	S^{17}	S^{15}
NV	I^{20}	I^{15}	R^{0}	R^{0}	R^{10}
GEN	S^{25}	S^{22}	S^{21}	S^{22}	S^{25}
K	I^{17}	I^{17}	S^{20}	S^{20}	S^{25}
TE	R^{10}	R^{10}	R^{10}	R^{11}	S^{20}
AS	R^{10}	R^{10}	R^{0}	R^{0}	R^{0}
VA	R^{14}	I^{15}	R^{0}	R^{0}	R^{0}
L	S^{24}	R^{0}	R^{0}	R^{0}	S^{17}

S – Sensitive, I – Intermediate, R- Resistant.

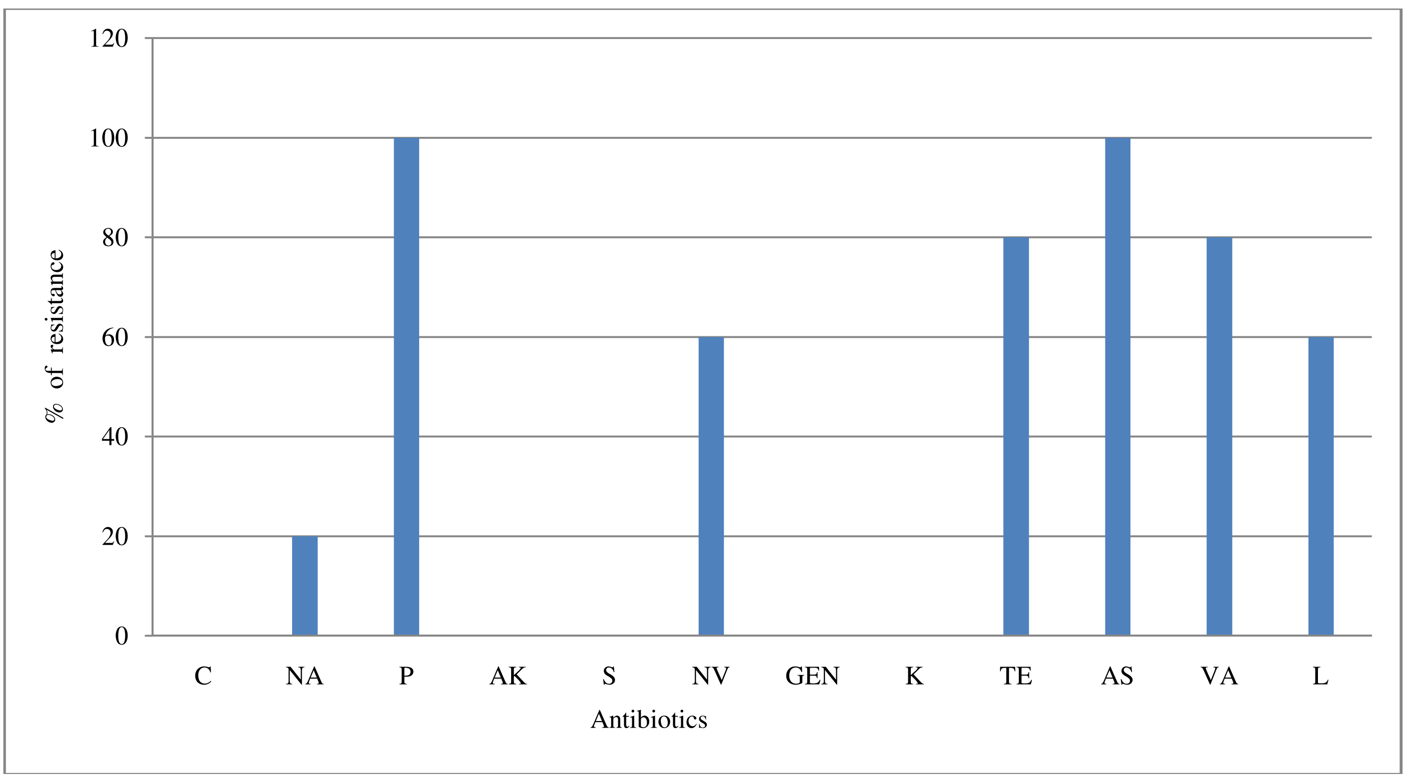

Figure-3: Percentage resistance of bacteria isolated from rhizosphere of water hyacinth.

Tabl–4: Multiple Antibiotic Resistance (MAR) index and resistance pattern of bacteria isolated from rhizosphere of Water hyacinth.

