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RESEARCH ARTICLE

COMPARATIVE ASSESSMENT OF BACTERIAL POPULATION AND PATHOGENIC RISK OF SAGO INDUSTRY WASTE WATER AND DRINKING WATER SOURCE OF ERODE DISTRICT (T.N) INDIA .

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Abstract

A comparative analysis was made to study the physico-chemical and microbial characterization of drinking water and sago effluent samples from various stations along the Cauvery river bank. Among many stations, Pallipalayam was found to be highly polluted. Among the observed bacterial genera(8) *E.coli* and *Pseudomonas* were present in all the samples and both of them contain virulence factors also. Thus the tapioca processing waste water causes damage to the aquatic bodies and most of the sago industries are found to be located on the Cauvery river bank. It poses a serious threat to the environment.

Introduction:-

Sago and starch production from the tapioca root crops is one of the major food industries in Southeast Asia. There are nearly about 1000 sago and starch processing factories in Salem and Namakkal district of Tamil Nadu, India. These sago industries release large amount of wastewater containing organic and inorganic solid wastes. This wastewater commonly referred as effluent which has obnoxious odour, irritating colour, lower pH, higher BOD and COD. It affects the health of soil, natural ecosystem, animals, plants and human beings (Saravanan *et al*., 2001,Ruban *et al* ., 2013).

Evaluations of polluted water for their physicochemical and biological parameters are essential for future pollution abatement programmes (Saradhamani *et al*., 2002 and Mohammad Subhan *et al.*, 2012). For this study, the sampling point (Pallipalayam region) was selected on the basis of its importance and an attempt has been made to check the water quality by physicochemical and microbiological analyses over a year.

Hence in the present study an attempt has been made to study the comparative account of river water and untreated sago effluent by investigating the physico chemical properties and microbial analysis. Up till now, many investigators have carried out studies on the physico chemical and biological characterization of river water and no report on the virulence and antibiotic resistance characterization of river water isolates was found.

Materials and Methods:-

Sample collection is a very important part of river study because conclusions drawn are based only on the testing of collected samples. Water samples from the sampling locations of Pallipalayam, Komarapalayam, Bhavani Kuduthurai, R.N. Pudur and Odapalli from March 2015 to August 2015(6 months) were collected for this study.

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Water samples for microbiological examination, were collected in non-reactive borosilicate glass bottles of 500 ml capacity each that had been cleansed and rinsed carefully, given a final rinse with distilled water and sterilized. After collection the samples were brought to the laboratory and stored in a refrigerator at 4°C till further processing.

The pH of the samples were measured using a digital pH meter (Elico) and the total dissolved solid was determined using a Conductivity meter. Enumeration of bacterial colonies was done using spread plate technique. The final results were expressed as colony forming unit (cfu/ml). Simultaneously, the colony morphology, size and color of the colonies were observed.

The isolated bacterial culture further identified and then characterized by using standard microbiology methods. The morphotypes were done for all the samples based on the Gram staining method to determine the likely organism present. The colonies of Gram-positive cocci in clusters were further confirmed using Indole test (I),Methyl red test(MR),Voges Proskauer test(VP),Citrate utilization test(C),Urease production test (U), Cytochrome oxidase activity, Catalase test,TSI agar test,Carbohydrate fermentation test.

The confirmed selected bacterial isolates were screened for their sensitivity/resistance to eight different antibiotics by Kirby-Bauer disk diffusion method on Mueller Hinton Agar. The antibiotic discs were obtained from Himedia, India. Plates were observed for a clear zone of growth inhibition and results were interpreted using reference table. The bacterial strains which showed resistance to two or more antibiotics (multidrug resistant) were further studied.

Biofilm Production was assayed using brain heart infusion agar supplemented with 5% sucrose and Congo red (0.08 g/l). The isolates were inoculated and incubated aerobically for 24 to 48 hours. This Biofilm production was indicated by black colonies with a dry crystalline consistency whereas biofilm non-producers remain pink, though occasional darkening at the center of the colony was observed.

