# Prevalence of Staphylococcus in raw meat samples in Southern Assam, India

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***Abstract:*** *The objective of the study is to find the prevalence of staphylococcus from raw meat samples in Southern Assam. In the present study 65 raw meat samples (chicken and goat) were collected from various regions in and around Southern Assam. Out of 65 samples analyzed 30 samples 17 from chicken and 13 from goat were positive for Staphylococci with the prevalence rate of 48.57% from Chicken and 43.33% from goat. Staphylococcal isolates were found variably resistant to the antibiotics tested. 80% of the isolates were positive for at least one of the antibiotics used in this study. The isolates showed maximum resistance for penicillin (73.33%) which is followed by Erythromycin(36.66%) Tetracyclin(26.66%) Oxacillin(23.33%) Ciprofloxacin (16.66%) Chloramphenicol(10%) Vancomycin(3.33%). The high prevalence of antibiotic resistant staphylococcal isolates found in raw meat samples in this study becomes a major health concern for the butchers as well as consumers.*
***Key words:*** *Antibiotics, staphylococci, vancomycin,*

## I.    Introduction

Food Borne Diseases (FBD) are defined by the World Health Organization (WHO) as diseases of infectious or toxic nature caused by, or thought to be caused by the consumption of food and water. The pathogenesis of bacteria causing food borne poisoning depends on their capacity to produce toxins after ingestion (in the digestive tract) or intoxication (ingestion of preformed toxins in food stuff) large numbers of bacteria are involved in causing food poisoning. Among the bacteria the most predominantly involved in these diseases is Staphylococcus species. Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food. (1)

Staphylococcal food poisoning includes symptoms such as sudden onset of nausea, vomiting, abdominal cramps and diarrhea (2). On heating at normal cooking temperature, the bacteria may be killed but the toxins remains active (3). The staphylococcal enterotoxins are highly heat resistant and are thought to be more heat resistant in food stuffs than in a laboratory culture medium (4). Besides these, enterotoxins producing Staphylococcus aureus are most dangerous and harmful for the human health. About 50% strains of these organisms are able to produce enterotoxins associated with food poisoning. Meat being a highly nutritious food, acts as a good material for bacterial growth; its quality depends on the initial bacterial contamination. This contamination causes meat deterioration, lowers quality and sometimes illness may be caused by bacterial pathogens or their toxins through meat and meat products. Studies have shown that meat can serve as a vehicle for transmitting Staphylococci from animal to cause the severe food borne intoxication in human (5, 6). Apart from pathogenicity, one more consideration in the context of food safety and health concern is the presence of transmissible antibiotic resistance markers. Staphylococci isolated from food are frequently resistant to one or more antibiotics (7, 8, 9) and hence could act as agents for spreading of antibiotic resistance genes (10). Besides, antibiotic residues in foods of animal origin affect the nutritive value of these foods. Recently there has been an enormous increase in the isolation of  Methicillin-resistant Staphylococcus aureus (MRSA) strains resulting from wide spread and prolonged use of methicillin in clinical settings and food animal production facilities (11, 12). Resistance to methicillin is mediated through the mec operon which is a part of the staphylococcal cassette chromosome mec (SCCmec) (13). The mecA gene codes for an altered penicillin-binding protein, PBP2a, which has a lower affinity for binding β-lactam antibiotics (14). S. aureus possesses several virulence factors responsible for its pathogenicity to the host.

During slaughtering processes, MRSA can be contaminated on carcasses (15), contributing to the high contamination rates of MRSA on retailed meats in fresh markets. The super antigens can be found in those MRSA strains. Therefore, meats and meat products act as a vehicle in transmission of MRSA to the butchers and consumers (16). The surveillance of MRSA in retailed meats was thought to be important. Although several studies of MRSA in meats have been documented in various countries (17, 18, 19, 20). But there is insufficient evidence available on the properties of Staphylococcus spp. isolated from meat samples in Southern Assam. In the present study, an attempt has been made to detect staphylococci in retailed meat samples collected from various regions in and around Southern Assam and to evaluate their antibiotic sensitivity patterns.

