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Isolation and Characterization of *Staphylococcus* sp., from Drinking Water Samples of Guntur, Krishna and Prakasham Districts, Andhra Pradesh, India

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ABSTRACT

Staphylococci are ubiquitous bacteria in the environment and it is widely distributed in different types of water including drinking water. The present study was undertaken to determine *Staphylococci sp*., in drinking water samples collected from Guntur, Krishna and Prakasam Dt., of Andhra Pradesh. 100 µl of each water samples are added onto the specific media (Mannitol Salt Agar) plates and incubated at 37˚C for 24 h and observed for the growth. After the incubation period, the colonies obtained on the specific media (Mannitol Salt Agar, Cetrimide Agar) are cultured using pure culture techniques and then confirmed by conducting morphological and biochemical tests like catalase, coagulase, oxidase, indole production test, methyl red test, Vogerproskauer test and mannitol tests for the bacterial strain. It is concluded that the bacterial species may be *Staphylococcus aureus.* However, further biochemical tests like DNase test and haemolysis tests have to be conducted to confirm the bacterial species.

Keywords: *Staphylococcus* sp., isolation, characterization, drinking water.

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INTRODUCTION

The staphylococci most frequently associated with human infection are *S. aureus, S. epidermidis* and *S. saprophyticus*[1-2]. *Staphylococcus* species are Gram-positive, non-motile, non-sporing cocci occurring singly, in pairs and in grape like irregular clusters: size may be variable. Colonies are opaque and may be white or cream and are occasionally yellow or orange. The optimum growth temperature is 30°C - 37°C. They are facultative anaerobes and have a fermentative metabolism. The genus *Staphylococcus* has at least 40 species, which are separated in to two major groups on the basis of their ability to clot (coagulate) blood plasma by the action of staphylocoagulase[3]. The coagulase-positive staphylococci (CoPS) include pathogenic species such as *Staphylococcus aureus*, while the coagulasenegative staphylococci (CoNS) include species that are part of the normal flora of the skin in humans such as *Staphylococcus epidermidis*[4].

Staphylococci are ubiquitous in the environment, and are found as normal flora in soil, different types of water [5-,6], skin and mucous membranes of humans and warm-blooded animals, and a wide range of foodstuffs such as dairy products and meat [7]. Multiple antibiotic-resistant CoNS were also reported from food, potable water and wastewater water[8]. Bacterial species associated with tap water, river water, pond water, and drinking water were characterized and counted by Amer *et al*. [9] and Salahuddin et al.,[10]. Similarly the bacterial contamination in drinking water samples collected from some rural habitats of northern Rajasthan, India [11] and the distribution of Staphylococci and their resistance trends in different types of water was also assessed [6]. The present study was undertaken to determine the *Staphylococcus* sp. in drinking water samples collected from Guntur, Krishna and Prakasam Dt., of Andhra Pradesh.

MATERIALS AND METHODS

Collection of Water Samples

Different sources of drinking water such as surface water and ground water from three districts i.e., Guntur, Krishna and Prakasam and well water from Prakasam Dt., are collected for the study and the list is mentioned in the Table- 1. Municipal water from three districts is also collected and is considered as controls. The water samples are collected in sterile capped containers and transported to the laboratory within 6h of water sample collection for further analysis by keeping them on the ice. The bacterial analysis included isolation, characterization and identification by following standard microbiological techniques like serial dilution, spread plate and streak plate. Characterization and identification was carried out by following morphological and biochemical tests. The chemicals are procured from Himedia.

Isolation of *Staphylococcus* sp.

To know the existence of *Staphylococcus* sp., in the collected samples of drinking water from Guntur, Krishna and Prakasam districts of A.P. the standard serial dilution method was followed. Then, 100 µl of each water samples are added onto the specific media (Mannitol Salt Agar) plates of *Staphylococcus sp.,* and incubated at 37˚C for 24 h and observed for the growth. After the incubation period, the colonies obtained on the specific media are cultured using pure culture technique (streak plate) and then confirmed by conducting the confirmatory tests for the bacterial strain.

Preparation of *Staphylococcus* sp. Pure Culture

To identify the species, loopful of culture grown from Singarayakonda ground water (G4) was inoculated on to mannitol broth and incubated for 24 h in orbital shaker. After 24 h of incubation, a loopful of broth was streaked on to the mannitol salt agar medium and incubated at 37 ^{0} C for 24 h and observed for the growth of single colonies.

Confirmatory Tests for *Staphylococcus sp.*

To confirm *Staphylococcus* sp., morphological characterization using Gram's staining and biochemical tests like catalase, coagulase, oxidase, indole production test, methyl red test, Vogerproskauer test and mannitol tests were conducted according to the standard microbiological techniques.

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RESULTS AND DISCUSSION

Staphylococcus mostly occurs as *S.aureus*or and *S.epidermidis.* In the present study, growth of *Staphylococcus* sp. was observed in all the collected drinking water samples of Guntur, Krishna and Prakasam Dt., of A.P. To identify the microbe, loopful of the broth culture was streaked on to the mannitol salt agar medium and incubated at 37 ^{0} C for 24 h and observed for the growth of single colonies. As shown in the Fig. No.1, after 24 h of incubation, formation of single colonies was observed. To identify the species of *Staphylococcus,* morphological characterization using Gram's staining and biochemical tests were carried out following standard microbiological techniques. Gram's staining indicated that the microbe is Gram positive organism (purple in color) and it is present in pairs or as irregular grape like clusters (Fig.No.2). It was also found that the colonies are opaque and are cream in color.