The β-lactamase production was assayed using the method of Lateef (2004). Broth culture of the test organism was spot inoculated on to starch agar containing penicillin and then incubated overnight at 37°C. The plates were then flooded with freshly prepared phosphate buffered saline containing potassium iodide, iodine. The presence of clear colourless zones around the bacterial growth is an indication of β-lactamase production.

The obtained results have been tabulated and presented.

Results and Discussion:-

The river in the Pallipalayam region which is used for drinking purpose was assessed for its quality through determinations of the physicochemical properties and enumeration of the microbial population of water samples. A descriptive summary of the physicochemical properties of the water samples was presented in Table.1. The temperature profile of the water lay within the range 24.1-25.5˚C. The highest temperature was observed in Pallipalayam sample. Hydroxyl ionic concentration at the sampling site corresponded to a pH 7.2 to 8.1. The maximum pH was observed in Komarapalayam and Odapalli (8.1).

The water sample was slightly alkaline throughout the duration of the study. Total alkalinity of the water was within the range 131-169mg/L, the highest alkalinity was observed in Pallipalayam water samples. The results showed TDS levels within the range 351-480 mg/L during the study period. The maximum TDS was present in Komarapalayam water sample. Chloride content in the present study was within the range 49-62 mg/L. Simultaneously 3 sago effluent water samples also analysed to determine the physicochemical properties. The result was summarized in Table.2. Water temperature during the study period ranged from 25˚C to 27˚C. The varied pH was observed from the 3 samples such as 4.1 to 5.6. Total alkalinity of the water was within the range 101 to 127 mg/L. The highest volume was observed in site II sample. The Chloride content in the present study was within the range 389 to 432mg/L. During the study period TDS levels as being within the range 1231 to 1458mg/L.

The microbiological quality of water is measured by the analysis and enumeration of bacteria. Among the 5 sampling sites of river samples, highest counts (65, 000000 CFU/ml) of bacteria were observed in Pallipalayam. In case of sago industrial effluent site I sample contain 92,000000CFU/ml. The results were tabulated in Table 3 and 4.

All five samples were utilized for the bacterial identification according to morphological and biochemical test. Following eight genera were isolated from river samples; *E.coli, Klebsiella spp, S.aureus, Salmonella spp,*

Pseudomonas spp, Vibrio spp, A.hydrophilia and Enterococcus spp. The highest percentage of isolates was observed in Komarapalayam and Pallipalayam water samples. Among the eight genera, *E.coli* and *Pseudomonas* were present in all places of samples. Following six types of genera were observed from the sago effluent samples; *E.coli, Klebsiella spp, S.aureus, Pseudomonas spp, A.hydrophila and Enterococcus spp.* Among them, *E.coli* was observed in all places of samples.

Antibiotic resistance pattern of river water isolates:-

In this study, 10 types of antibiotics were utilized for the determination of resistance isolates. Among the 10 antibiotics, Novobiocin was resisted by 83.3% of isolates and followed by Ciproflaxcin and Amikacin (79.1%). Totally 64.5% of antibiotic resistant isolates were observed and each isolate showed resistance to at least 4 antibiotics. The results were depicted in Fig.1.

Among the eight genera, *E.coli* (75%) was highly resistance to all antibiotics and followed by *Aeromonas spp* (72.5%), *Klebsiella* and *Pseudomonas* (70%). In this study no one isolate was resistance to all antibiotics and same time no one was sensitive to all antibiotics.(Fig.2).

Antibiotic resistance pattern of sago effluent isolates:-

Among the sago effluent isolates, 60% of antibiotic resistance isolates were observed against 10 antibiotics. Our isolates were highly resistance to Chloramphenicol (76.5%) and followed by Tetracyclin (71%) Ciproflaxcin and Erythromycin (64.2%). None of the isolates was sensitive to all antibiotics (Table 4 and Fig.3). The highest resistance was observed in *Enterococcus spp* (65%) and second most in *E.coli* (63.3%) isolates and followed by *Pseudomonas* and *Klebsiella* (60%). This was shown in Fig.3.