## II.   Materials and Methods:

**2.1 Collection of samples and screening of Staphylococcus isolates:**

Fiftysix raw meat samples of chicken and goat were collected from different retailers of various regions in and around Southern Assam. Sampling box containing ice pads were used for carrying the samples from market to laboratory maintaining low temperature. The meat samples were brought to the laboratory within two hours for processing. Ten grams of meats were mixed with 90 ml of staphylococcus enrichment broth and homogenized for 1 min. The liquid portion was incubated at 37ºC without shaking for 6 h. Subsequently, one ml of bacterial culture was diluted as dilution of $10^{-1}$ and $10^{-2}$ and plated on Mannitol Salt agar (MSA) and Baird-Parker Agar (BPA). The plates were then incubated at 37ºC for 24-48 h.  Screening of staphylococcal isolates was done on the basis of morphology, Gram's stain, catalase, coagulase and mannitol fermentation tests according to standard protocols.

**2.1.1Morphological characteristics:**

The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's method of staining. The stained smear was observed under microscope.

**2.2. Biochemical characterization of the isolates:**

After morphological characterization, the isolates were also investigated for five different biochemical characteristics namely catalase test, oxidase test, mannitol fermentation test, starch hydrolysis test and coagulase test.

**2.2.1. Catalase test:** A single colony from a pure culture plate was picked using a sterile loop and mixed with 3% $H_2O_2$ on a clean glass slide. Liberation of oxygen in the form of bubbles within a few seconds was taken as a positive test.

**2.2.2. Oxidase test**: A single colony from a pure culture plate was picked using a sterile loop. Culture was then streaked on oxidase disk and wait for 20 sec to observe colour change if blue colour appears it indicates positive result.

**2.2.3. Mannitol fermentation test:** The isolates grown on MSA were classified as either positive or negative for mannitol fermentation depending on their ability to ferment mannitol resulting in change of pH of the medium. This pH change is indicated in the form of discolouration of the medium from red to yellow. The isolates growing on MSA indicated the growth of salt tolerant staphylococci. Discolouration of the medium from red to yellow was taken as positive result while no change in medium colour was recorded as negative result for mannitol fermentation.

**2.2.4 Starch Hydrolysis test:** Starch agar medium was prepared in conical flask and autoclaved at $121^{o}$c for 15 mins. Media was then poured on petri plates and allowed to solidify. Isolates were streaked on the plates and kept at $28^{0}$c for 48 hours. Plates were then flooded with iodine solution and result was observed. Formation of clear zone indicated positive result.

**2.2.5 Coagulase test:** The tube coagulase test was performed in sterile tubes by adding 0.5 ml of broth culture of the selected isolates to 0.5 ml of citrated rabbit plasma. After mixing the contents, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of uninoculated sterile broth and 0.5 ml of citrated rabbit plasma. The tubes were monitored for clot formation at 30 minutes interval for the first 4 hours and then after 24 hours incubation. The reaction was considered positive if a clot was visible within the tube and negative if no degree of clotting was visible.
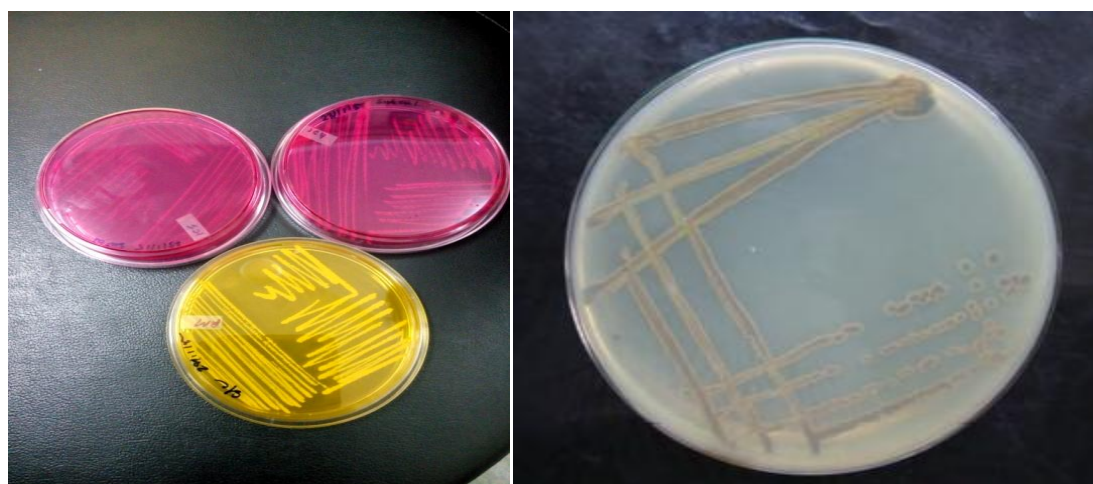
**2.3 Antibiotic sensitivity test:**

