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Research Article

INCREASED PREVALENCE OF COAGULASE-POSITIVE *STAPHYLOCOCCUS AUREUS* IN MUNICIPAL DRINKING WATER SUPPLIES

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ABSTRACT: The current study was designed to study the occurrence, enumeration, seasonal prevalence, and characterization of *S.aureus* isolates recovered from municipal drinking water supplies of Neemuch, Madhya Pradesh. Towards this, samples were taken from three selected sites; *Dam* (n=24), *Treatment Plant* (n=24), and *Municipal Tap* (n=24), fortnightly for a period of 1 year. The samples were examined for the presence and enumeration of *S.aureus* on Mannitol salt agar by ***Direct Plating Method.*** Confirmation of presumptive *S.aureus* isolates was carried out by means of gram staining technique, and production of catalase and coagulase. Coagulase positive *S.aureus* was recovered from 100% of raw, 95.8% of treated and 100% of tap water samples analyzed, in numbers significantly different from the standard set by WHO and BIS ($P<0.05$). The presence and percentage of Coagulase positive *S.aureus* in water distributed to the consumers is alarming and presents a serious health concern. The quality of water that reaches the end-point users must be identical to that which left the treatment plant, however, in the present study the microbiological quality in terms of *S.aureus* counts, at the point of consumption was worse than at the plant ($P<0.05$). The results of present study suggest that water purification systems in developing countries need to be efficient in the manner that they produce a "safe product" at least, if not sterile and must ensure safe delivery of water at the tap of consumers.
Key words: Characteristics, Drinking water quality, Potable water, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus in potable water may be regarded as a natural flora or one of the genera that are commonly found in water supplies known as heterotrophic plate count (HPC) bacteria. Despite the suggestion that it is not possible to establish health-based standards for the presence of HPC bacteria in drinking water, the incidence of high concentration of *S.aureus* in water intended for human consumption may represent potential health hazards. Although *S.aureus* is a common member of the human microflora, intake of water contaminated with *S.aureus* can result in manifestations such as boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis and pneumonia. Gastrointestinal disease (enterocolitis or food poisoning) is caused by a heat-stable staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids. This paper reports the results of an investigation on the presence of Coagulase-positive *S.aureus* in municipal drinking water supplies of Neemuch.

MATERIAL AND METHODS

Study Area

Residents of Neemuch use drinking water supplied from *Jaju Sagar Dam* (Fig 1) after being treated through traditional method of alum flocculation and chlorine disinfection at Hingoria Treatment Plant. The treatment plant distributes treated water directly to the community, without any routine bacteriological testing. Hence, the investigation intended to sample the three kinds of supplies (Raw water, Treated water & Tap water) fortnightly, in order to evaluate complete removal of *S.aureus* and measure post-treatment changes.

Isolation and Enumeration

A total of 72 samples comprising 24 samples each of raw water, treated water and tap water were examined for the presence of *S.aureus*. Isolation and enumeration of *S.aureus* was done by *direct plating method* on Mannitol salt agar (Himedia, Mumbai, Maharashtra, India) in triplicates. Typical *S.aureus* colonies (yellow colonies with yellow zones) were counted after being confirmed by coagulase and catalase tests.

Confirmation of Isolates

Tube method was used for coagulase test in which 2-3 isolated pure colonies of *S.aureus* was inoculated in 2 ml of human plasma to give a milky suspension. Tube was incubated at 35^{o}C in ambient air for 4 hours. After incubation tube was checked for clot formation. For testing catalase activity, *S.aureus* culture growing on nutrient agar slant was taken on a slide and 0.5 ml of 3% hydrogen peroxide was added on the culture. Positive results were observed through bubbles forming in response to microbial activity.

