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Identification of Toxic Shock Syndrome Toxin-1 (TSST-1) gene in *Staphylococcus aureus* isolated from potable water, Namakkal

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Abstract
Concern over exposure to drinking water contaminants and the resultant adverse effect on human health has prompted several studies evaluating the quality of potable water sources. The present study is aimed at assessing the quality of potable water sources in and around Namakkal area and identified the TSST-1 gene producing *Staphylococcus aureus*. A totally 33 water samples comprising of 6 River water, 7 Pond water, 10 Municipality water and 10 Well water samples were collected aseptically. The physiochemical and the microbiological studies are most important regions by which we were able to test the potability of water. The collected samples were processed for bacterial isolation using the Mannitol Salt Agar (MSA agar) (Hi-media, Mumbai).The suspected pure colonies of isolates were identified and characterized using standard biochemical tests. In AST test, totally 18 isolates were subjected in that most of the *S.aureus* showed sensitive to Kanamycin, Co-trimazaxole and Amphicillin. In MAR index, MSa 14,WSa15 showed 83.3% of resistance to all drugs and 66.6% to RSa1,WSa18.Based on the virulence characters 18(100%) isolates of *S.aureus* produced serum resistance, 8(44.44%) for coagulase positive and 6(33.33%) for haemolysis. In PCR assay, only two water sources such as River (RSa3) and Pond (PSa9) isolates of *S.aureus* expressed the virulence gene of TSST-1. From this study it was concluded that the water is commonly contaminated with pathogenic *S.aureus* and this contamination may be played a role in the transmission of potentially harmful infection to human beings.

Keywords: Water sources, Water borne diseases, *S.aureus*, Antibiotic resistance, Virulence, TSST-1 gene

Introduction
Water is a basic element to life and health; over 1 billion people worldwide have no access to safe drinking water and insurance of its quality and have been a very important issue from the beginning. Water is a good that must serve for the development of the whole person and of every living thing. Water may be contaminated in many ways. The different forms of contamination come from different sources and are dealt with in different ways. The water contamination mainly occurred in three forms such as physical, biological and chemical. Water has played a significant role in the transmission of human diseases and the indicator organisms have been used to suggest the presence of pathogens [1]. Water-borne diseases constitute one of the major public health hazards in developing countries. Worldwide, in 1995 contaminated water and food caused more than three million deaths; of which more than eighty percentages were among children under age five. Besides the conventional pathogens, which are transmitted by water, several emerging water-borne pathogens have become increasingly important during the last decade. In India, more than seventy percentage of the epidemic emergencies are either water-borne or water related materials. A substantial amount of work has been carried out on common water-borne pathogens in India [2].

A common hazard of household water is contamination by potentially harmful bacteria and other microorganisms. Short term gastrointestinal disorders and illnesses such as gastro-enteritis, giardiasis, typhoid, dysentery, and cholera; have been linked to water contaminated by microorganisms such as, *Staphylococcus aureus, Campylobacter jejuni, Escherichia coli, Salmonella spp., Vibrio cholerae, Yersinia enterocolitica, Shigella species* etc. Such bacteria have also been isolated from even chlorinated water [3]. Most of the water borne organisms cause diarrhea disease; it is an important cause of morbidity and mortality in developing countries, particularly in infants and elders. Bacterial infections are responsible for twenty to forty percent of diarrhea illness, and several bacterial species have been frequently ascribed to diarrhea [4].

Staphylococcus, a genus of Gram-positive bacteria derived its name from Greek ʻstaphyleʼ meaning ʻbunch of grapesʼ and ʻkokkosʼ meaning ʻgranuleʼ. When viewed under microscope the organisms exhibit grape-like appearance. *Staphylococcus aureus* is an aerobic or anaerobic, non-motile, non-spore-forming, catalase and coagulase-positive, Gram-positive coccus, usually arranged in grape like irregular clusters. The catalase test used to identify *S.aureus* also differentiates enterococci and streptococci. When exposed, *S.aureus* is converts hydrogen peroxide (H_2O_2) to water and oxygen resulting in a positive catalase test. A small percentage of *S.aureus* can be differentiated from most other *Staphylococci* by the coagulase test. *S.aureus* produces the enzyme "coagulase" that forms clot formation differentiating with most other *Staphylococcus* species that are coagulase-negative. Most *Staphylococcus aureus* strains isolated from patients with toxic shock syndrome (TSS), a severe acute illness that rapidly leads to multi-organ system failure, produce a toxin known as toxic