Antibiotic sensitivity was performed using the Kirby-Bauer disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). The screened staphylococcal isolates were tested with a panel of 7 antibiotics namely penicillin G (10 units), oxacillin (1 mcg), chloramphenicol (30 mcg), ciprofloxacin (30 mcg), erythromycin (15mcg), tetracyclin (25mcg) and vancomycin (30mcg), Results were recorded after 24 hours of incubation at 35°C on Mueller Hinton agar and interpreted as per NCCLS (2009) (21)standards.

## III. Results:

A total of 56 meat (chicken and goat) samples were collected from different retailers of Southern Assam. Out of these 56 isolates 30 probable Staphylococcal isolates were selected after observing their morphology on Braid Parker agar medium (black colonies on Baird-Parker Agar),  Mannitol Salt agar medium (creamy or yellow colonies on Mannitol Salt Agar) and gram's staining characteristics (gram positive, spherical cells arranged in regular clusters resembling to branch of grapes.)

**Table 1: Showing prevalence of Staphylococcus in collected samples**

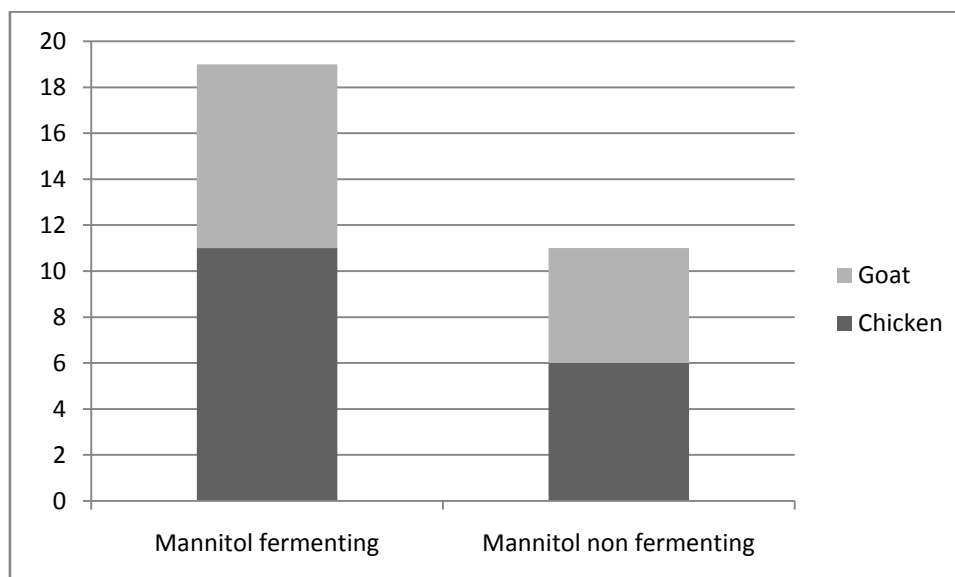| Meat type | No. of sample | No. of isolates | No.(%) of Staphylococcal positive isolates |
|---|---|---|---|
| Chicken | 31 | 31 | 17(48.57) |
| Goat | 25 | 25 | 13(43.33) |
| Total | 56 | 56 | 30(46.15) |