Characterization

Morphological characteristics of the isolates were studied by using conventional microbiological techniques viz. light microscopic observations of gram-stained smears. Motility was tested by stabbing the culture into deep tubes of mannitol motility test medium (Himedia, Mumbai, Maharashtra, India), appearance of cloudiness was evident for motility. To study cultural characteristics, colony morphology was observed on mannitol salt agar as well as on nutrient agar plates. Type of growth on nutrient agar slant and in nutrient broth was also studied. Oxygen requirement was determined by inoculating *S.aureus* culture in fluid thioglycollate medium (Himedia, Mumbai, Maharashtra, India) deep tubes. To study the physiology of *S.aureus* isolates, a series of 16 biochemical tests (Table 2) were performed.

Figure 1 (i): Map of the study area showing Jaju Sagar Dam **(ii) Jaju Sagar Dam**

RESULTS AND DISCUSSION

Occurrence, Enumeration and Seasonal Prevalence

S.aureus is ubiquitous with widespread distribution in the environment including human body, and their presence in aquatic environments is well established. Although *S.aureus* can be found among the genera that normally exist in potable water as HPC bacteria [4], there are many reasons for potential concern when *S.aureus* are present in drinking water supplies. Common food preparation practices such as washing boiled potatoes, pasta, shelfish, and cooling of boiled eggs could possibly leave these food items contaminated with *S.aureus*. If these food items used for preparation of salads are left at room temperature, or improperly refrigerated, the possibility of staphylococcal food intoxication certainly exists if these *S.aureus* contaminants were toxigenic. In the present study, *S.aureus* showed 100% frequency of occurrence in raw and tap water samples and 95.8% in treated water samples examined, throughout the year and the counts obtained, were significantly higher than the prescribed WHO and BIS standard ($P<0.05$).

In accordance to which, there is no tolerable lower limit for pathogens in water intended for consumption, preparing food, drink or for personal hygiene; it should contain no agents pathogenic to humans. All the isolated strains were found to produce coagulase enzyme. Production of coagulase enzyme by the isolates indicates their pathogenic nature. Coagulase enzyme is involved in clotting of the blood converting the soluble fibrinogen into insoluble fibrin, which enables *S.aureus* to resist phagocytosis and other host defence mechanisms. The presence and percentage of these pathogenic forms of *S.aureus* in potable water would greatly harm health of those inhabitants who completely rely on this unsafe municipal water. Log concentration of the isolates in raw, treated and tap water samples are summarized in Table 1 and the annual trend in *S.aureus* densities in the three supplies is depicted in Figure 2. Statistical analysis proved significant differences in log counts among raw, treated and tap water samples throughout the year (P<0.05).

All the 24 samples collected from *Jaju Sagar dam* contained high densities of *S.aureus* throughout the sampling months, which ranged from 1.98 log cfu/ml (Jan, S2) to 2.59 (Aug, S1). Seasonal studies of the counts (Fig 3) showed that the highest counts were observed in the rainy season followed by summer and winter. Seasons were categorized as summer: March-June, rainy: July-October, and winter: November to February. The average log count during rainy season was 2.43, whereas the average log count during winter was recorded to be 2.19. During summer months, the average count was 2.41, higher than colder months. The origin of *S.aureus* in *Jaju Sagar Dam* may be due to the animals and human activities; because human and animals are the primary way the bacteria are transported through the environment. *S.aureus* is present in the nasal passages, the throat, and on the hair and skin of 50% or more of healthy individuals. Other possible source of *S.aureus* might be environmental because it is widely distributed in nature and not fecally transmitted as the other intestinal pathogens do. *S.aureus* exists in air, dust, sewage, water, soil, plants and environmental surfaces. The extremely hazardous sources of contamination in *Jaju Sagar Dam* may be excessive monsoon rains, floods, herbicides, fungicides, untreated municipal waste, medical waste and coastal water pollution due to waste discharges and oil spills. The results of present study are supported by a number of studies by many researchers who also reported *S.aureus* occurrence in raw source waters. Hussain et al. [11] isolated *S.aureus* in different drinking water sources studied from Kohat, Pakistan. Osman et al. [18] studied occurrence of total staphylococci in all the raw water samples examined from Nile river, Egypt. Suthar et al. [21] observed that the drinking water samples collected from some rural habitations of Northern Rajasthan were contaminated with *S.aureus*. Abdel-Rahim et al. [1] obtained *S.aureus* in all of the tested sources of the drinking water in Al Gedarif city (raw and treated waters of Atbara River, main reservoirs and zeer waters of all sources). Lechevallier and Seidler [14] also isolated Coagulase positive *S.aureus* from over 6% of 320 rural drinking water specimens from well. Mihdhdir et al. [16] also detected S.*aureus* in three out of five drinking water stations in Makkah Al-Mokarama. Kolawole et al. [12] isolated *S.aureus* from raw water of Oyun river, chlorinated tank, storage tank, male hostel tap, and female hostel tap. Chandra et al. [8] obtained *S.aureus* in Gola river water, Uttaranchal, India. Ahmed et al. [3] also recovered *S.aureus* from water samples of different dams and related filtration plants of Rawalpindi, Islamabad region in Pakistan. Shittu et al. [19] isolated *S.aureus* from drinking water of Abeokuta, Nigeria. Baig et al. [6] tested drinking water of northern Pakistan and found that water samples from all the sampling sites except one site were contaminated with *S.aureus*. Sila et al. [20] also recovered *S.aureus* from the Lamingo Dam Jos Nigeria, its water filter tanks and water taps but in contrast to the findings from present investigation, he concluded that tap water samples showed the highest frequency of occurrence followed by the dam water samples, while the treated water samples showed the least. Lamka et al. [13] identified *S.aureus* from standard plate count agar in rural drinking water supplies. From all these findings, it may be inferred that *S.aureus* is the normal inhabitant of drinking water sources and other aquatic bodies.