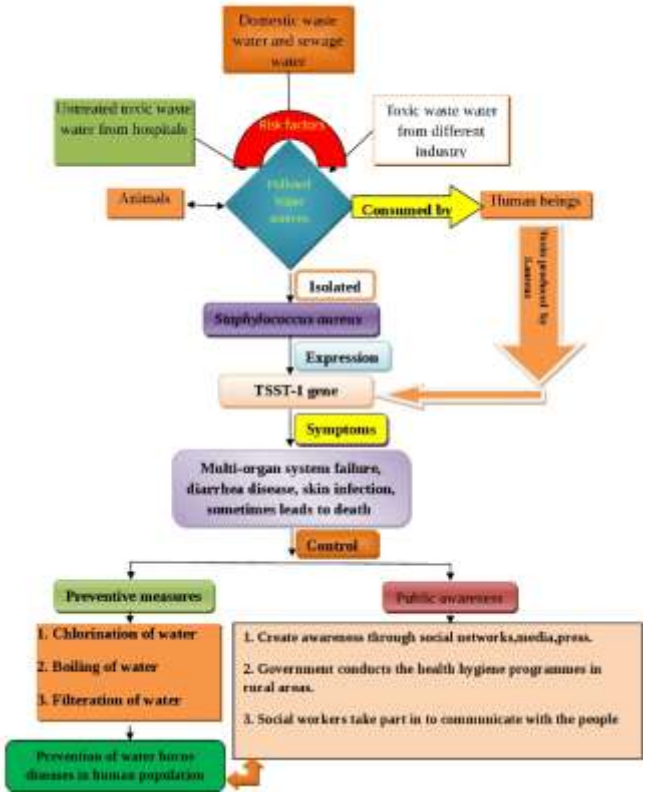
shock syndrome toxin-1 (TSST-1). Higher temperature offers an ideal environment for the multiplication of *S. aureus* and the release of toxins. The consumption of water containing *S. aureus* toxins can lead to enterotoxin poisoning within a few hours [5].

Staphylococcus aureus produce a remarkable range of secreted virulence factors that facilitate their pathogenicity, such as toxic shock syndrome toxin-1 (TSST-1). TSST-1 is known as a super antigen for its ability to non-specifically stimulate activation of T lymphocytes. TSST-1 is encoded by *tstH*, which is located on the bacterial chromosome within a 15.2-kb mobile genetic element; it has been associated with several acute or chronic human diseases, including toxic shock syndrome (TSS). TSS is an acute and potentially fatal illness characterized by high fever, diffuse erythematous rash, desquamation of the skin 1 to 2 weeks after the onset (if not fatal before this time), hypotension, and involvement of three or more organ systems [6]. Hemolytic test otherwise known as Blood Agar culturing method is used for identification of forms of hemolysis from pathogenic microorganisms. Generally pathogenic microbes secrete an enzyme known as "Hemolysin", an exotoxin by nature and disrupt membrane of the host likely erythrocytes. The mechanism of action of hemolysin is that it disrupts RBC"s and increases the content of free iron and is also involved in dermonecrosis and vasoconstriction. The coagulase-positive *Staphylococcus aureus* is a major cause of various community and hospital acquired infections. Antibiotic resistant *staphylococci* are major public health concern since the bacteria can be easily circulated in the environment. In drinking water, *staphylococci* may be regarded as one of the genera that are commonly found in water supplies Heterotrophic Plate Count (HPC) bacteria, multiple antibiotic-resistant *Staphylococcus aureus* were also recovered from food, potable water and wastewater [7].

Although it was suggested that it is not possible to establish health-based standards for the presence of HPC bacteria in drinking water, the presence of large abundance of *Staphylococcus aureus* in water intended for human consumption, may represent potential health hazards, especially if these strains possess determinants of antibiotic resistance and enterotoxin production [8]. The *S.aureus* isolated from drinking water was the most virulent and resistant to multi-antibiotics [9]. Water is one of the most important requirements for survival of life on the earth. Now a days, the demand of the water increase due to the increase of human population in cities. The main problem how before the world is that of safe drinking water, which is fast assuming alarming properties problem related to storage, misuse and pollution of water are wide spread both in the rural areas. The problem is becoming increasingly complex with growing population, industrialization urbanization [10].