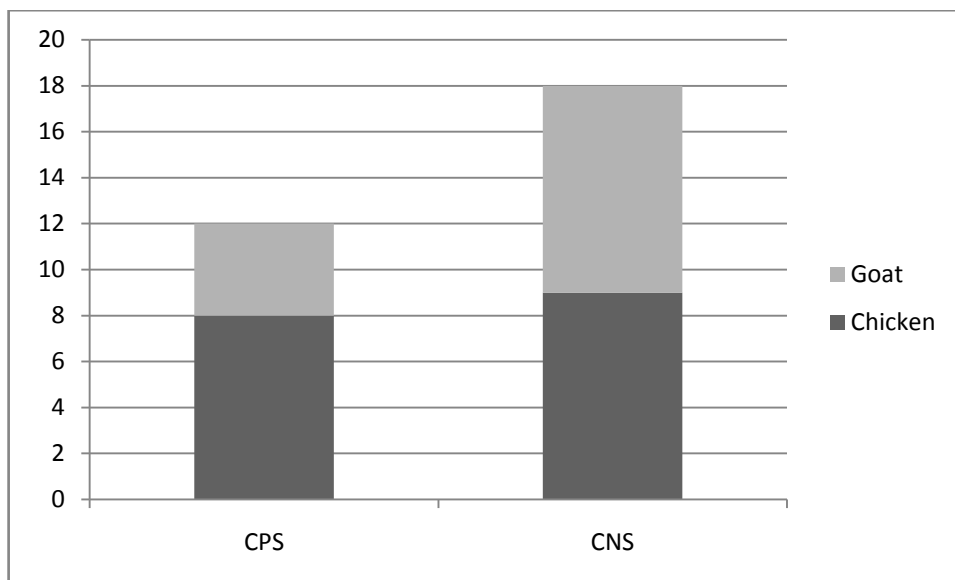**Fig1: Colonies of Staphylococcus on Braid Parker agar and Mannitol Salt agar**

In the present study almost all the 30 isolates were positive for catalase test and negative for oxidase and starch hydrolysis, but shows variation in the results of mannitol fermentation, coagulase test. The percentage of coagulase negative Staphylococcus (CNS) was higher (60%) than that of coagulase positive Staphylococcus (CPS) (40%).

**Table2: No. and Rate percentage of positive and negative isolates for Mannitol Fermentation and Coagulase test.**

| Meat type | No of isolate | Mannitol fermentation | | | | Coagulase test | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | positive | | negative | | positive | | Negative | |
| | | No | % | No | % | No | % | No | % |
| Chicken | 17 | 11 | 64.70 | 6 | 35.29 | 8 | 47.05 | 9 | 52.94 |
| Goat | 13 | 8 | 61.53 | 5 | 38.46 | 4 | 30.76 | 9 | 69.23 |
| Total | 30 | 19 | 63.33 | 11 | 36.66 | 12 | 40 | 18 | 60 |



**Fig2: Distribution of Mannitol fermenting and Mannitol non fermenting in the collected samples**

**Fig 3: Distribution of CNS and CPS in the collected samples**

The staphylococcal isolates detected in this study showed the following frequencies of resistance to the 7 antibiotics used. 24 out of the 30 isolates (80%) were resistant to at least one of the antibiotics used. High rate of resistance was observed for penicillin with 73.33% isolates showing resistance. Among the 30 isolates, 22 , 7, 3, 5, 11, 8 and 1were resistant to penicillin, oxacillin, chloramphenicol, ciprofloxacin, erythromycin, tetracycline and vancomycin respectively.

