

## OCCURRENCE AND ANTIBIOTIC SENSITIVITY PROFILING OF *Staphylococcus aureus* ISOLATED FROM RAW CHEVON

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### ABSTRACT

The chevon is an animal source protein that is delicious, nutritious and a very well-known food for all. This study aimed to determine the prevalence as well as the antibiotic sensitivity of *Staphylococcus aureus* in chevon sold at the different wet markets in Dhaka City. A total of 120 samples of raw chevon were collected from some selected areas. The cultural characteristics, staining and biochemical properties were used to isolate and identify the *S. aureus* bacteria. The highest and lowest Total Viable Count (TVC) in the supplied sample were  $\log_{10}9.22$  CFU/gm and  $\log_{10}9.04$  CFU/gm, respectively. The overall prevalence of *S. aureus* in Dhaka City was 47.5%. The highest prevalence was recorded at BNP Bazar (65%) of the Dhaka City. Isolates were investigated for antibiotic sensitivity profile using a Kirby-Bauer disc diffusion assay against five common antibiotics used in goats. The *S. aureus* showed the highest sensitivity to gentamicin (57.9%), followed by ciprofloxacin (56.14%), and the highest resistance pattern was shown against amoxicillin (100%), followed by ampicillin (92.98%), and tetracycline (63.15%). These bacterial contaminations in the chevon may be mitigated following proper hygienic management, proper boiling or cooking, and public health awareness.

**Keywords:** *Staphylococcus aureus*, detection, prevalence, antibiotic sensitivity

### INTRODUCTION

Goats were the first animals that humans domesticated (Saeid *et al.*, 2008). The world's goat population is currently around one billion, which is less than cattle (1.5 billion) and sheep (1.2 billion). On the other hand, Asia accounts for 54.4 % of the world's goat population (FAOSTAT, 2020). The goat is one of the most popular and commonly raised meat animals in Asian countries (Mazhangara *et al.*, 2019). In Bangladesh, there are about 26.6 million goats, of which most of them are Black Bengal Goats (DLS, 2020). Goat meat, often known as chevon, is the meat of domestic goats (*Capra hircus*). Because of its unique taste and lack of religious barriers goat meat has occupied an acceptable and sustained place in the diet as a source of animal protein among diverse red meats. Meat is highly important in maintaining the human body's strength to produce energy, health, and vigor (Das and Saikia, 2017).

Many researchers had isolated and identified *Staphylococcus aureus* (*S. aureus*) bacteria from raw chevon. The *S. aureus* cells are Gram-positive and appear in a spherical shape. They are often in clusters resembling a bunch of grapes when observed under a light microscope after Gram staining. The name 'Staphylococcus' was derived from Greek, meaning a bunch of grapes (staphyle) and berry (kokkos). The diameter of the cells ranges from 0.5 to 1.0  $\mu\text{m}$  (Foster, 1996). The *S. aureus* is a commensal and opportunistic pathogen that causes a wide spectrum of infections, superficial skin infections, fatal and invasive diseases (Abebe 2020). Because of a combination of "toxin-mediated pathogenicity, invasiveness, and antibiotic resistance" this ubiquitous bacterium is a significant pathogen. The *S. aureus* is a desiccation-resistant organism with the ability to thrive in potentially dry and stressful habitats, such as the human nose and on the skin and inanimate surfaces such as clothing surfaces (Chaibenjawong and Foster, 2011).

Antimicrobial resistance (AMR) is another common reason for antimicrobial therapy failure. It occurs when microbes evolve mechanisms that protect them from the effects of antimicrobials (WHO, 2014). The introduction of new antibiotics to counter this pathogen has frequently been closely followed by the emergence of resistant strains. Most notably, isolates of *S. aureus* resistant to  $\beta$ -lactams have

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become frequent, and many of these are also resistant to  $\beta$ -lactamase-resistant penicillin (Schito, 2006). Methicillin-resistant *S. aureus* (MRSA) connected with livestock is on the rise, and there is a considerable chance that it will spread zoonotic diseases. Professionals in the agricultural sector are particularly at risk for infection (Guardabassi *et al.*, 2013), and probably the community through the food chain (Kluytmans, 2010).

Chevon is one of the world's most important meat sources, especially for the Indian subcontinent (Mazhangara *et al.*, 2019). In Bangladesh, the goat contributes to a major part of its economy. The economic aspect of goat disease, as well as their mortality and morbidity due to bacterial infection, is of great concern to livestock owners and the government. Due to a lack of awareness and research, bacteria specially *Staphylococcus aureus* present in chevon and mutton and the consequences of these on human health remain unknown. The region related recent research data were not available in Bangladesh for developing the control and prevention program as well as further research. On the above circumstances, the present study was conducted with the objectives of identification and occurrences of the *Staphylococcus aureus* in raw chevon along with an assessment of antibiotic resistance pattern of the isolates in the Dhaka City.