The world health organization informs that every year more than 3.4 million people die as a result of water related diseases around the world. The ingestion of contaminated water is an important mode of pathogen transmission. Even if disinfection is practiced in water supply systems, due to the failure of disinfection system could result in serious health hazard and contaminations may occur. When compare with nature, the human beings are the major risk factors to create the contaminated water in the environment from different aspects. Finally that will be reflecting their negative reactions against human population and it will be leads to the death or severe illness. So preventing the water borne diseases we are only the responsible to regenerate the water sources for future generation (Flow chart 1). Public health it is highly appreciated that potable water system should be safe. Potable water system can be polluted with coliforms and pathogenic bacteria from normal diseased or carrier human and animal excrete. Microbiological examination of water should routinely be carried out to monitor and control the quality and safety of drinking water. In the present investigation on isolation and identification of TSST-1 producing *Staphylococcus aureus* from various potable water systems in and around Namakkal district was carried out.

Flow chart 1: Prevention and control of *Staphylococcus aureus* from polluted water sources



Materials and Methods

Study area

Residents of in and around Namakkal area used drinking water from different sources such as River, Pond, Well and Municipality water. These water sources were used for the day today activities of human beings in Namakkal area. Water quality mainly depends on the microbial load in the water sources. So, the analysis of water quality and microbial in these areas that will be safe for people to prevent the water borne diseases. Based on this aspect this study was carryout.

Sample collection

A Total of 33 samples were collected from the four different sources in and around the Namakkal district, such as River water, pond water, well water and Municipality water. The samples were collected individually in sterile polyethylene bags and transported to the laboratory. The samples were processed within 2 hours.

Analysis of sample pH and temperature

All samples were tested for the pH, temperature with the use of instruments such as pH meter (ELICO make; model LI 120), and Thermometer.

Isolation of *Staphylococcus aureus*

The water samples were directly streaked on the surface of Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hours. After incubation the plates were observed. Golden yellow colonies were presumptively identified as *Staphylococcus* spp. The presumptive isolates were confirmed as *Staphylococcus aureus* using the cultural and standard biochemical characteristics. The individuals colonies from the streaked plates were selected and were continuously streaked onto the nutrient agar slant test tubes. The test tubes were incubated at 37ºC for 16-24 hrs and then stored under the refrigerator for further study.

Antibacterial susceptibility test

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial profiles of the *S.aureus* against nine antimicrobial agents such as Co-trimazaxole, Erythromycin, Vancomycin, Amphicillin, Kanamycin and Trimethoprim, The nutrient broth was prepared and sterilized at 121°C and inoculated the isolates then incubated at 37°C for 24 hours. After incubation period the broth culture were inoculated into surface of the Mueller-Hinton agar plates and antibiotic discs were placed 30 mm, then Plates were incubated at 37°C for 18 to 20 hours. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; sensitive, Intermediate and resistant according to the Clinical and Laboratory Standards Institute (CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [11].

Determination of virulence characters

Haemolytic activity

The haemolytic activity of the *S.aureus* was determined by blood agar plate assay. Overnight culture broth was inoculated in surface of the blood agar medium. The plates were incubated at 37°C for 24 hours. The zone of inhibition around the colonies consider as a positive result [12].

Serum inactivation assay

Serum inactivation assay was performed by the method of Dharmathikari *et al.*, 2009. Isolates were inoculated into glucose phosphate broth containing bromothymol blue and 2% human serum and incubated at 37°C for 24 hrs. The resistance was observed by change in colour from green to yellow [13].

Coagulase tube test

The tube is filled with 0.5 ml of 1 in 10 diluted rabbit plasma. To the tube, 0.1 ml of overnight broth culture of test bacteria is added. All the tubes are incubated at 37ºC and observed up to four hours. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube. Clumping (due to coagulation) of the organisms in 10 seconds indicate positive result. This was done for the plate suspected to be *Staphylococcus aureus* [14].