Fig.1:- Resistance patterns of bacterial isolates of River and Sago water samples

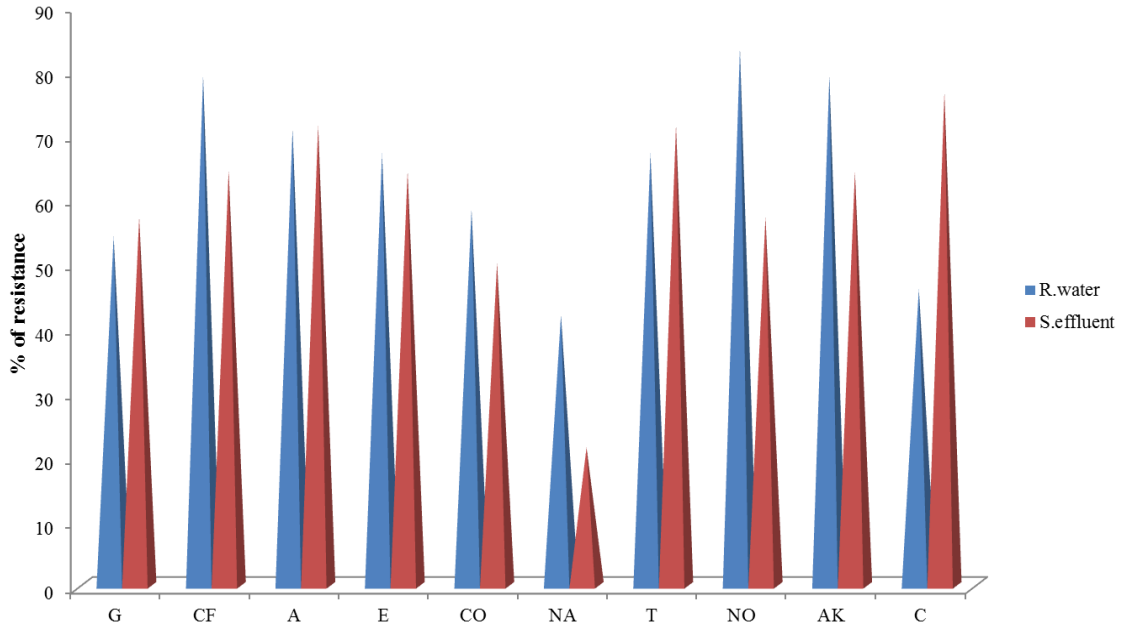


Fig.2:- Percentage of antibiotic resistance of river water isolates.

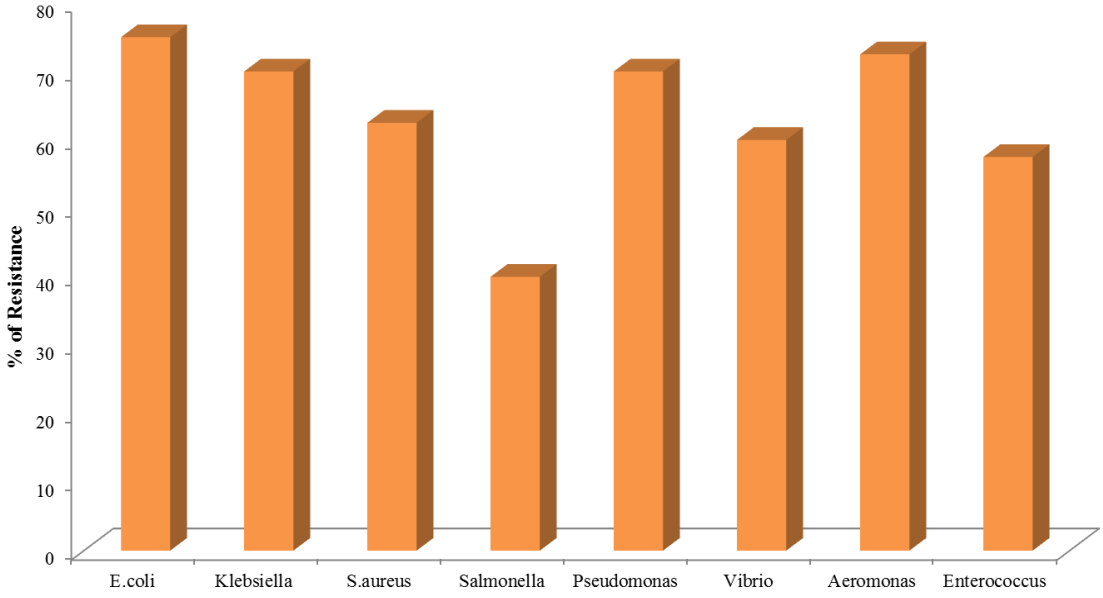


Fig.3:- Percentage of antibiotic resistance of sago effluent isolates.

