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***Research Article** (Open Access)*

ISOLATION AND EVALUATION OF ANTIBIOTIC SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* IN FRESH PRAWNS FROM DIFFERENT MARKET AREAS OF CHENNAI

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ABSTRACT
Prawns are shrimp like shellfish in the biological group of marine animals known as the decapods with muscle cellularity as key indicator for its growth. *Staphylococcus aureus* is the human pathogen among the staphylococci. The main aim of the study is to isolate and evaluate antibiotic sensitivity of *Staphylococcus aureus* in fresh Prawn from different market areas of Chennai to be done. The samples I, II, III were collected from three different market areas of Chennai. All samples were kept for isolation of *Staphylococcus aureus* in MSA for 48 hours and the isolates were tested by staining method. Gram positive cocci awere seen in clusters. The antibiotic sensitivity testing of the samples was carried out by Kirby Bauer disc diffusion method and graphically depicted by using mean and t- test. From the results, Chloramphenicol, Gentamycin, Erythromycin, Ciprofloaxin, Rifampicin shows significant level with increased sensitivity ($p>0.3$) at the level of 30% when compared to the standard Zone diameter Interpretation chart. Vancomycin showed no significance. Thus, the prevalence of the *Staphylococcus aureus* in the edible prawns from three market areas of Chennai is evaluated to create awareness on the seriousness of the infection of *Staphylococcus aureus* which lead to food borne disease through edible fresh prawns.

Keywords: *Prawns, Staphylococcus aureus, Isolation, Antibiotic sensitivity*

INTRODUCTION

Prawn

Prawns are a shrimp-like shellfish in the biological group of marine animals known as the decapods in classification. Despite some anatomical differences between true shrimp and prawns, these closely related creatures are nearly identical from a nutritional perspective, due to similar feeding habits and body composition. Prawns are an excellent source of high-quality protein and several important vitamins and minerals that support good health for humans. They are basically low in calories which is made up of extremely healthy cholesterol. In fact, according to the American Journal of Clinical Nutrition in 2010, eating prawns is part of a heart healthy diet. And because they are common throughout the world, there are healthy prawn dishes within almost every style or type of cuisine in the world. Prawns are basically low in calories and contain no carbohydrates. Although the cholesterol content of prawns is significant, these shellfish also contain heart-healthy, omega-3 fatty acids (Faribha Khaloodooz *et al.*, 2010).

Muscle of prawn

Prawn growth is regulated by fundamental process of molting associated with complex biochemical activity of specific proteins that helps withdrawal of exoskeleton from the carapace during muscle growth and development. Muscle cellularity is the key indicator mechanism behind the skeletal growth of particular growth of particular organism that involves the contrasting balance of hyperlaxia and hypertrophy of the muscle fibre. The delicate balance may vary depending upon various internal and external factors of the growth of prawn. Research findings reveal that diet is one of the significant extrinsic environmental factors governing the muscle growth of fish thereby causing such variation in fibre size and number.

Nutritional benefits

Eating prawns regularly will provide nutritional benefits as eating prawns provides a complete protein which means it includes amino acids in the proper proportion for the body to function properly to engulf nutrients. Prawns are a great source of nutrients such as Vitamins B-6, B-12 and Niacin, which helps the human body to produce energy, build muscle and replenish red blood cells. Prawns contain significant amounts of iron, a mineral that is essential for the body to distribute oxygen. And because it is in only a few types of food, iron deficiencies that cause severe exhaustion are surprisingly widespread, especially for women.