Isolation of genomic DNA

Procedure:

- Take 1.5 ml of overnight broth culture in to 2 ml micro centrifuge tubes.
- The tubes were centrifuged at 8000 rpm for 5 minutes.
- After centrifugation, the supernatant was discarded and the pellet was collected. The pellet was suspended in 200µl of 1X TE buffer + 100µl of 10% SDS and mixed by overtexing.
- The tubes were kept in water bath at 60°C for 20 minutes.
- Then added with 300µl of Phenol: Chloroform: Isoamyl alcohol mixture to extract the DNA and mixed completely by vortexing.
- The tubes were then centrifuged at 10000 rpm for 10 minutes to separate the phases.
- The aqueous phase containing the DNA was carefully removed and transferred to new tubes.
- Equal volume of Isopropanol was added to the tubes containing the aqueous phase.
- It was mixed by inverting the tubes 3 to 4 times.
- The tubes were then centrifuged at 10000 rpm for 10 minutes to pellet the DNA.
- The supernatant was discarded and the pellet was collected.
- To the pellet, 200µl of 70% ethanol was added and centrifuged at 10000 rpm for 10 minutes.
- Then Ethanol was decanted completely and the pellet was air-dried to give purified DNA.
- Re-suspended the dried DNA pellet in 20 µl of TE buffer and dissolved by tapping.
- DNA solutions were stored at 4°C for further work.

Confirmation of DNA by agarose gel electrophoresis:

Agarose gel electrophoresis is carried out in a horizontal submarine electrophoresis unit. Thirty ml of 1 % Agarose gel was prepared with 1X TBE buffer (do not mix) and heated the content to get up to clear solution for casting Agarose gel. After cooling the solution, 7 µl of staining dye solution was added into the casting system. The gel was allowed to solidify, and then carefully disassembled from the casting system without disturbing the wells and placed in 1X TBE buffer filled electrophoresis tank (the buffer level should be above gel). 5 µl of genomic sample DNA mixed with 2 µl of gel loading dye and then loaded to gel and simultaneously loaded 3 µl of DNA marker provided in the nearby well. The power card terminals was connected at respective positions, run the gel at 50 V, till the gel loading dye migrate more than half the length of gel. Then switched off the unit and visualized the isolated DNA under UV Transilluminator.

PCR (Polymerase chain reaction)

All isolates of *Staphylococcus aureus* were subjected in PCR assay according to Abdulmula *et al*, 2006 procedure with some modification. The primers was obtained from Sigma, India and used in the PCR comprised Primer 1 5'-ATGGCAGCATCAGCTTGATA-'3 and 5'-TTTCCAATAACCACCCGTTT-'3. Each PCR reaction mixture (20 µl) contained 1 µl of template DNA (Genomic DNA), 2 µl of 10 X PCR buffer, 0.5 µl of 2.0 mM of each primers, 1 µl of 25 mM of each deoxynucleotide triphosphate and 0.5 µl of Taq DNA polymerase (Con. 5U/ µl) and 15.5 µl of molecular grade water. A brief spin was given to settle down the materials than tubes were kept in theromocycler (Genei). After initial denaturation at 94°C for 5 min, the

samples were subjected to 30 cycles of denaturation at 94°C for 2 min, annealing at 55°C for 2 min and extension at 72°C for 1min. A final extension was performed at 72°C for 7 min. Following PCR, the reaction mixtures were analyzed by electrophoresis on a 2 % Agarose gel, containing ethidium bromide (0.2 mg/ml), in the presence of an appropriate DNA molecular weight marker. Then observed the amplification bands under UV Transilluminater and determinate the genetic diversity from *Staphylococcus aureus* [15].

Results

A totally of 33 samples were collected from four different water samples namely river water, pond water, municipality water, well water in and around the Namakkal area, Tamilnadu, India. The quality of the collected water samples were evaluated by pathogenic bacteria in four different water sources. In this study totally 18(54.54%) samples showed positive results for *Staphylococcus* spp.,

Physicochemical properties

All samples were subjected into analysis of physicochemical properties such as pH, Temperature. A summarized account of the results obtained for above parameters tabled in (Table 1 to 4). The pH value varying from 6.99 – 8.65 depending on the types of water samples.

River Water

Among the 6 river water samples the highest pH value is 8.1 (Rw3) and lowest pH value is (7.6 Rw1) and highest Temperature 31°C was observed in Rw3.

Pond Water

In7 pond water samples, highest pH 8.1 and lowest pH value is 7.5. The highest temperature of the pond water 30°C was observed in Pw2,6,7.

Municipality Water

Among the 10 samples, the highest pH 7.45 (Mw7) and lowest pH 6.99 (Mw9) were obtained from municipality water samples. The highest Temperature was 30°C in number of samples.