In the present study, the isolation rate of *S.aureus* in treated water samples reduced to 95.8% and the log counts ranged from 0.51 (Feb, S1) to 2.01 (Aug, S2) (Table 2). Analysis of Variance showed that throughout the sampling months, treated water samples showed significantly decreased amounts of counts as compared to that of raw water samples (P<0.05), but the counts were significantly higher than the drinking water standard limit of zero count per ml [22,7] (P<0.05). These results put a question on the working efficiency of the treatment plant in complete removal of pathogens from the raw water. The log cfu/ml counts in samples from the tap ranged from 1.52 (May, S2) to 2.16 (Dec, S2) which also crossed the standard limit of zero count, throughout the year. Moreover, these counts at point of use exceeded the counts obtained in the outlet samples (P<0.05), which showed continuous deterioration of the water quality within the distribution system. The decline in microbial water quality within the distribution system may be attributed to the recovery and subsequent growth of sub-lethally damaged bacteria, to system deficiencies such as cross connections, broken water mains and contamination during bulk storage and repairs, lack of residual chlorine and to the presence of biofilms within the distribution system from which cells may be released into the flow.

Drinking water distribution systems provide an oligotrophic environment, and post-treatment recovery and growth of bacteria is therefore a concern because of the effect the environment can have on public health. Similar and higher percentages of *S.aureus* occurrence in chlorinated potable water were reported worldwide [10,9], Manji et al. [15] reported incidence of *S.aureus*, in treated tap water samples of Calabar South Local Government Area. Lechevallier et al. [14] also identified *S.aureus* from SPC bacteria isolated from distribution water. Rahim et al. [1] found *S.aureus* in all the tested treated waters of Atbara River, of Al Gedarif city, Sudan. Ojo et al. [17] also studied occurrence of *S.aureus* in potable public water supply within Lagos University, Ojo. Lamka et al. [13] analyzed drinking water supplies in a Modern Rural Neighborhood. In his study, 8% of the 78 sampled households harbored *S.aureus*. Abulreesh et al. [2] also isolated *S.aureus* from potable water samples. Occurrence of *S.aureus* in distribution system or any kind of potable water is of great concern due to its pathogenic nature. In addition, Staphylococci are well known for their ability to produce biofilm formation on different surfaces such as distribution pipelines. The presence of *S.aureus* in biofilm formation within drinking water distribution system may, in part, cause aesthetic and hygienic problems as staphylococci within the biofilm consortia can inherit resistance to disinfectants, and their long term persistence, together with other HPC bacteria, can deteriorate the overall microbiological quality of potable water. Further potential waterborne pathogens may take refuge within the biofilm formation and survive for longer periods, with the possibility of acquiring resistance to antibiotics due to transferrable resistance genes.