Staphylococcus aureus

The name Staphylococcus comes from the Greek staphyle, meaning a bunch of grapes, and coccus, meaning berry, and that is what staph bacteria look like under the microscope, like a bunch of grapes or little round berries. The genus is divided into coagulase-positive staphylococci and coagulase-negative staphylococci based on their ability to coagulase plasma (Pyorala *et al*.,2011). *Staphylococcus aureus* is the most significant human pathogen among the staphylococci, as it is ubiquitous spherical bacterium, Gram positive and facultative anaerobic. It can grow in wide range of pH (between 4.2 and 9.3), temperatures (between 7oC and 48.5oC) and in high concentration of sodium chloride (15%) (Le loir *et al*.,2003,). These characteristics favour the growth of this bacterium in many food products. Indeed previous studies have isolated *S.aureus* from various food origin. Staphylococci can be found normally in the nose and on the skin (and less commonly in other locations) of around 25%-30% of healthy adults and in 25% of hospital or medical workers. In the majority of cases, the bacteria do not cause disease but , a cut, abrasion, or other damage to the skin or other injury may allow the bacteria to overcome the natural protective mechanisms of the body that leads to infection (Le loir *et al*.,2003) Transmission is typically from direct contact. In a study worldwide, food poisoning is caused by *S. aureus*. In the United States, *S. aureus* is considered one of the top five pathogens causing domestically acquired foodborne diseases and it is responsible for an estimate of 241,000 illnesses per year in the study. A study in Morocco showed that *S. aureus* was responsible for 72% of foodborne outbreaks in the country (Ed- dra *et al*.,2017). Staphylococcus epidermidis must rely primarily on cell-surface polymers and the ability to form a bio film to survive in the host the bacteria depends on (Ed- dra *et al.*,2017).

Antibiotics

Antibiotics are any substance that inhibits the growth and replication of a bacterium or kills it outright Antibiotics are a type of antimicrobial designed to target bacterial infections within (or on) the body. Antiseptics and disinfectant are used on non-living surfaces, for example in hospitals of course, bacteria are not the only microbes that can be harmful to humans. Some microbes, produce substances specifically to kill other nearby bacteria in order to gain an advantage for food, water or other limited resources. In laboratory antibiotics are produced by some microbes. Antibiotics have saved millions of lives and were also known as miracle drug in the past which were no longer the ultimate way for the treatment of the infections because bacteria have continued to develop multiple resistance towards many different type of classes (Waters *et al*.,2005). Antibiotic sensitivity analysis is a test that determines the “sensitivity” of bacteria to an antibiotic which determines the ability of the drug to kill the bacteria. The results of the test helps to determine which drugs are likely to be most effective in treating the infection.

MATERIALS AND METHODS

Sample collection and processing:

Fresh prawns were collected from three different places in Chennai. Sample I from Ennore, Sample II from Korrukupet, Sample III from Chintadripet. The samples were freshly collected early morning from the market and stored in plastic box and it was kept in refrigerator. The muscle of prawn from Sample I was taken and it was cut using sterile scissor and it was nicely homogenized with few drops of saline using sterile mortar and pestle. The procedure was repeated for other two samples.

***Microbiological analysis**:*
Isolation of staphylococci:
From the homogenized mixture using a sterile loop the mixture was taken as it was inoculated on Mannitol Salt Agar (MSA). The plates were incubated at 37ºC for 24 hours. Next day the colonies were picked up and gram staining was done. Gram positive cocci was observed in clusters and it was picked up for further study.
***Antibiotic sensitivity testing**:*
The antibiotic sensitivity testing was carried out by Kirby Bauer disc diffusion method (Bauer *et al.*, 1966).Standard suspension of the isolates were made and the turbidity was matched to Mac Farland standard 0.5. The inoculum was spread evenly on the agar surface using a sterile cotton swab and allowed to dry for 5-10 minutes. Antibiotic disks were placed on the Mueller Hinton agar surface with appropriate spacing. Plates were inverted and incubated at 37°C. After the incubation, the diameter of the zone of inhibition was measured and interpreted according to the CLSI zone size interpretive chart.

RESULT AND DISCUSSION

In the present study, *Staphylococcus aureus* was isolated from fresh prawn and antibiotic sensitivity was analyzed.
Isolation:
In the present study , *Staphylococcus aureus* is isolated from all the three samples (Figure 1).