Well Water

In 10 well water samples, highest pH 8.65 (Ww6) and lowest 7.20 (Ww2) and highest Temperature was 31°C in Ww4. Among the 4 types of water samples the highest pH value 8.65 were obtained from well water. The moderate Temperature was obtained from the water samples 28°C - 31°C. The highest Temperature (31°C) was showed by well, river and pond water.

Table 1: Physiochemical Properties of River Water

S. No	Sample name	Area	Mean p^H	T°	Occurrence of *Staphylococcus spp.,*	%
1	Rw1	Velur	7.6±0.0088	30	+	
2	Rw2	Mohanur	7.83±0.0120	29	+	
3	Rw3	Pallipalayam 1	8.1±0.04414	31	+	
4	Rw4	Pallipalayam 2	7.83±0.0120	30	+	
5	Rw5	Pallipalayam 3	7.3±0.0120	29	+	**100%**
6	Rw6	Velur	7.4±0.0084	30	+	

Note: Rw- River water, T°- Temperature, %- Percentage

Table 2: Physiochemical Properties of Pond Water

S. No	Sample name	Area	Mean p^H	T°	Occurrence of *Staphylococcus spp.,*	%
1	Pw1	Vattampadi 1	7.5±.23094	29	+	
2	Pw2	Muthukapatti 1	8.1±.24664	30	+	
3	Pw3	Pallayapalayam1	8.0±.26034	29	+	
4	Pw4	kosavampatti	7.65±.25981	29	-	
5	Pw5	Thuthikulam	7.76±.28061	29	-	50%
6	Pw6	kalappanayakanpatti	7.85±.37676	30	+	
7	Pw7	Vattampadi 2	7.99±.37551	30	+	

Note: Pw- Pond water, T°- Temperature, %- Percentage

Table 3: Physiochemical Properties of Municipality Water

S. No	Sample name	Area	Mean p^H	T°	Occurrence of *Staphylococcus spp.,*	%
1	Mw1	Namakkal fort	7.3±.2603	30	-	
2	Mw2	Mohanur Road	7.1±.2309	29	+	
3	Mw3	S.pudhur	7.0±.2027	28	-	
4	Mw4	Trichy Road	7.4±.2631	29	-	
5	Mw5	E.B colony	7.1±.2603	30	+	
6	Mw6	Tiruchangode	7.2±.2309	29	-	
7	Mw7	Namakkal fort	7.45±.1946	28	+	
8	Mw8	Mohanur Road	7.38±.2757	29	-	30%
9	Mw9	Trichy Road	6.99±.2470	30	-	
10	Mw10	E.B colony	7.26±.3175	30	-	

Note: Mw- Municipality water, T°- Temperature, %- Percentage

Table 4: Physiochemical Properties of Well Water

S. No	Sample Name	Area	Mean p^H	T^O	Occurrence of *Staphylococcus spp.*,	%
1	Ww1	Namakkal 1	8.62±.1946	29	-	40%
2	Ww2	Trichy road 2	7.20±.21058	28	+	
3	Ww3	Salem road 1	7.40±.2027	30	-	
4	Ww4	Mohanur road 1	7.60±.2271	31	+	
5	Ww5	Mohanur road 2	7.41±.2666	30	-	
6	Ww6	Mohanur road 3	8.65±..2592	29	-	
7	Ww7	Salem road 2	7.82±.26909	29	+	
8	Ww8	Salem road 2	8.50±.2333	30	+	
9	Ww9	Namakkal 2	8.10±.1757	29	-	
10	Ww10	Namakkal 3	7.73±.2349	28	-	

Note: Ww- Well water, T°- Temperature, %- Percentage

Isolation and identification

Among the 33 samples, 18 (54.5%) were showed positive on mannitol salt agar. The isolates were observed by cultural and colony morphological characterization. The rounded and golden yellow colonies were observed from the MSA agar (Figure 1).The genus level identification was done by preliminary tests. Further species level identification was done by standard biochemical tests (Table 5).

Antibiotic sensitivity test

All 18 isolates of *S. aureus* was subjected to antibiotic stability test with 6 antibiotics. Each isolates were resistance to at least one antibiotic. Most of the *S. aureus* showed sensitive to Kanamycin, Co-trimazaxole and Amphicillin. In resistance patterns, 11(61.11%) isolates showed resistance to Vancomycin and Erythromycin. 8(44.44%) isolates for Amphicillin, 7(38.88%) for Trimethoprim (Table 6).Different types of resistance patterns were observed. Based on the multidrug resistance (MDR) index 3 isolates of Msa14, Wsa15 and Wsa17 showed 83.3% to all drugs. 2 isolates of Rsa1, Wsa18 showed 66.6% and RSa2, PSa8, MSa12 showed 50% (Table 7). The highest antibiotic resistances were observed from municipality water (83.3%) second most river water (66.6%).