To further identify the organism, biochemical tests like catalase, coagulase, oxidase, indole production test, methyl red test, Vogerproskauer test and mannitol tests were conducted. The results are represented in Table No.2, Figure No. 3 and 4. It was observed that catalase, coagulase, methyl red test, Vogerproskauer test and mannitol tests are positive and oxidase, indole production test are negative for all the tested drinking water samples collected from Guntur, Krishna and Prakasam Dt., of A.P. Similarly, the bacterial contamination in drinking water samples collected from some rural habitats of northern Rajasthan, India [11] and the distribution of staphylococci and their resistance trends in different types of water was also assessed [6]. Tortora et al., [12] reported that in general *Staphylococcus* occurs in water that contains organic pollutants i.e., mineral ions, and organic matter contents. The organic matter content may provide a better environment for the development of this bacterium in water sources. The occurrence of this bacterium in drinking water samples may indicate the mixing of runoff water in water resources as suggested by Tortora et al., [12]. It is concluded that based on the morphological and biochemical characterization that the tested organism may be *Staphylococcus aureus*. However, further biochemical tests like DNase and haemolysis tests have to be conducted to confirm it.

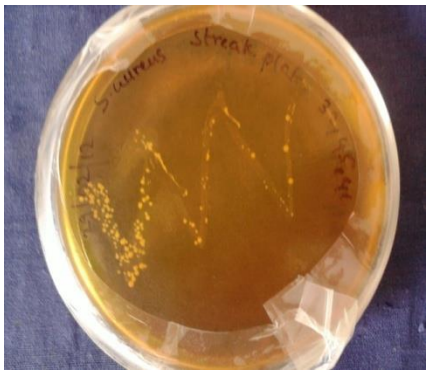

Figure 1:Streak Plate of *Staphylococcus* sp.

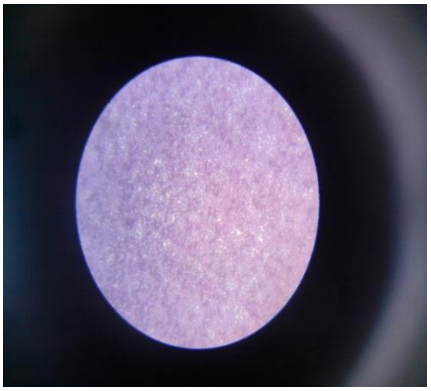

Figure 2: Microscopic image of *Staphylococcus* sp.

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Table 1: List of water samples collected from Guntur, Krishna and Prakasam districts of Andhra Pradesh.

S. No.	Source of Water	Area of Collection	Label of the Water Sample
1	Surface water	Sekuru, near Sangamjagarlamudi, Guntur dist.,	S1
2	Surface water	Gudiwada, Krishna dist.,	S2
3	Surface water	Ongole, Prakasam dist.,	S3
4	Ground water	Guntur, Guntur dist.,	G1(a)
5	Ground water	Godavvaru, Guntur dist.,	G1(b)
6	Ground water	Vijayawada, Krishna dist.,	G2
7	Ground water	Ongole, Prakasam dist.,	G3
8	Ground water	Singaraikonda, Prakasam dis.,	G4
9	Well water	Ongole, Prakasam dist.,	1
10	Well water	Singaraikonda, Prakasam dist.,	W2
11	Municipal water	Guntur,Guntur dist.,	C1
12	Municipal water	Gudiwada Krishna dist.,	C2(a)
13	Municipal water	Vizayawada Krishna dist.,	C2 (b)
14	Municipal water	Ongole, Prakasam dist.,	C3
15	Municipal water	SingaraikondaPrakasam dist.,	C4

Table 2 : Biochemical Characterization of *Staphylococcus* sp.

Sample No.	Catalyse test	Coagulase test	Oxidase test	Indole production test	Methyl red test	Vogerproskauer test	Mannitol test
S1	+	+	-	-	+	+	+
S2	+	+	-	-	+	+	+
S3	+	+	-	-	+	+	+
G1(a)	+	+	-	-	+	+	+
G1(b)	+	+	-	-	+	+	+
G2	+	+	-	-	+	+	+
G3	+	+	-	-	+	+	+
G4	+	+	-	-	+	+	+
W1	+	+	-	-	+	+	+
W2	+	+	-	-	+	+	+
C1	+	+	-	-	+	+	+
C2(a)	+	+	-	-	+	+	+
C2 (b)	+	+	-	-	+	+	+
C3	+	+	-	-	+	+	+
C4	+	+	-	-	+	+	+

Figure3: Catalase Test for *Staphylococcus* sp.

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Figure 4: Mannitol Test for *Staphylococcus* sp.

CONCLUSIONS

The present study was undertaken to determine *Staphylococci* sp., in drinking water samples collected from Guntur, Krishna and Prakasam Dt., of Andhra Pradesh. The isolation, identification and characterization of the bacterium were carried out by following the standard microbiological tests like streak plate, Gram's staining and biochemical tests. The results indicated that catalase, coagulase, methyl red test, Vogerproskauer test and mannitol tests are positive and oxidase, indole production test are negative for all the tested drinking water samples collected from Guntur, Krishna and Prakasam Dt., of A.P. From these findings it was concluded that the tested organism may be *Staphylococcus aureus*. However, further biochemical tests like DNase and haemolysis tests have to be conducted to confirm it.

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