## MATERIALS AND METHODS

The present study was conducted during the period of January 2021 to June 2021. The samples were collected from the different retail shops of the Kacha Bazar in Dhaka City. Then collected samples were brought to the Laboratory of the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University for further analysis. A total of 120 raw chevon (goat meat) samples were collected from six different markets in Dhaka City namely Krishi market, Geneva camp, Taltola bazar, BNP bazar, Agargaon kacha bazar, and Mohammadpur town hall market. The net weight of the each sample was 500 gm. The samples were promptly stored in the icebox, and transferred to the Departmental Laboratory of this University.

The stock culture of the collected chevon was prepared using phosphate buffer solution (PBS). At first, the stock culture was made by mixing of the 1 ml of PBS with 1 ml of pure culture in nutrient broth and storing it at  $-20^{\circ}\text{C}$ . The 10-fold dilution was made for total viable count (TVC). Then the bacteria were cultured on nutrient broth and incubated at  $37^{\circ}\text{C}$  for 24 hours. The bacteria were detected based on cultural properties on different culture media (Nutrient agar, Blood agar, Mannitol salt agar). Morphological characterization was done using gram staining method. The biochemical tests of isolates were also done for observing the biochemical properties of the isolates. Antibiotic sensitivity profiling of the isolated bacteria was shown using disc diffusion methods. The procedure suggested by Carter (1986) and Thaker *et al.* (2013) was followed throughout the experiment for isolation and identification of bacteria.

### Antibiotic discs

The antibiotics susceptibility pattern was determined using commercially available antibiotic discs (OXOID Limited, Canada). After placing the discs on the plate, the plates were inverted and incubated at  $37^{\circ}\text{C}$  for 16 to 18 hours. The diameter of the full inhibitory zones (including the diameter of the disc) was measured and recorded in millimeters after incubation. Without opening the lid, measurements were taken with a ruler on the underside of the plate. The zones of growth inhibition were compared to the Clinical and Laboratory Standards Institute's zone-size interpretive (CLSI, 2007). According to CLSI, zone diameter interpretation standards, antimicrobial testing findings were classified as sensitive, intermediate, or resistant on Table 1.

### Statistical analysis

Data were entered into the Microsoft Office Excel 2021 spreadsheet. The recorded data were analyzed using SPSS software (version 20.0). The means value and standard deviations (mean $\pm$ SD) were determined. The  $P$  ( $< 0.05$ ) value was calculated using chi-square test.

**Table 1. Drugs with their disc concentration for the Enterobacteriaceae family**

Name of Antibiotic	Disc conc. ( $\mu\text{g}$ /disc)	Zone Diameter Interpretive Standard (mm)		
		Resistant	Intermediate	Susceptible
Gentamicin (GM)	10	$\leq 13$	14-17	$\geq 18$
Tetracycline (TE)	30	$\leq 14$	15-18	$\geq 19$
Amoxicillin (AMX)	10	$\leq 13$	14-17	$\geq 18$
Ampicillin (AMP)	10	$\leq 13$	14-16	$\geq 17$
Ciprofloxacin (CIP)	5	$\leq 15$	16-20	$\geq 21$

## RESULTS AND DISCUSSION

The results presented below demonstrated the isolation and identification of the *S. aureus* bacteria from raw chevon samples at surrounding area of the Dhaka City. The results also indicated the prevalence and antibiotic sensitivity resistant pattern of the isolates to different antibiotics.

### Determination of TVC

**Table 2. The TVC of supplied chevon sample sold at different wet markets in Dhaka City**

Name of themarkets	TVC logcfu/ml (Mean $\pm$ SD)	P-value
Krishi Market	9.06 $\pm$ 0.02	0.01
Geneva Camp	<b>9.23 <math>\pm</math> 0.01</b>	0.01
Taltola Bazar	9.12 $\pm$ 0.02	0.01
BNP Bazar	9.18 $\pm$ 0.01	0.01
Agargaon Bazar	9.13 $\pm$ 0.02	0.01
Town Hall market	<b>9.02 <math>\pm</math> 0.03</b>	0.01

The assessment of total viable bacterial counts using aerobic plate count was shown in Table 2. The average microbial load on fresh meat from several markets ranged from  $\log_{10}9.02 \pm 0.03$  CFU/gm to  $\log_{10}9.23 \pm 0.01$  CFU/gm. Geneva camp had the greatest bacterial load ( $\log_{10}9.23 \pm 0.01$  CFU/gm), followed by the lowest observation ( $\log_{10}9.02 \pm 0.03$  CFU/gm) in Town Hall Kacha Bazar. According to Mukhopadhyaya *et al.*, (2009), the presence of a high number of microorganisms ( $\text{APC} > 10^7 \text{cfu/cm}^2$ ) accelerates the deterioration of meat. In a TVC column were found to be statistically significant ( $P=0.01$ ).

