OCCURRENCE AND ANTIBIOTIC SENSITIVITY PROFILING OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RAW CHEVON

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This is to certify that the thesis entitled "**Occurrence and Antibiotic Sensitivity Profiling of *Staphylococcus aureus* Isolated from Raw Chevon**" submitted to the Department of Microbiology and Parasitology, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, in partial fulfillment of the requirements for the degree of **Master of Science (MS) in Microbiology**, embodies the result of a piece of bona fide research work carried out by **Md Nehal Hasnain**, Registration no. : **14-06044**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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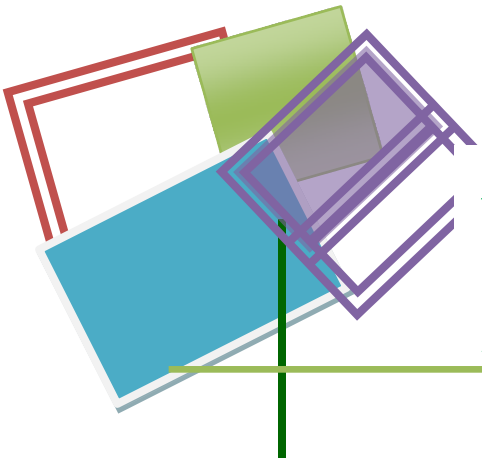

Dedicated
To My
Beloved Parents

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATION	FULL WORD
AMP	Ampicillin
AMX	Amoxicillin
Approx.	Approximately
BG	Brilliant Green
CFU	Colony Forming Unit
CIP	Ciprofloxacin
DNA	Deoxyribonucleic acid
EMB	Eosin Methylene Blue
et al.	and others
ESBL	Extended-Spectrum Beta-Lactamase
E.coli	*Escherichia coli*
etc.	Etcetra
Fig.	Figure
GM	Gentamicin
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen Sulphide
hrs.	Hours
IN	Intermediate
Lbs.	Pound
Ltd.	Limited
Mg	Milligram

LIST OF ABBREVIATION AND SYMBOLS (CONT'D)

ABBREVIATION	FULL WORD
MH	Muller Hinton
Ml	Millilitre
Mm	Milimeter
mon.	Month
Min.	Minute
MR	Methyl Red
NCCLS	National Committee for Clinical Laboratory Standard
NM	Non motile
No.	Number
PCR	Polymerase chain Reaction
PBS	Phosphate buffered solution
R	Resistant
S	Sensitive
SAU	Sher-e-Bangla Agricultural University
Sp	Species
SLT	Shiga-like toxin
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
TET	Tetracycline
TSI	Tripple Sugar Iron
UTI	Urinary tract infection
VP	Voges-Proskauer
yrs.	Years
ºC	Degree Celsius
%	Percentage
~	Tilde
®	Registard trade mark
µg	Microgram

Occurrence and Antibiotic Sensitivity Profiling of *Staphylococcus Aureus* Isolated from Raw Chevon

ABSTRACT

This study aimed to determine the prevalence as well as the antibiotic sensitivity of *Staphylococcus aureus* in market meat (chevon) sold at the different wet markets in Dhaka city. A total of 120 samples of raw chevon were collected from the selected area. *S. aureus* was isolated from the samples using standard microbiological methods with their Total Viable Count (TVC). The highest TVC found on a sample was $\log_{10}$ 9.22 CFU/gm and the lowest TVC was $\log_{10}$ 9.04 CFU/gm. Cultural characteristics, biochemical testing, and gram staining were used to isolate and identify bacteria. Prevalence rate of *S. aureus* ware 47.5%. Isolates were investigated for antibiotic sensitivity profiling by using a Kirby-Bauer disc diffusion assay against five commonly used antibiotics used in goats. *S. aureus* showed the highest sensitivity to gentamycin (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%). According to the findings of this study, chevon contains multidrug-resistant *S. aureus* pathogens on market meat. The presence of multidrug-resistant *S. aureus* bacteria in market meat raises serious public health concerns, which can be transmitted to humans through direct contact or the food chain, posing a public health risk.