**Table 3: showing antibiotic sensitivity patterns of the staphylococcal isolates**

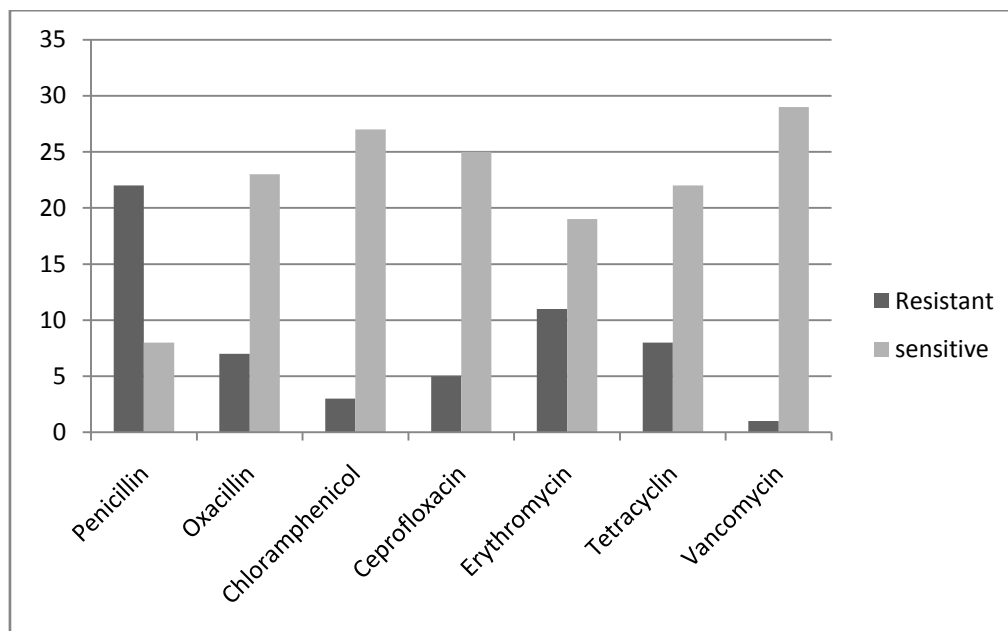| Antibiotics | Disc Content (mcg/disc) | Resistant | | Sensetive | |
|---|---|---|---|---|---|
| | | Number | **%** | Number | **%** |
| Penicillin | 10 units | 22 | 73.33 | 8 | 26.66 |
| Oxacillin | 1 units | 7 | 23.33 | 23 | 76.66 |
| Chloramphenicol | 30 units | 3 | 10 | 27 | 90 |
| Ciprofloxacin | 30 units | 5 | 16.66 | 25 | 83.33 |
| Erythromycin | 15 units | 11 | 36.66 | 19 | 63.33 |
| Tetracyclin | 25 units | 8 | 26.66 | 22 | 73.33 |
| Vancomycin | 30 units | 1 | 3.33 | 29 | 96.66 |

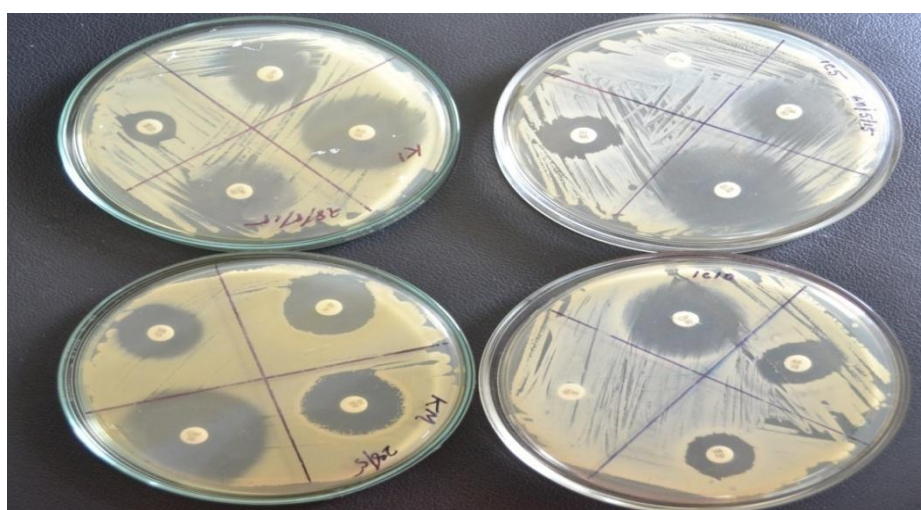**Fig 4: Antibiotic Sensitivity pattern of the Staphylococcal isolates**