Table 1: Prevalence of *Staphylococcus aureus* in municipal supplies

Months		Raw water samples				Treated water samples				Tap water samples			
		*Actual CFU/ml	Log CFU/ml	Mean Log CFU	SD	*Actual CFU/ml	Log CFU/ml	Mean Log CFU	SD	*Actual CFU/ml	Log CFU/ml	Mean Log CFU	SD
January	S1	13.0	2.11	2.04	0.09	5.00	1.69	1.50	0.28	9.00	1.95	1.81	0.20
	S2	9.66	1.98			2.00	1.30			4.66	1.66		
February	S1	16.3	2.21	2.25	0.06	0.33	0.51	0.99	0.67	8.33	1.92	1.76	0.22
	S2	20.0	2.30			3.00	1.47			4.00	1.60		
March	S1	28.6	2.45	2.41	0.05	8.33	1.92	1.83	0.11	10.0	2.00	1.84	0.21
	S2	24.0	2.38			5.66	1.75			5.00	1.69		
April	S1	32.0	2.50	2.43	0.10	4.33	1.63	1.42	0.29	8.00	1.90	1.97	0.10
	S2	23.0	2.36			1.66	1.22			11.3	2.05		
May	S1	20.0	2.30	2.36	0.09	2.66	1.42	0.71	1.00	7.00	1.84	1.63	0.22
	S2	27.3	2.43			0.00	0.00			3.33	1.52		
June	S1	26.0	2.41	2.43	0.03	4.00	1.60	1.30	0.42	6.66	1.82	1.77	0.06
	S2	29.0	2.46			1.00	1.00			5.33	1.72		
July	S1	37.0	2.56	2.53	0.04	8.00	1.90	1.93	0.04	10.6	2.02	1.97	0.07
	S2	31.6	2.50			9.33	1.96			8.33	1.92		
August	S1	39.3	2.59	2.55	0.05	7.00	1.84	1.92	0.11	12.6	2.10	1.98	0.16
	S2	33.0	2.51			10.3	2.01			7.33	1.86		
September	S1	19.6	2.29	2.26	0.04	6.66	1.82	1.69	0.18	4.00	1.60	1.77	0.24
	S2	17.0	2.23			3.66	1.56			9.00	1.95		
October	S1	22.0	2.34	2.37	0.04	2.00	1.30	1.59	0.41	8.00	1.90	1.73	0.24
	S2	25.3	2.40			7.66	1.88			3.66	1.56		
November	S1	15.0	2.17	2.12	0.07	4.66	1.66	1.81	0.20	13.6	2.13	2.06	0.10
	S2	11.6	2.06			9.00	1.95			9.66	1.98		
December	S1	21.0	2.32	2.37	0.06	5.00	1.69	1.76	0.08	7.33	1.86	2.01	0.21
	S2	26.3	2.42			6.66	1.82			14.6	2.16		
Frequency of Isolation (n=24)		100%				95.8%				100%			
Range (Log CFU)		1.98-2.59				0.51-2.01				1.52-2.16			
% of samples crossed detection limit (0 CFU/ml)		100%				95.8%				100%			

*Mean of triplicates, SD = Standard deviation S1- First fortnight sample, S2- Second fortnight sample