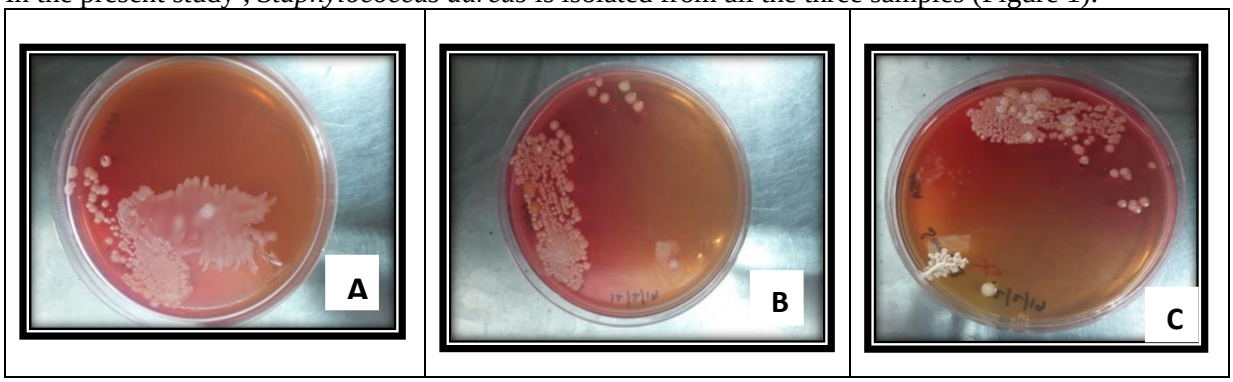

Figure 1: Isolation Of *Staphylococcus aureus* from different market areas in Chennai **A.** Ennore **B.** Korukupet **C.** Chintadaripet
Antibiotic sensitivity:
In the present study, antibiotic sensitivity of *Staphylococcus aureus* was analysed (Figure 2).

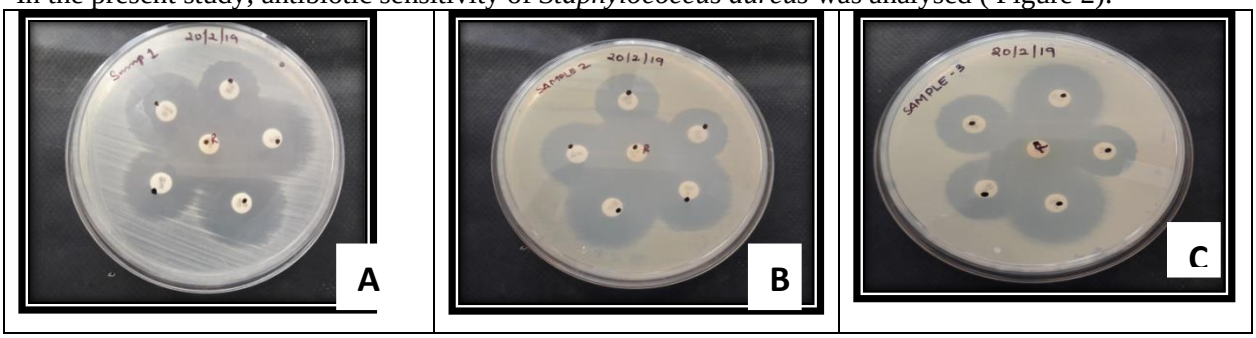

Figure 2: Antibiotic Sensitivity Of *Staphlococcus aureus* By Disc Diffusion Method **A.** Sample I **B.** Sample II **C.** Sample III

I.Chlorampenicol
In the Sample I (29), Sample II (29) it has shown more sensitivity when it is compared to the sample II (22). Whereas, the prawn collected from all the three market area has shown significantly (P<0.3)

increased sensitivity at the level of 30% by t-test interpreted by standard interpretation chart (18) (Figure 3 (A)).

II. Gentamycin

In the Sample I (27), it has shown **more sensitivity** when it is compared to the sample II (20) and III (16). Whereas, the prawn collected from all the three market area has shown significantly (P<0.3) increased sensitivity at the level of 30% by t-test interpreted by standard interpretation chart (15) (Figure 3 (A)).