Isolates	MAR Index	Resistance Pattern
Chromobacterium	0.3	P As Va T
Vibrio	0.4	P As L T Na
Bacillus	0.5	P Nv As Va L T
Listeria	0.4	P Nv As Va L T
Pseudomonas	0.3	P Nv As Va

Table–5: Examples for the occurrence of potentially human pathogenic species of bacteria in the rhizosphere of diverse plants.

Identified species	Source	Diseases	Reference
Aeromonasveronii, Alcaligenes xylosoxidans, Enterobacter cloacae, Ochrobactrum anthropi, Pseudomonas aeruginosa, Serratia marcescens	Rhizosphere of rice (*Oryzasativa*)	diarrhoea, septicemia, pneumonia, Urinary tract and respiratory infections, infective endocarditis, osteomyelitis, conjunctivitis, keratitis, endophthalmitis.	Mehnaz et al.[31], Gyaneshwar et al.[54], Tripathi et al.[32]
Escherichia coli, Vibrio cholerae, Vibrio parahaemolyticus, Salmonella typhi	Rhizosphere of water hyacinth (*Eichhornia crassipes*)	Food poisoning, diarrhoea, vomiting, acute gastroenteritis, diarrhoea, meningitis.	Pratap[34]
Burkholderia cepacia, Enterobacter agglomerans, Ochrobactrum anthropi, Ochrobactrum tritici, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Stenotrophomonas maltophilia, Streptococcus pyogenes	Rhizosphere of wheat (*Triticum sativum*)	Pneumonia, cystic fibrosis,septic arthritis, endophthalmitis, periostitis, endocarditis, osteomyelitis, infective endocarditis, blood stream and biliary tract infection, cystic fibrosis, traumatic burns, gastro enteritis, skin infection, food poisoning, pharyngitis, rheumatic fever.	Morales et al.[55], Germida and Siciliano[56]
Burkholderia cepacia, Klebsiella pneumoniae, Serratia liquefaciens, Sphingomonas paucimobilis, Stenotrophomonas maltophilia	Rhizosphere of maize (*Zea mays*)	Pneumonia, nosocomial infection, septic arthritis, osteomyelitis, urinary tract infection, bloodstream infection.	Lambert et al.[57], Dalmastri et al.[58], Chelius and Triplett[59]

The occurrence of antibiotic and heavy metal resistance in bacteria from industry, sewage, mining, agriculture etc. were already reported[60-63]. In bacteria, tolerance to antibiotics and metals occurs concurrently when the genes specifying resistant phenotypes are located together on the same genetic element such as a plasmid, transposon, or integron[64-67]. As the genes of heavy metal and antibiotic resistance are found on the same mobile genetic element, metal pollution can promote the emergence of antibiotic resistances in exposed organisms[68].

Most of the antibiotics reach the water bodies through land runoff[69]. Besides, in fish farming, antibiotics are added directly to the water[70,71]. These situations can cause the emergence of antibiotic resistant bacteria in aquatic environments. Since animals or poultry have often been treated with antibiotics, the waste generated after their butchery contains high levels of antibiotics[72]. In addition, antibiotics added to the aerial part of infected plants reached the aquatic system, even though the amount of antibiotics used in agriculture is low than human, veterinary medicine and animal production[73]. MAR strains may serve as a reservoir for antibiotic resistant genes in the aquatic environment and pose a greater risk by transfer this resistance to other pathogenic bacteria[74]. From Vietnam, the presence of antibiotic resistance and antibiotic resistance genes (ARGs) in *Escherichia coli* isolates from hospital wastewater even after the post-treatment were reported[75]. Similar results were found by Alam et al.[76] in the hospital waste water from Aligarh, Uttar Pradesh and Turolla et al.[77] from a wastewater treatment plant. Hong et al.[78] discussed the problems associated with the usage of reclaimed water which contain antibiotics residue leads to the emergence of antibiotic resistant bacteria that harbour mobile genetic elements. Studies have shown that antibiotics can accumulate in the environment and can persist up to a year[79-81]. MAR bacteria are fit than its non resistant counterpart and are for able to survive under harsh conditions[82]. There are "green" waste water treatment techniques like artificial wetlands which use plants that sometimes also include water hyacinth. Since mats of water hyacinth provide a habitat for the multidrug resistant and pathogenic bacteria, there are possibilities to flourish these bacteria near urban areas and cause danger to human and animals.