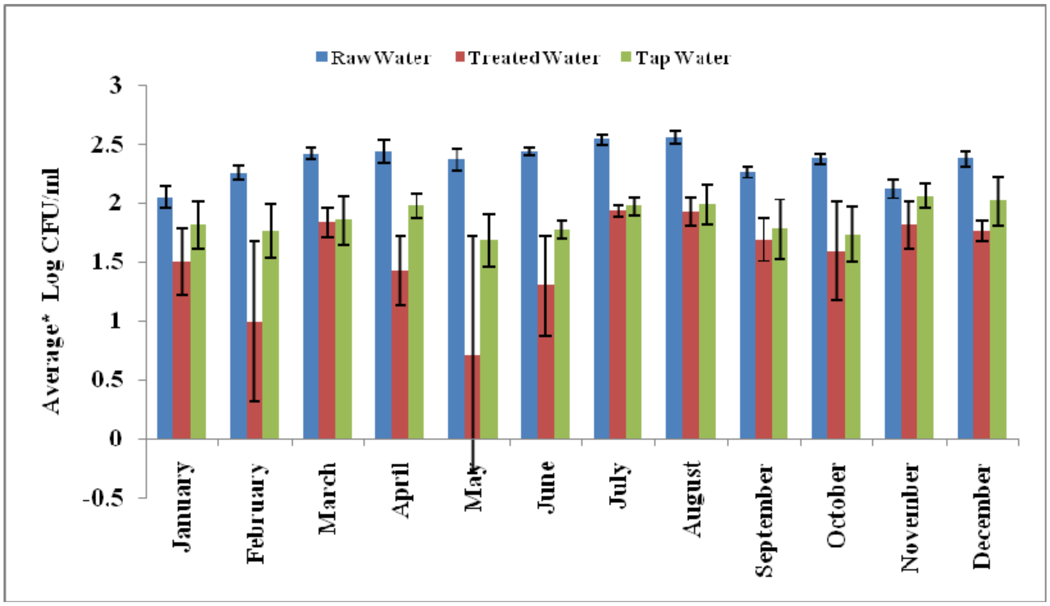


Figure 2: Annual trend in *S.aureus* counts in the three kinds of supply

*Mean of S1 & S2, the bars show standard deviation

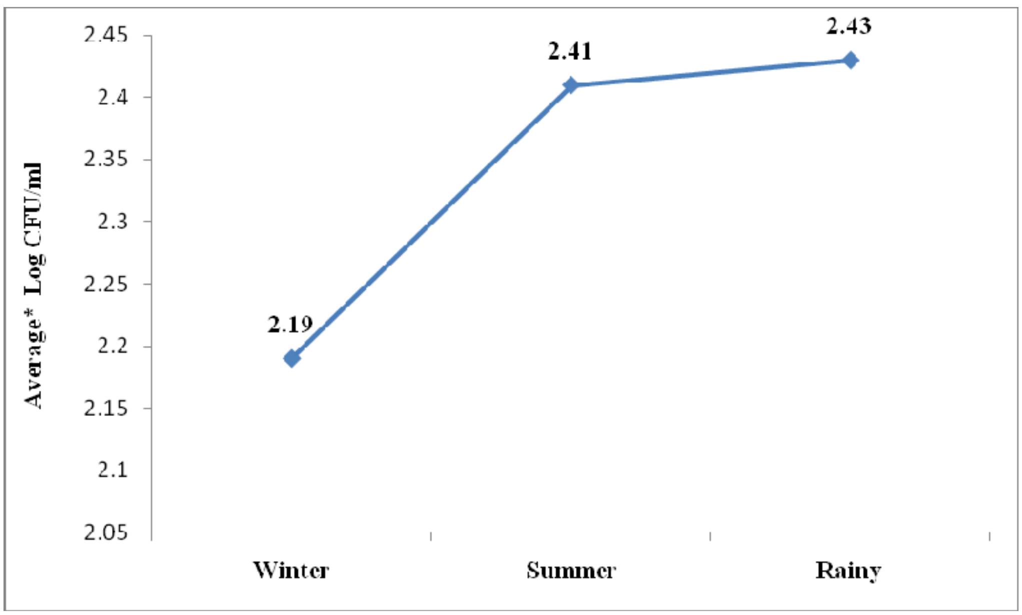


Figure 3: Seasonal fluctuations in *S.aureus* counts in dam

*Mean of four reading

Morphological, Cultural and Physiological Characteristics

Table 2 summarizes morphological and cultural characteristics studied for the isolates. Gram staining of the isolates showed short, spherical-shaped gram positive cocci arranged in clumps. Lack of thin spreading filaments and growth limited to the line of inoculation in mannitol motility test medium confirmed the cells as non-motile. Overnight growth (18 to 24 hours) of *S.aureus* on mannitol salt agar produced small to large (2 to 8mm in diameter) round, yellow colonies with yellow zones. Isolates showed three types of colony morphology predominantly; round with entire margin, round with scalloped margin and round with raised margin. On nutrient agar plates the colonies were white, opaque, round with entire margin and convex elevation. Isolates showed filiform type of growth on nutrient agar slant and in nutrient broth it produced membranous type of surface growth, granular type of sub-surface and flocculent type of sediment growth. The results of physiological characterization (Fig 4) are tabulated in Table 3.