III.Erythromycin

In the Sample II (23), it has shown **more sensitivity** when it is compared to the sample I (8) and Sample III (15). Whereas, the prawn collected from all the three market area has shown significantly (P<0.3) increased sensitivity at the level of 30% by t-test interpreted by standard interpretation chart (23) (Figure 3 (B)).

IV. Ciprofloxacin

In the Sample II (28) and III (25), has shown **more sensitivity** when it is compared to the sample I (25). Whereas, the prawn collected from all the three market area has shown significantly (P<0.3) increased sensitivity at the level of 30% by t-test interpreted by standard interpretation chart (21) (Figure 3 (C)).

V.Vancomycin

In the Sample I (20), it has shown **more sensitivity** when it is compared to the sample II(12) and III (6). Whereas, the prawn collected from all the three market area has shown significantly (P<0.3) increased sensitivity at the level of 30% by t-test interpreted by standard interpretation chart (17) (Figure 3 (A)).

VI.Rifampicin

In the Sample I (25), it has shown **more sensitivity** when it is compared to the sample II (21) and III (21). Whereas, the prawn collected from all the three market area has shown significantly (P<0.3) increased sensitivity at the level of 30% by t-test interpreted by standard interpretation chart (20) (Figure 3 (A)).

Table 1: Antibiotic Sensitivity of *Staphylococcus aureus*

S. No.	ANTIMICROBIAL AGENT	t- test OF ANTIMICROBIAL AGENT		
		SAMPLE I (mm)	SAMPLE II (mm)	SAMPLE III (mm)
1.	**CHLORAMPHENICOL**	29±3.83	29±*3.83*	22±*3.83*
2.	**GENTAMYCIN**	27±*1.86*	20±*1.86*	16±*1.86*
3.	**ERYTHROMYCIN**	8±1.84	23 1.84	15 1.84
4.	**CIPROFLAXIN**	25± 5	28± 5	25± 5
5.	**VANCOMYCIN**	20± 0.98*	12± 0.98*	6± 0.98*
6.	**RIFAMPICIN**	25± 1.47	21± 1.47	21 ±1.47

Significant at the level of 30% (P<0.3) in t- test
** not significant at the level of 30% (P<0.3) in t- test*