Human activities and sewage discharge to the Kuttanad wetland ecosystem may be the reason behind the occurrence of human pathogenic bacteria. The high prevalence of these bacteria in the wetland designates their possible occurrence in fish and other edible products derived from this source[83]. Studies reported that the sun's UV-A, red, and infrared radiations have the ability to inactivate pathogens[84-88]. One of the factors affecting the ability of solar disinfection is the quality of the water and wastewater. Another important factor affecting the natural disinfection is the dense growth of the macrophytes which interfere with the entry of sunlight in to the water.

In the present study area, water hyacinth spread like a mat which cover the water bodies and have a thick, heavily branched, dark, fibrous root system prevents the sunlight in to the water, thereby restrict the solar disinfection and the microbes present in the aquatic ecosystem remain uninterrupted and flourished. Abhirosh[89] studied the detrimental effect of natural sunlight and UV light on the survival of *E. coli*, *S. paratyphi* and *V. parahaemolyticus* in natural waters. The high organic load and frequent recreational activity is likely to reduce the effectiveness of sunlight on the inactivation of microorganisms.

The present study area, Kuttanad receives large amount of treated and untreated wastes through human activities including agriculture and poultry farming and it also acquires waste from the five major river systems which is flowing to it. It is also one the major rice producing zone in Kerala and variety of antibiotics may used to prevent the pathogen attack and these antibiotics reach the water through runoff. Unscientific constructions such as roads, bridges etc across the rivers and canals in Kuttanad, arresting the water flow and have resulted in the creation of large pools of stagnant and contaminated water that act as breeding ground for the multidrug pathogenic bacteria. The uncontrolled use of pesticides in agriculture and the disposal of untreated urban and domestic wastes including animal / poultry husbandry into the Kuttanad wetland might increase the emergence of metal and multiple antibiotic resistant bacteria[90].

The fast growing water hyacinth mats also provide habitat for MAR bacterial strains for further colonization. The presence of pathogenic rhizosphere bacteria of water hyacinth might pose health risk to the people who use the water body for fishing, collection of clam, sand mining and recreation. Constant monitoring and legal measures to check the entry of pollutant is essential to safeguard the people against health risk posed by multidrug resistant pathogenic bacteria.

Conclusion

The occurrence of metal tolerant and multiple antibiotic resistant rhizosphere bacteria in water hyacinth collected from Kuttanad wetland ecosystem shows that the water hyacinth is acting as a anchoring niche for metal resistant and MAR bacteria. The microbial load in rhizosphere were 67×10^3CFU/ml and the same of associated water and sediment system were 2.21×10^2 CFU/ml and 2.56×10^3 CFU/ml. A total of five different genus of bacteria were identified from the rhizosphere of water hyacinth which belongs to *Chromobacterium, Bacillus, Listeria, Pseudomonas,* and *Vibrio*. The pattern of heavy metal resistance by *Chromobacterium* - Pb>Zn≥Cu>Cd, *Vibrio* - Pb>Zn>Cu> Cd, *Bacillus* -Pb>Cu>Zn>Cd, *Listeria* - Pb> Cu>Zn>Cd, and *Pseudomonas* - Pb>Zn>Cu>Cd. The pattern of antibiotic susceptibility showed that all the bacterial isolates were sensitive towards Chloramphenicol, Amikacin, Gentamicin and resistant towards Penicillin and Ampicillin. The percentage resistance of antibiotics showed that Penicillin and Ampicillin have maximum resistance (100%) and Nalidixic acid(20%) showed the minimum. The most frequent resistant pattern observed was P Nv As Va L T (Penicillin-Novobiocin-Ampicillin – Vancomycin- Lincomycin- Tetracycline) which was exhibited by *Bacillus* and *Listeria*. All the isolates have a MAR index value higher than 0.2 which shows the high-risk source of contamination. *Pseudomonas* and *Vibrio* were identified as the pathogenic strains. The rhizosphere of water hyacinth harbour pathogenic MAR bacteria and could cause the public health risks since the Kuttanad wetland is being used by millions of people for their livelihood activities. The wetland also receives untreated waste water from the nearby domestic and agriculture areas, the presence of pathogenic microbes must be monitored. The spread of water hyacinth and MAR bacteria can be control by the proper management of wetlands. So far there have been no studies reported on the metal tolerance and antibiotic resistance of bacteria from the rhizosphere of water hyacinth from Kuttanad wetland ecosystem. However, further studies are necessary to recognize the exact resistance determinants involved in the metal and antibiotic resistance.

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