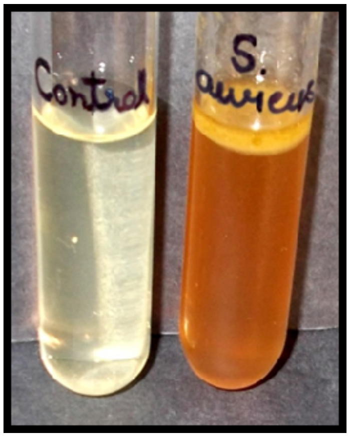
Table 2: Morphological and Cultural characteristics of *S.aureus* isolates

Morphological Characteristics		
Cell Shape & Arrangement		Coccus, clumps
Motility		-
Gram's Reaction		+
Presence of Capsule		-
Presence of Spores		-
Acid Fast Reaction		-
Cultural Characteristics		
Type of growth on NA slant-	Amount of growth	Abundant
	Color	Golden
	Opacity	Opaque
	Form	Filiform
Oxygen requirement		Facultative Anaerobic
Colony on Nutrient agar plate-	Form	Round
	Color	White
	Margin	Entire
	Elevation	Convex
Growth in nutrient broth-	Amount of growth	Moderate
	Surface growth	Membranous
	Sub-surface growth	Granular
	Sediment growth	Flocculent
Colony on MSA plate-	Form	Round
	Color	Yellow
	Margin	Entire
	Elevation	Convex

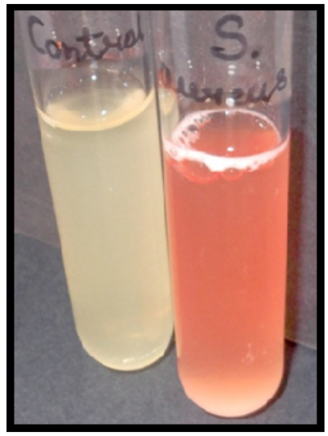
Table 3: Biochemical characteristics of *S.aureus* isolates and type strain

Biochemical Characteristics	*S.aureus* Type strain	*S.aureus* Isolated strains
Indole Test	-	-
Methyl Red Test (MR)	+	+
Voges–proskauer Test (VP)	-	V
Simmon Citrate Test (SC)	-	-
Oxidase Test	-	-
Catalase Test	+	+
Oxidative-Fermentative Test (OF)	F	F
Nitrate Reduction Test (NR)	+	+
Mannitol Fermentation Test	+	+
Amylase Test	-	-
Gelatinase Test	+	+
Urease Test	-	-
Coagulase Test	+	+
Triple Sugar Iron Agar Test (TSIA)		
1.Glucose	+	+
2. Gas from Glucose	-	-
3. Sucrose	+	+
4. Lactose	+	+
5. H_2S	-	-

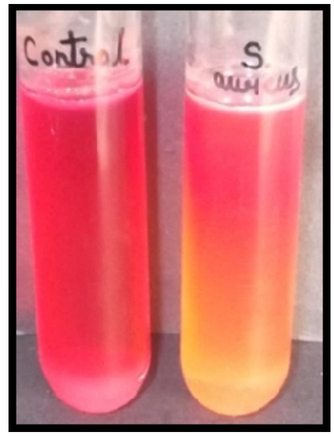
+ = 90 to 100% of the isolates were positive;
- = 0 to 10% of the isolates were positive; V = variable reaction