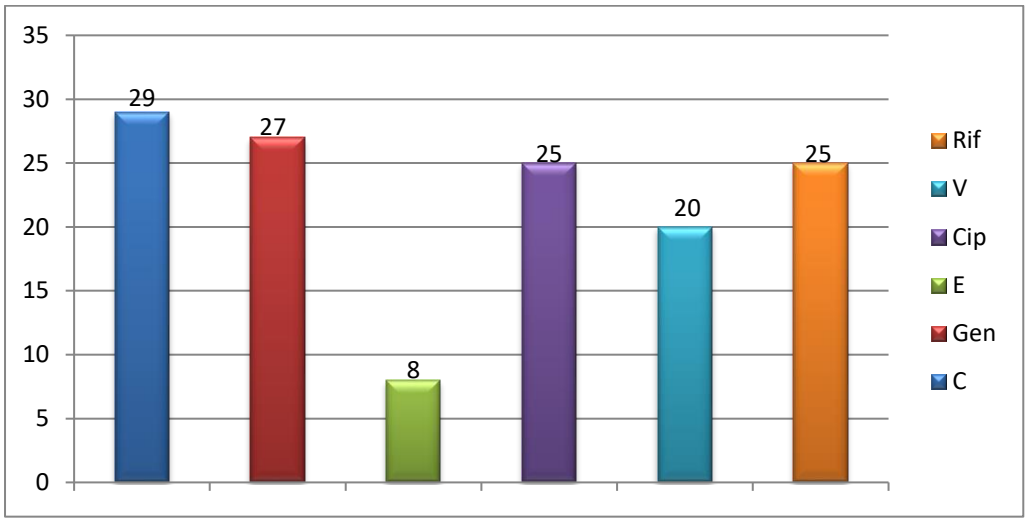

Figure 3 (A): Graphical representation of antibiotic sensitivity in Sample I

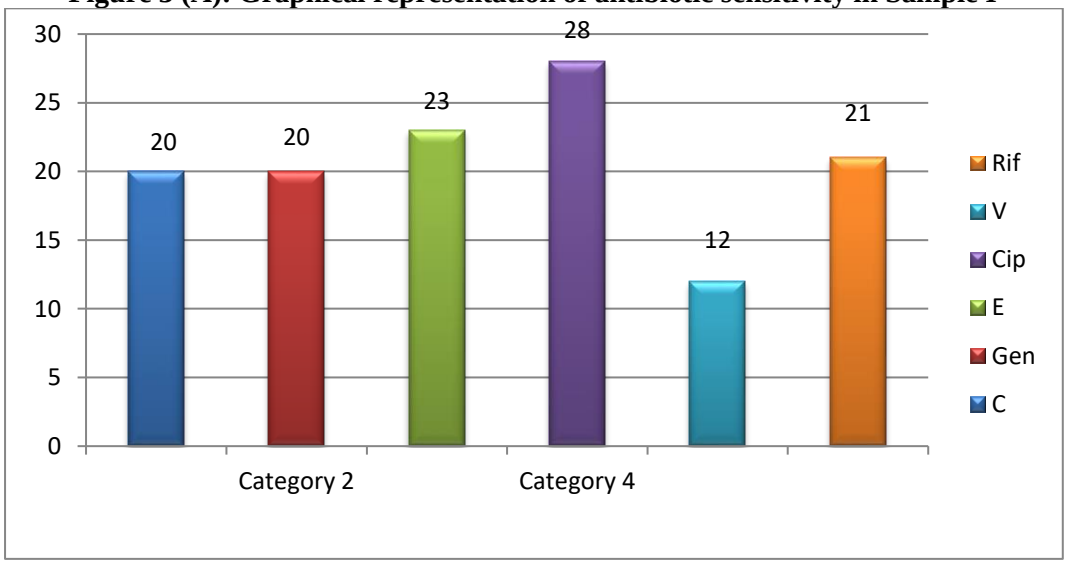

Figure 3 (B): Graphical representation of antibiotic sensitivity in Sample II

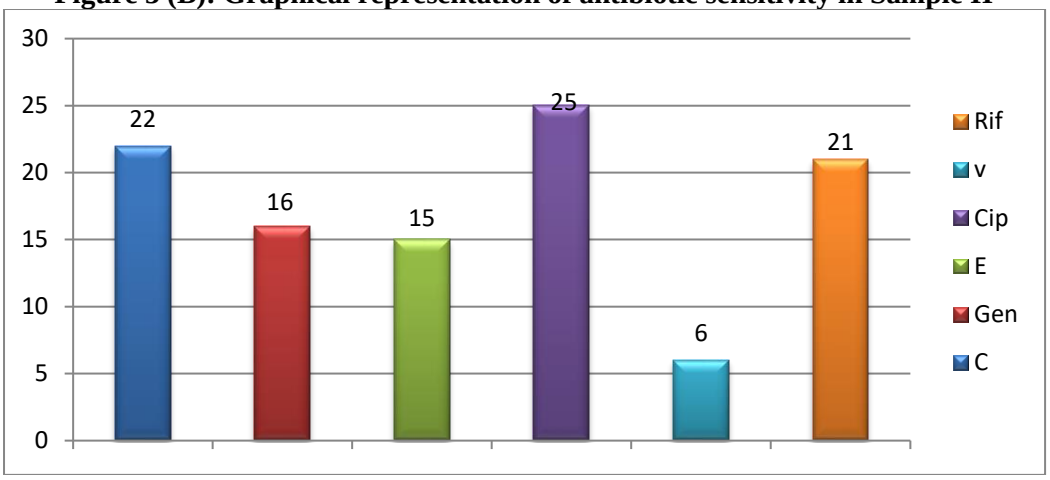

Figure 3 (C): Graphical representation of antibiotic sensitivity in Sample III

Staphylococcus infections are caused by staphylococcus bacteria, types of germs were commonly found on the skin or in the nose of even healthy individuals. The bacteria cause no problems or result in relatively minor skin infections. But staphylococcus infections can turn deadly if the bacteria invade deeper into the body, entering the bloodstream, joints, bones, lungs or heart. A growing number of otherwise healthy people are developing life-threatening staph infections as per the research. Treatment usually involves antibiotics and drainage of the infected area in general. Some staphylococcus infections no longer respond to common antibiotics.