Table 5: Biochemical Characters for *S.aureus*

S. No	Name Of The Biochemicel Tests	Results
1.	Glucose Fermentation	A^+
2.	Sucrose Fermentation	A^+
3.	Lactose Fermentation	A^+
4.	Maltose Fermentation	A^+
5.	Mannitol Fermentation	A^+
6.	Triple sugar iron	A/A
7.	Indole production Test	-
8.	Methyl red Test	+
9.	Voges –proskauer Test	-
10.	Citrate utilization Test	-
11.	Catalase Test	+
12.	Oxidase Test	-
13.	Urease	

Table 6: Antibiotic resistance/sensitivity patterns of *S.aureus*

S. No.	Strain Name	Antibiotics resistance/sensitive patterns					
		COT	E	VA	AMP	K	TR
1.	RSA1	R	R	S	R	S	R
2.	RSA2	S	R	R	R	I	S
3.	RSA3	S	I	S	S	S	S
4.	RSA4	I	R	I	I	S	S
5.	RSA5	S	S	R	S	S	S
6.	RSA6	S	R	R	I	I	S
7.	PSA7	S	R	R	I	I	S
8.	PSA8	S	R	R	R	I	S
9.	PSA9	S	R	R	I	I	I
10.	PSA10	S	I	R	I	S	I
11.	PSA11	S	S	R	R	S	I
12.	MSA12	S	R	R	R	S	S
13.	MSA13	I	S	S	S	S	R
14.	MSA14	R	R	S	R	R	R
15.	WSA15	S	R	S	R	S	R
16.	WSA16	S	S	S	S	S	R
17.	WSA17	S	I	R	R	S	R
18.	WSA18	R	R	R	I	S	R

Note: R-Resistant, S- Sensitive, I- Intermediate

Table 7: Multi Antibiotic Resistance (MAR) index of *S.aureus*

S. No.	Strain Name	Percentage of *S.aureus*		
		Resistant %	Intermediate %	Sensitive %
1.	RSA1	66.6	-	33.3
2.	RSA2	50	16.6	33.3
3.	RSA3	-	16.6	83.3
4.	RSA4	16.6	50	33.3
5.	RSA5	16.6	-	83.3
6.	RSA6	33.3	33.3	33.3
7.	PSA7	33.3	33.3	33.3
8.	PSA8	50	16.6	33.3
9.	PSA9	33.3	50	16.6
10.	PSA10	16.6	50	33.3
11.	PSA11	33.3	16.6	50
12.	MSA12	50	-	50
13.	MSA13	16.6	16.6	66.6
14.	MSA14	83.3	-	16.6
15.	WSA15	83.3	-	16.6
16.	WSA16	16.6	-	83.3
17.	WSA17	83.3	16.6	-
18.	WSA18	66.6	16.6	16.6

Determination of virulence factors

In this study, totally 18 isolates of *S.aureus* were subjected to characterize the virulence factors such as serum resistance, coagulase and haemolysis. These results were shown in the (Table 8).

Table 8: Virulence characterization of *S.aureus*

S.No.	Strain name	Virulence factors		
		Haemolysis	Serum resistant	Coagulase
1.	RSA1	-	+	-
2.	RSA2	-	+	+
3.	RSA3	+	+	+
4.	RSA4	-	+	+
5.	RSA5	-	+	-
6.	RSA6	-	+	+
7.	PSA7	+	+	+
8.	PSA8	+	+	+
9.	PSA9	-	+	+
10.	PSA10	-	+	-
11.	PSA11	-	+	-
12.	MSA12	+	+	-
13.	MSA13	+	+	+
14.	MSA14	-	+	-
15.	WSA15	-	+	-
16.	WSA16	-	+	-
17.	WSA17	-	+	-
18.	WSA18	+	+	-

Serum resistant test

All isolates of *S.aureus* were showed resistance to human serum that indicates by turns the green colour of medium to yellow (Figure 2).