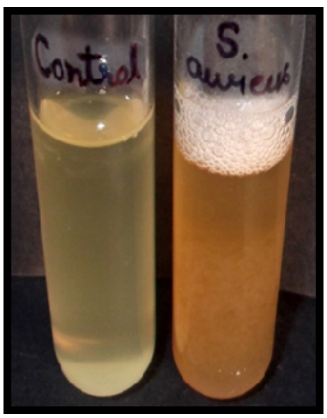
Negative Indole Test



Positive MR Test



Positive Mannitol Test

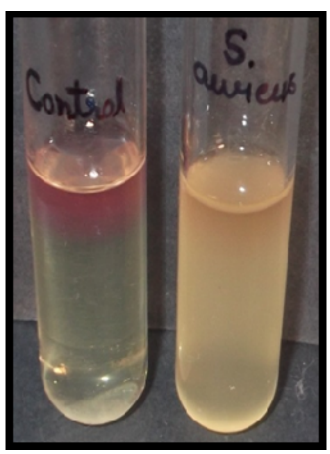


Negative VP Test

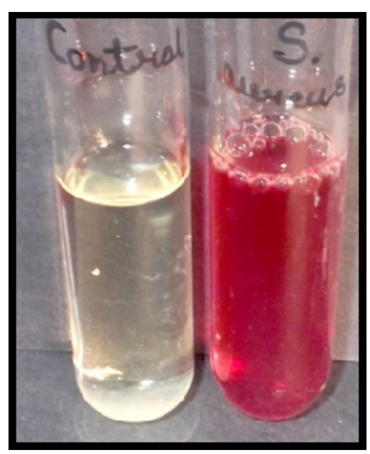
Negative SC Test

Negative Urease Test

Positive TSIA Test

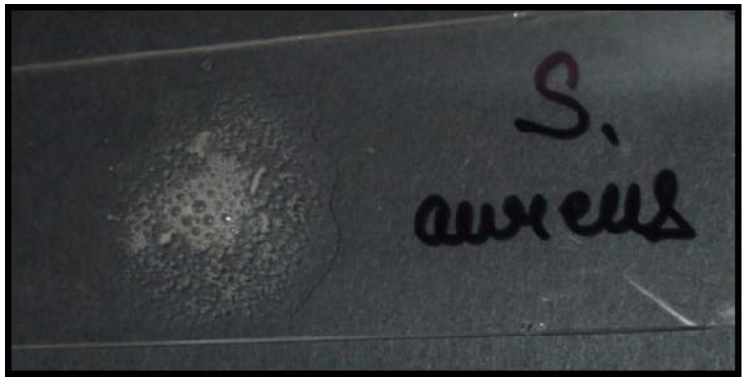


FTG Test–Facultative Anaerobic

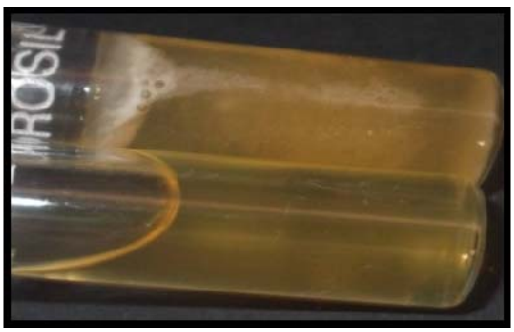


Positive NR Test

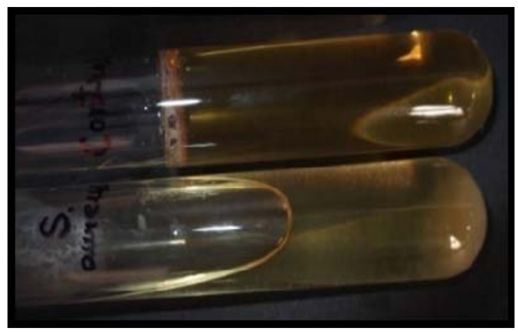
Negative Oxidase Test



Positive Catalase Test



Positive Coagulase Test



Positive Gelatinase Test

OF Test- Fermentative

Negative Starch Test

Figure 4: Biochemical Test Results of *S.aureus*

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