**Fig 5: Showing zone of inhibition of Staphylococcal isolates on Muller Hinton Agar**

## IV. Discussion

Staphylococci have been frequently isolated from raw meat samples with reports of significant toxin production implicated in food poisoning cases. In the present study Staphylococcal isolates were detected with the prevalence rate of 48.57% from chicken and 43.33% from goat meat.

Many researchers have reported the presence of staphylococci in meat samples with varying prevalence rates. Similar results were also reported by Suk-kyung et al., 43.3 % (22), Shareef et al., 52.04 %(23), Kozacinski et al., 46.15 % (24), Citak and Duman 47.2 % (25) and Lee et al., 50 % (26) regarding the prevalence of Staphylococcus in chicken. Ebrahim et al.,(27)reported 47.5%  prevalence rate of staphylococci in goat meat which was in accordance with our study. In our study the percentage of CNS was higher 60% compared to that of CPS 40%. Goja et al.,(28) Yurdakul et al.,(29) also reported the higher percentage of CNS from meat samples. The high number of CNS isolated in this study could be justified by the fact that CNS are found abundantly in the normal teat skin flora and mucosa of humans and animals while some are free living in the environment (30).  The contamination of meat by Staphylococcus may directly occur due to skin lesions or sneezing and coughing (31) or indirectly through working surface or slaughtering equipments (32). Antibiotic sensitivity test of the isolates revealed maximum resistance for penicillin 73.33% which is in accordance with previous studies by Lee et al.,(33) Mohan et al.,(34), Aslantas et al.,(35).  In our study one of the isolate from goat meat showed resistant to all the 7 antibiotics used in the study. This finding supports the earlier report by Ziad W Jaradat et al., (36)  The resistance pattern of other antibiotics are as follows:  Erythromycin (36.66%),

Tetracyclin(26.66%) Oxacillin(23.33%) Ciprofloxacin (16.66%) Chloramphenicol(10%) Vancomycin(3.33%). This result showed closest similarity with the study by Yurdakul et al., (29) .But the resistance for Vancomycin was higher compared to that of our study. The isolates showed high susceptibility for chloramphenicol and ciprofloxacin as reported earlier by Umaru et al., (37) and Lee (32). However, antibiotic resistance development among the Staphylococcal isolates poses a problem of concern. Such a trend of resistance to antibiotics can be attributed to random use of antibiotics, dry period treatments and different treatment choices in farms of this region (34).

## V. Conclusion:

Staphylococcal food poisoning is a major concern in public health programs worldwide.  In our study we isolated both coagulase positive and coagulase negative Staphylococcal isolates from raw meat samples. Among these isolation of staphylococci, coagulase negative staphylococci were the major isolates. This mean that all these species were associated with human specially on the skin and mucous membrane and considered as normal human flora. Therefore, proper handling of raw meat, adequate cleaning of hands, surfaces, equipments, disinfection of  slaughter houses, vehicles and good personal hygiene can reduce spreading of Staphylococcus through meat. The high prevalence of Antibiotic resistant Staphylococcus in raw meat samples in this region becomes a major health concern among the population. Therefore, further molecular based studies are necessary to identify the Staphylococcal isolates and their toxin-producing potential for improved management of food and to decrease human diseases.

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