In another study conducted by Haque *et al.* in 2008, they found a  $\log_{10} 6.03$  CFU/gm in slaughter yards and  $\log_{10} 6.53$  CFU/gm in meat stall samples respectively in Mymensingh town. The different TVC values in various time intervals were observed in another study conducted by Parvin *et al.*, in 2017. She observed TVC values of  $5.17 \pm 0.28$  log CFU/gm (0 hrs),  $6.64 \pm 0.05$  log CFU/gm (2 hrs), and  $8.47 \pm 1.27$  log CFU/gm (5hrs) respectively. In Tripura of India, another study was conducted by Shapna *et al.* (2018) and found a TVC of  $6.84 \pm 0.42$  log CFU/gm, which was significantly lower than the current study. This discrepancy could be attributed to differences in season, environmental variation, management, biosecurity of the slaughter house or different study methods.

### Cultural properties in different media

After 24 hours of aerobic incubation at  $37^{\circ}\text{C}$ , the nutrient broth revealed the growth of bacteria as indicated by the turbidity. In this current study, *Staphylococcus* species produced turbidity in nutrient broth. The *Staphylococcus* species produced gray to whitish colored colonies in nutrient agar plates after 24 hours of aerobic incubation at  $37^{\circ}\text{C}$ . In blood agar media, the large, creamy white, beta-hemolytic colonies were found. The growth revealed golden yellow colored colony in mannitol salt agar (Fig. 1). The findings of this research were consistent with the report of MCL and Sborough (2005); and Cheesbrough (2006).

**Table 3. Morphological and cultural properties of *Staphylococcus* species from chevon**

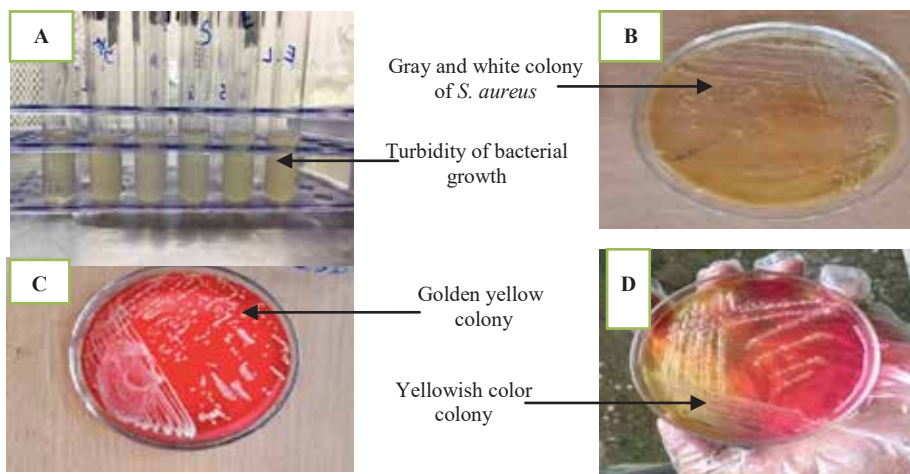
Feature	Appearance
Nutrient agar	Gray, white, or yellowish colony
Blood agar	White to golden yellow colony
Mannitol salt agar	Yellow colour colony
Staining properties	Gram positive, cocci arranged in grapes like clusters

**Gram’s staining properties of the *Staphylococcus* species**

The bacterial smears were examined under microscope which revealed Gram-positive, cocci shaped and arranged in grapes like clusters.

**Biochemical properties of the *Staphylococcus* species**

The biochemical properties of the *Staphylococcus* species were observed and showed in Table 4. These bacteria fermented all the five basic sugars and produced only acid. It showed a positive reaction in the cases of catalase, coagulase tests and MR-VP tested but negative results were found in the case of Indole reaction. The coagulase test was used to distinguish pathogenic *S. aureus* from non-pathogenic *S. aureus*. The biochemical properties of these bacteria were also supported by Cheesbrough (2006) and OIE Manual (2000).



**Fig. 1. Turbidity of bacterial growth was seen in nutrient broth (A); Gray and white colony of *S. aureus* in Nutrient agar (B); Golden yellow colony in Blood agar (C) and Yellowish color colony in Mannitol salt agar**

**Table 4. Biochemical properties of *Staphylococcus aureus***

Fermentation properties with five basic sugars					Indole test	VP Test	MR Test	Catalase test	Coagulase Test
DX	ML	L	S	MN					
A	A	A	A	A	-	+	+	+	+

**Legends:** DX= Dextrose; ML = Maltose; L =Lactose; S = Sucrose; MN = Mannitol;  
A = Acid production; + = Positive reaction; - = Negative reaction.