Keywords: *S. aureus*, Prevalence, TVC, Antibiotic Sensitivity, Chevon, Dhaka city

CHAPTER 1

INTRODUCTION

Goats were among 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) (FAOSTAT, 2020). Asia, on the other hand, accounts for 54.4 % of the world's goat population (Mazhangara *et al.*, 2019). 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 (Das and Saikia, 2017). Meat is highly important in maintaining the human body's strength to produce energy, health, and vigor (Das and Saikia, 2017). Food animals are the primary reservoirs for many food-borne zoonotic bacterial diseases, and animal-derived food products are the primary mode of transmission (Abebe *et al.*, 2020). Several meat-borne outbreaks have been recorded in recent years, and they are regarded as one of the world's major public health threats (Komba *et al.,* 2012). Abattoirs, storage at the retailer's shop, heavily infected utensils, and meat-handling benches are all potential sources of meat infection. During subsequent handling, processing, preparation, and distribution of carcasses and meat products, cross-contamination occurs (Dave and Ghaly, 2011).

Many researchers had isolated and identified *Staphylococcus aureus* (*S. aureus*) bacteria from raw chevon. *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 μM (Foster, 1996). The *S. aureus* is a commensal and opportunistic pathogen that can cause a wide spectrum of infections, from superficial skin infections to severe, and potentially fatal, invasive diseases (Lowy 1998). 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). These properties promote organism growth in various food products (Loir *et al.,*2003). The *S. aureus* can survive on hands and environmental surfaces for extended periods of time after initial contact (Scott and Bloomfield, 1990).

Staphylococcal food-borne disease is one of the most frequent food-borne disorders globally, caused by *S. aureus* enterotoxins in food (Kadariya *et al.,* 2014). However, multiple studies have found *S. aureus* in a variety of food products, including raw retail meat, indicating that consumers may be at risk of *S. aureus* colonization and subsequent infection. The *S. aureus* produces a wide variety of toxins. Staphylococcal enterotoxins are a group of nine major serological types of heat stable enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ) that are part of a larger family of pyrogenic toxin superantigens (Balaban and Rasooly 2002; Argudín *et al.,* 2010). These toxins are responsible for food poisoning in animals and can cause a variety of infections, including skin infections and mastitis in cows, goats, and sheep (Bierowiec *et al.,* 2016). Clinical and sub-clinical mastitis in ruminants can cause severe financial losses in the dairy industry by lowering milk quality and quantity (Bergonier *et al.,* 2003).

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 β-lactams have become frequent, and many of these are also resistant to β-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). The development of resistance to several antibiotics and other compounds with antimicrobial action exacerbates the pathogenesis of S. aureus. These strains cause therapeutic failures and hence restrict the options for treating major illnesses, increasing the expense of medical and preventive care (Cuny *et al.,* 2013).

As a result, studying the susceptibility and resistance patterns of *S. aureus* organisms to various antibiotics is critical for the better treatment of human and animal diseases. 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, the presence of bacteria in goat meat and the consequences of this on human health remain unknown.

Considering the above information, the current study was designed with the following objectives in mind:

I. To isolate and identify the *Staphylococcus aureus* from raw chevon
II. To demonstrate the occurrence of Staphylococcal contamination of chevon in the study area
III. To determine the antimicrobial resistance pattern of isolated *S. aureus*.

CHAPTER 2

REVIEW OF LITERATURE

Isolation, identification, characterization, prevalence and antibiogram determination of the bacteria isolated from raw chevon from different wet market in Dhaka city was performed using the information gained from the following related review of literature.

2.1. Isolation, Identification of pathogenic *Staphylococcus aureus* bacteria

Şanlıbaba, P. (2022), A study was conducted to extract and identify *S. aureus* from retail raw red meat samples in Ankara. The study also assessed their enterotoxin gene and antibiotic resistance profiles. Between July 2019 and November 2020, 452 retail raw meat samples, comprising beef (n=200), sheep (n=125), and lamb (n=127), were randomly purchased from various supermarkets and butchers in Ankara and analyzed for the presence of *S. aureus*. Morphological and molecular (16S rRNA and nuc gene) approaches were used to identify the *S. aureus* strain.