Coagulase test

All isolates were carryout to coagulase test with human plasma, out of 18 isolates, 10(55.55%) isolates were showed coagulase negative and 8(44.44%) isolates showed positive. Among the 4 types of sample highest percentage was occur in river and pond water (Figure 3).

FIGURE: 2 Serum resistance

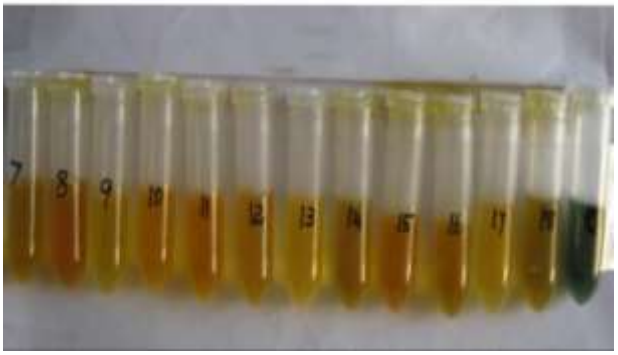


FIGURE: 3 Coagulase test

Hemolysis test

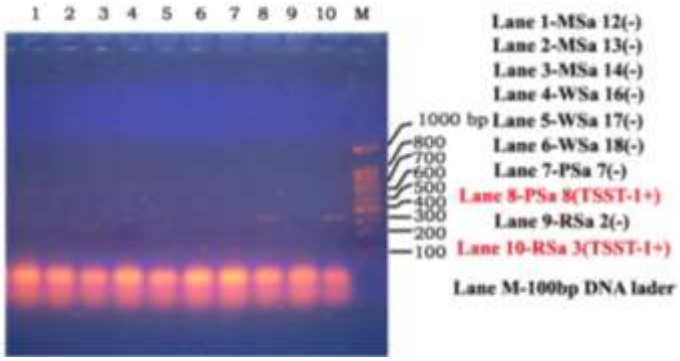
Haemolysis was performed with human blood. Out of 18, 6(33.3%) of isolates were showed the hemolytic activity. The result was indicated by the zone of clearance in around the colony. The highest activity was observed from municipality water (66.67%) second most pond water (40%) was shown (Figure 4).

FIGURE; 4 Haemolysis test

Amplification of TSST-1 gene from *Staphylococcus aureus*

In this PCR assay ten isolates of *Staphylococcus aureus* was selected based on the virulence producing isolates and subjected according to the previous study. Among the 10 isolates of *Staphylococcus aureus* the 2 isolates produce the TSST-1gene (Figure 5).

FIGURE: 5 Amplification of TSST-1 gene from *S.aureus*



Discussion

Contaminated drinking water has become a cause of numerous waterborne disease outbreaks in the developing area. Many studies have found these microorganisms to be those most frequently discovered in drinking water. In our present study all samples were collected from 4 different types of sources among them river water had highest prevalence of *staphylococcus aureus*. Same time all samples were utilized for physic chemical characters like pH and Temperature. The drinking water resources were severely contaminated in this region. The presence of *Staphylococci* in different types of water, particularly those designated for human consumption and/or recreation has been documented [16, 7]. In drinking water, *Staphylococci*, may be regarded as one of the genera that are commonly found in water supplies as Heterotrophic Plate Count (HPC) bacteria. Although it was suggested that it is not possible to establish health-based standards for the presence of HPC bacteria in drinking water, the presence of large abundance of *Staphylococcus aureus* in water intended for human consumption, may represent potential health hazards, especially if these strains possess determinants of antibiotic resistance and enterotoxin production [9]. *Staphylococcus aureus* isolated from drinking water were the most virulent and resistant to multi-antibiotics among all of the HPC bacteria recovered from water supplies in South Africa. Furthermore, water contaminated with *Staphylococcus aureus* was reported to cause food poisoning when used to cool boiled eggs [17].