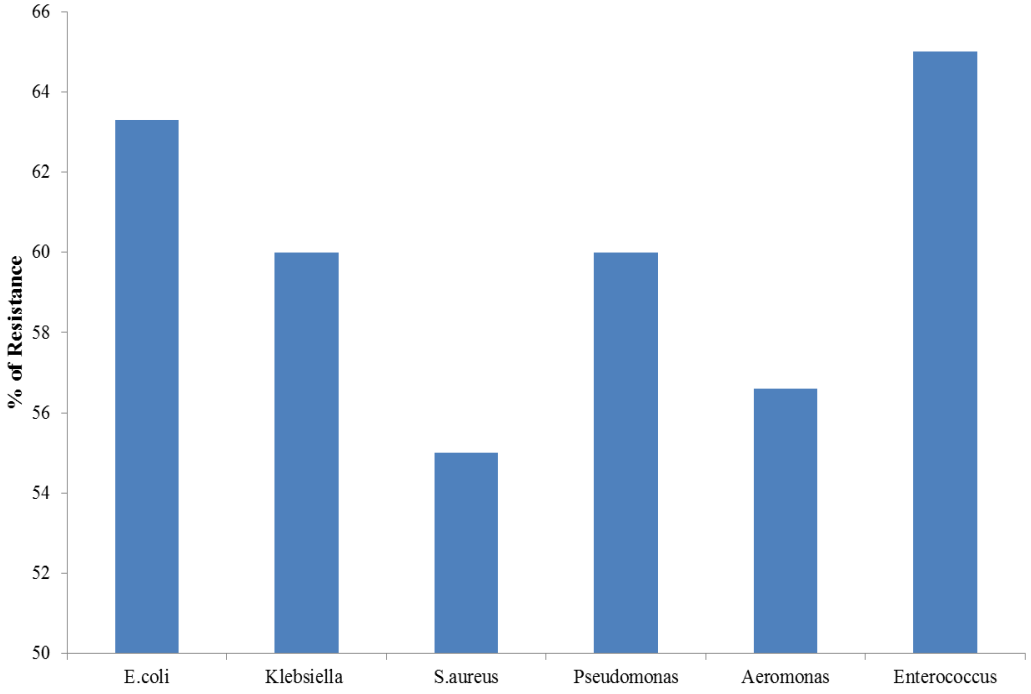


Table 1:- Physicochemical characterization of river water samples.

S. No	Parameters	Samples (n =6)				
		Komarapalayam	Bhavani Kuduthurai	R.N. Pudur	Pallipalayam	Odapalli
1.	Temperature (^{0}c)	24.3	24.9	22.5	25.5	24.1
2.	pH	8.1	7.5	7.2	7.8	8.1
3.	Total dissolved solids (mg/ml)	480	366	459	470	351
4.	Alkalinity (mg/L)	131	156	145	169	154
5.	Chloride (mg/L)	155	149	131	169	162

Table 2:- Physicochemical characterization of Sago effluent

S.No	Parameters	Sago effluent samples (n=6)		
		Station 1	Station 2	Station 3
1.	Temperature^{o}C	27	25	27
2.	pH	5.6	5.5	4.1
3.	Total dissolved solid (mg/mL)	1231	1458	1268
4.	Alkalinity(mg/L)	101	127	119
5.	Chloride(mg/L)	432	389	412

Table 3:- Total Bacterial population of River water samples.