Staphylococcus infections ranged from minor skin problems to endocarditis, a life-threatening infection of the inner lining of the heart (endocardium). As a result, signs and symptoms of staphylococcus infections vary widely, depending on the location and severity of the infection. *Staphylococcus aureus* is found in sea food as the most important pathogen according to various studies.

It is necessary to know *Staphylococcus aureus* and the antibiotics which are sensitive to Prawn. The massive use of antibiotics in feed to promote growth and the in appropriate use of antimicrobial agent in vetenary and human medicine are considered to the major contributors to the emergence of resistance. Moreover *Staphylococcus aureus* is notorious for its ability to become resistance to antimicrobials due to their capacity to produce exopolysaccaride barrier and because of their location within microbes which limit the action of drugs.

After isolating staphylococci, staining method was proceeded to observe *Staphylococcus aureus*. Christian gram devised a method to differentiate two types of bacteria based on their structural differences. In the test bacteria that retain the crystal violet dye because of the thick layer in peptidoglycon are called as Gram positive bacteria. Since the outer membrane is absent in gram positive bacteria, the peptidoglycon layer absorbs the crystal violet stain and shows the shape of the bacteria. In the present observation, by using Gram staining method Staphylococci are seen in clusters and appearance of crystal violet confirms the *Staphylococcus aureus*.

From the present study, it is found that all the three samples which has been collected from three different market area has *Staphylococcus aureus* in the muscle of prawn. The strains which are isolated from three samples are all sensitive to Chloramphenicol (100%), Gentamycin (100%), Rifampicin (100%), Ciproflaxin (100%). Out of three isolates, two isolates was sensitive to Vancomycin. Out of three isolates one isolate was sensitive to Erythromycin.

Due to the indiscriminate and uncontrolled use of antibiotics *Staphylococcus aureus* strains have lost the sensitivity against the common antibiotics. The antibiotic resistant organisms present in fishery products pose a threat in public health activities and clinical practices (Sanjeev *et al.,* 1985).

The findings of study revealed over consumption of prawn from the respected area as a potential risk of food borne poisonings because of its contamination with the strains of *Staphylococcus aureus*. Moreover, contamination is related to the season, sampling sites and the origin of the raw material. It also creates awareness to the people who consumes the prawn regularly. Since *Staphylococcus aureus* infection also enters into the blood stream when it's severe, the consumption of the prawn can be reduced (Scallen *et al., 2*011).

Furthermore, the study also enumerates evaluation of *Staphylococcus aureus* species in fresh prawns from three different areas from Chennai and comprehent the prevalence of *Staphylococcus aureus* in fresh prawns from Chennai. It was found that Chloramphenicol, Gentamycin, Erythromycin, Ciprofloxacin, Rifampicin showed sensitivity in all the three samples. Vancomycin showed resistance in sample III (Figure 3 (A-C))

The safety of fish and shrimp could be directly influenced by the lack of hygienic habits of fish handlers and contact with contaminated work surfaces, including benches, tables and unwashed knives (Albuquerque *et al.,* 2007). Various factors impinge upon seafood safety that ranges from contamination originating from the environment where it is caught to contamination caused by the consumer prior to eating (Abdulla *et al.,* 2003).

Thus, the identification of the sensitive antibiotics will be useful for the treatment of *Staphylococcus aureus* infection from the edible prawns and the prevalence of the *Staphylococcus aureus* in the edible prawns from the market areas of Chennai will help to further be cautious of the intake of the prawns inclusive to clean environment to understand and create awareness on the seriousness of the infection which leads to foodborne disease and determination of antibiotic sensitivity of *Staphylococcus aureus* in edible prawns will help to treat the patients those are infected from the bacteria.

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