**Occurrence of isolated bacteria**

Occurrence of *Staphylococcus aureus* found in this study area in wide range (Table 5). The overall occurrence of these bacteria in the Dhaka City was 47.5%. The highest (65%) outbreak was recorded in

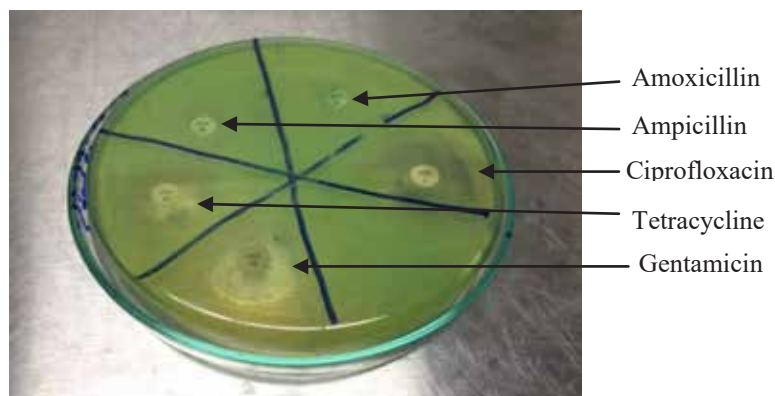
the BNP Bazar. lowest (35%) observation was found in both Krishi market and Taltola Bazar. The researcher, Das and Saikia (2017) found 43.33% prevalence in goat meat in Assam, which was slightly lower than the current study. It might be due to environmental or managerial variation.

**Table 5. Occurrences of the *Staphylococcus aureus* from meat samples in different markets**

Location	No. of Sample	No. of positive sample	Occurrence (%)
Krishi Market	20	7	35
Geneva Camp	20	12	60
Taltola Bazar	20	7	35
BNP Bazar	20	13	65
Agargaon Kacha Bazar	20	10	50
Town Hall Market	20	8	40
<b>Total</b>	<b>120</b>	<b>57</b>	<b>47.5</b>

#### Antibiotic sensitivity profiling of *Staphylococcus aureus*

A total of 57 positive samples of *Staphylococcus aureus* from chevon samples were detected based on cultural properties, staining properties and biochemical properties. The cultural sensitivity test (CS test) was done for knowing the antibiotic sensitivity pattern (Fig. 2 and Table 6). All 57-sample were resistant to amoxicillin. The gentamicin and ciprofloxacin were comparatively sensitive drugs against these bacteria. All isolated bacteria showed 100% resistant to amoxicillin, followed by ampicillin (92.98%), gentamicin (63.15%), and almost similar resistant against tetracycline (57.9%), and ciprofloxacin (56.14%).



**Fig. 2. Antibiotic sensitivity test of *Staphylococcus aureus***

Earlier observations by Hassan *et al.*, (2021) was that Tetracycline (100%) and Ampicillin (90%) resistant to *S. aureus* and 100% sensitive to ciprofloxacin. The scientist Bantawa *et al.*, (2019) in Nepal found 100% amoxicillin resistant. Isolates from raw chevon were sensitive to gentamicin (63.15%) followed by ciprofloxacin (56.14%). The results strengthen the earlier observations of Zehra *et al.*, (2019) who found 54.86% to gentamicin and 38.2% sensitive to ciprofloxacin on meat samples in Panjab, which was slightly lower than the current study. The discrepancy in antibiotic sensitivity was slightly lower probably due to genetic variation of the pathogen, environmental variation, or study methods.

**Table 6. Antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from chevon**

SN.	Antibiotics used	Resistant	Intermediate	Susceptible
1	Amoxicillin	57 (100%)	-	-
2	Ampicillin	53 (92.98%)	4 (7.02%)	-
3	Gentamicin	7 (12.28%)	17 (29.82%)	33 (57.89%)
4	Tetracycline	36 (63.15%)	12 (21.05%)	9 (15.8%)
5	Ciprofloxacin	6 (10.53%)	19 (33.33%)	32 (56.14%)

## CONCLUSION

The current study was conducted from January to June 2021 to detect the *Staphylococcus aureus* along-with antibiotic sensitivity pattern of these bacteria from raw chevon in different wet markets of Dhaka North City Corporation. A total of 120 samples were collected from 6 different wet markets. The prevalence of *Staphylococcus aureus* contamination in the raw chevon was 47.5%. The highest TVC ( $\log_{10}$  9.22±0.01 CFU/ml) was found in the Geneva Camp and the lowest was recorded in Town Hall Kacha Bazar ( $\log_{10}$  9.02±0.03 CFU/ml). The amoxicillin showed 100% resistant. The gentamicin and ciprofloxacin were found as moderate sensitive against these bacteria. Some variation in the results from previous findings might be due to the environmental or managerial variation, types of the cultural media procedures and genetic variation of the bacteria.

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