Sample name	Dilution factor	Total colonies	CFU/ml
Komarapalayam	10^{-6}	35	35,000000
Bhavani Kuduthurai	10^{-6}	35	35,000000
R. N. Pudur	10^{-6}	58	58,000000
Pallipalayam	10^{-6}	65	65,000000
Odapalli	10^{-6}	39	39,000000

Table 4:- Total bacterial population of sago water samples.

Sample name	Dilution factor	Total colonies	CFU/ml
Station 1	10^{-6}	92	92,000000
Station 2	10^{-6}	12	12,000000
Station 3	10^{-6}	14	14,000000

Virulence factors of river water isolates:-

The result was identified by black color formation of the colony on the media. Out of the 24 isolates, 16 (66.6%) of them were biofilm producers. Among the 8 types of genera, highest number of biofilm producers as *Aeromonas spp* (100%) and followed by *E.coli* and *Enterococcus spp* (75%).

The clear zone of inhibition was observed around the colonies which were as a positive result of betalactamase. Out of the 24 isolates, 12 (50%) were betalactamase producers. Among the eight types of genera, 100% of producers as *Klebsiella* and *Pseudomonas* and followed by E.coli and Enterococcus (75%). The result was depicted in Fig.4.

Shigella spp *Aeromonas spp*

Fig 4:- Percentage of river water isolates producing virulence factors.

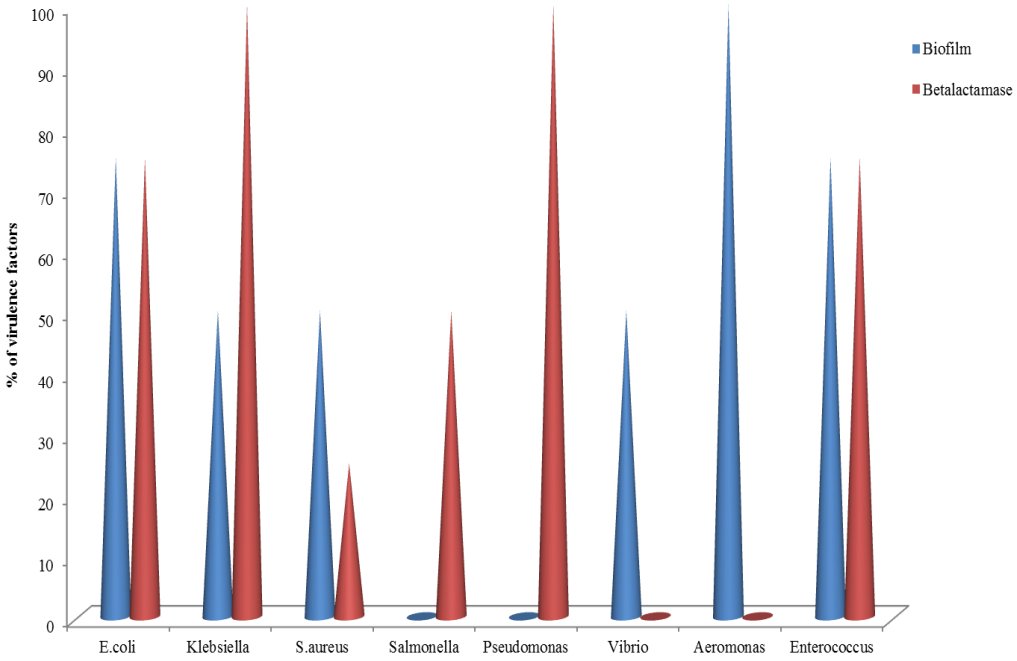
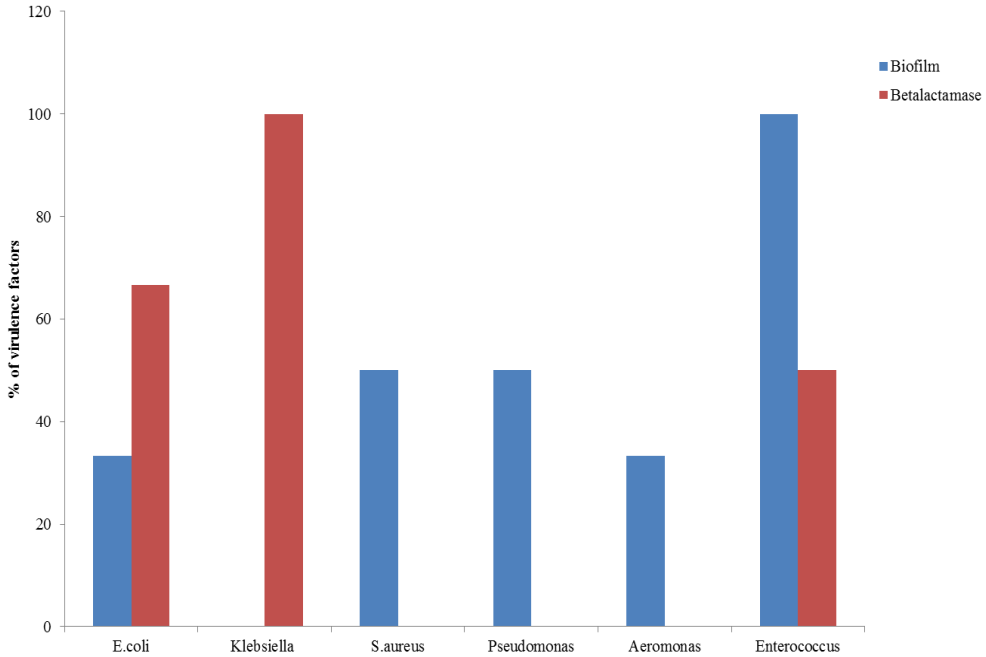