Water is one of the most relevant vehicles of bacterial propagation and dissemination. The presence of antibiotic resistance bacteria or resistance determinants in drinking water has been reported sporadically for more than 20 years in different countries [9]. The present study aimed is assessing the distribution of *Staphylococci* and their resistance trends in different types of water. Most of the *S.aureus* showed sensitive to Kanamycin, Co-trimazaxole and Amphicillin. In resistance patterns, 11(61.11%) isolates showed resistance to Vancomycin and Erythromycin. 8(44.44%) isolates for Amphicillin, 7(38.88%) for Trimethoprim. Different types of resistance patterns were observed. The colonizing distribution network, may pose health threats considering their ability to form biofilm formation, which may harbour potential waterborne pathogens, as well as their role as a reservoir for transferable resistance genes to other potential bacterial species [16, 7]. At present study out of 33 samples 18(54.54%) of isolates were showed coagulase positive. Among the 4 types of samples, the highest percentage was observed on river and pond water sources. These results were correlate with the previous work described that *Staphylococcus aureus* producing coagulase was performed using a total of 4 *Staphylococcus* spp. to determine whether the organism was pathogenic or non-pathogenic. It was found that the isolated *Staphylococcus* spp. was coagulase positive i.e. it was the pathogenic *Staphylococcus aureus* [18].

Diseases caused by *Staphylococc*i are the result of a synthesis of several virulence factors including the different hemolysins, which are important for virulence of the *S.aureus* and other species. *Staphylococcal* haemolysin are identified as an important virulence factor that contributes for bacterial invasion and to escape from the host immune response. Alpha-haemolysin or alpha toxin considered being a main pathogenicity factor because of its haemolytic, dermonecrotic and neurotoxic effects. Additionally, beta haemolysin contains sphingomyelinase that is more active against sheep and bovine erythrocytes [19]. Several studies indicated that haemolysins of *S.aureus* correlated well with source of samples. In this study out of 18 samples 33.3% of isolates produced the hemolytic activity. The *Staphylococcal* secreted toxins include cytotoxins, superantigens, proteases, lipase and coagulase. Cytotoxins are a group of toxins that can lyse host cells and include α-toxin (Hla), β-toxin (Hlb), γ-toxin (Hld), and leukocidins (LukF-PV).These toxins not only lyse host cells, but also alter the host immune response such as inducing caspase dependent and caspase-independent apoptosis [20]. Superantigens are a class of protein toxins that can cause nonspecific T-cell activation and massive cytokines released by *S.aureus* superantigens include several enterotoxins (SEs), toxic shock toxin-1 (TSST-1), and exfoliative toxins (ETs). Enterotoxins cause food poisoning, whereas toxic shock toxin is responsible for toxic shock syndrome [21].

TSST is an acute multisystem disease characterized by high fever, hypotension, vomiting, diarrhea, myalgias, nonfocal neurologic abnormalities, conjunctival hyperemia, strawberry tongue, and an erythematous rash with subsequent desquamation on the hands and feet. TSST-1-producing strains of *Staphylococcus aureus*. TSS, however, also occurs in children, non menstruating women, and men. Non menstrual TSS has been associated with wound infection, nasal packing, sinusitis, tracheitis, pneumonia, abscesses, burns, osteomyelitis, and primary bacteremia. Several studies [22] have shown that the TSST-1 gene is more prevalent in MRSA than in methicillin-susceptible *S.aureus.* In the present study, 20% of isolates have TSST producing TSST-1 gene was identified by PCR. Only two isolates expressed these toxin gene but this is not a final in future this few counts increase the large population due to the environmental stress, drug resistance and other virulence factors. So starting stage of control that will be reduce the risks when compare with the highly outbreak in epidemics. *Staphylococcus aureus* causes a broad range of illnesses, from minor skin infections and abscesses to life-threatening diseases such as pneumonia, meningitis, endocarditis, septicemia and Toxic Shock Syndrome (TSS). Toxic Shock Syndrome Toxin-1 (TSST-1) is a *Staphylococcal* secreted exotoxin that is responsible for TSS, since it leads to

non-specific binding of MHC II with T cell receptors, resulting in polyclonal T cell activation. TSST-1 also plays a role in the pathogenesis of several autoimmune and allergic diseases associated with B cell hyperactivity, and it produces antagonistic effects on IL-4-induced IgE synthesis. Symptoms of TSS include high fever, accompanied by low blood pressure, malaise and confusion, which can rapidly progress to stupor, coma and multi-organ failure [23]. According to a report by planning commission india (2002) the risk of water contamination resulting in water borne diseases is higher in rural areas under the following conditions; inadequate availability of water, poor quality of water at sources, ill maintained water pipeline and sewer lines, open air defecation is rampant, lack of proper disposal if human, animal and household wastes and lack of awareness of good sanitation and personal hygienic practices. Total result of the present study suggests that drinking water of Namakkal surrounding area has low quality and nutritional value due to contamination of microbes.

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Conflict of Interest
The authors declared that they have no competing interests.

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