Fig 5:- Percentage of sago effluent isolates producing virulence factors.



Virulence factors of sago effluent isolates:-

Out of the 14 isolates, 11(42.5%) were biofilm producers. In this present studies, all isolates of *Enterococcus* was produce the biofilm and second most *P.aeruginosa* and *S.aureus* (50%). Out of the 14 isolates, 36% of isolates was betalactamase producers. Among the six types of genera, highest production was observed in *Klebsiella* and *E.coli* isolates (100%). *S.aureus*, *Pseudomonas* and *Aeromonas* was not producing the betalactamase production. The result was depicted in Fig.5.

Bacteria are the most commonly used microbial tracers because they grow well in aqueous media and are easily detectable. Water bacteriology in distribution systems have received less importance in developing countries, as much time is spent on supplying water in quantity and microbiological water quality is not given importance in quality assessment as much as physical and chemical parameters.

The river in the Pallipalayam region was assessed for its quality through determinations of the physicochemical properties and enumeration of the microbial population of water samples from the riverbank areas.

The recognized bacterial indicators most commonly used for assessing water quality are bacteria of the *Enterobacteriaceae* family defined as the total coliform bacteria and the fecal coliform bacteria. Biofilm causing isolates were very difficult to treat because they were highly resistant to antibiotics. It's a serious global threat and challenge to health care professionals (Saravana *et al.,* 2011). During the production of biofilm, isolates expressed several virulence factors and an increased resistance against phagocytosis and other host defense mechanisms (Costerton *et al.,* 1999).

In this present study, 66.6% and 42.5% of biofilm producers were observed from river water and sago water samples respectively. The previous studies of Thenmozhi *et al.,* 2014 observed the biofilm producing isolates of *Aeromonas spp* from Cauvery river water samples. For our knowledge no report was observed on the biofilm producing isolates from Cauvery River water in Erode area. This biofilm formation was high in river water samples compared than sago water which containing chemicals and toxic materials suppressed the biofilm formation.

Beta-lactamases that inactivate all the penicillins and cephalosporins including the extended spectrum cephalosporins are termed Extended Spectrum Beta-Lactamases, (ESBLs). In this present study, 50% and 36% of betalactamase producing isolates were observed from river water and sago water samples respectively. In our knowledge this is the first report from our area.

From the above it can be concluded that tapioca processing wastewater causes heavy damage to the receiving area due to the high pollution strength. Most of the starch companies are located near the banks of rivers or lakes and it has become customary to discharge the wastewater from tapioca factories into these bodies of water. It poses a very serious threat to the environment and quality of life in rural areas.

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