Frederick *et al.,* (2020), A total of 200 swabs of ready-to-eat meats were tested (50 each of grilled chevon, mutton, hog, and guinea fowl). Microbial load and *S. aureus* analyses were performed using a modified method from the USA-FDA Bacteriological Analytical Manual. The disc diffusion method was used to determine antibiotic susceptibility. The microbial load of the ready-to-eat meats ranged from 4.02 to 4.85 log cfu/cm^2, with 34.0% positive for *S. aureus* on average. Grilled guinea fowl had the highest incidence (46%), while grilled chevon and pork had the lowest (24% each).

Zehra *et al*., (2019), reported the prevalence of methicillin-resistant *S. aureus* (MRSA) in retail meat from Punjab, India, is reported in this study. The *S. aureus* isolates were isolated and identified using traditional microbiological methods. Isolates also underwent Etest. PCR and sequencing were used to identify and characterize antimicrobial resistance genes. The following tests were carried out: MLST, SCCmec, and spa typing. S. aureus was isolated from 408 meat samples and 101 swab samples. Penicillin had the highest phenotypic resistance (90.97%), followed by ciprofloxacin (61.80%), tetracycline (45.14%), and erythromycin (11.11%). Chicken isolates had considerably higher MICs for tetracycline than

chevon and pork samples, as well as significantly higher MICs for trimethoprim/sulfamethoxazole and gentamicin than chevon and swab samples ($P<0.05$).

Wu *et al.,* (2018), The purpose of this study was to determine the prevalence and characteristics of *S. aureus* in 1,850 retail meat and meat products in China between July 2011 and June 2016. The samples were taken from most China's provincial capitals and included 604 raw meat, 601 quick-frozen meat, and 645 ready to eat meat. *S. aureus* was discovered in 35.0% (647/1,850) of the samples using the qualitative and quantitative methods. The levels of *S. aureus* in retail meat showed that the MPN value of most of the positive samples ranged from 0.3 to 100 MPN/g.

Namir *et al*., (2018), The current study aims to determine the percentage of *S. aureus* in frozen and fresh meat gathered from retail outlets in the Karbala region, Iraq, as well as the presence of *Staphylococcus* enterotoxin. Polymerase Chain Reaction (PCR) technology is used to detect a gene. The results showed that *S. aureus* was discovered in 57 (57%) of 100 frozen and fresh bovine meat samples. The highest incidence of *S. aureus* isolation was found in frozen meat (64%), followed by fresh meat (50%), with no significant variations ($P>0.05$) in the rate of *S. aureus* isolation across the different samples. A PCR assay revealed that 20 (35%) of 57 *S. aureus* isolates from bovine foods carried the sea gene of SEA, 12 (37.5%) isolates from frozen meat samples, and 8 (32%) isolates from fresh meat samples.

Pu *et al.,* (2009), A total of 120 raw meat product samples (pork, n=90; beef, n=30) were gathered at random from 30 retail grocery stores owned by seven supermarket brands in Baton Rouge, Louisiana. For six weeks, sampling trips were done once a week (February to March 2008). After cleaning the exterior of the package with % alcohol-soaked paper towels, the meats were mixed with equal volumes of buffered peptone water (BD Diagnostic Systems, Sparks, MD) and aseptically massaged for 5 minutes. An equivalent volume of double-strength enrichment broth was added to a 50-ml portion of the rinse (Trypticase soy broth supplemented with 10 % NaCl and 1 % sodium pyruvate). After 24 hours at 35°C incubation, the enrichment broth was streaked in duplicate on Baird-Parker (BP) medium and spread-plated with cefoxitin (4 g/ml). After 48 hours, three to six putative *S. aureus* and MRSA colonies per meat sample (black colonies surrounded by 2 to 5 mm clear zones) were transferred to Trypticase soy agar plates and confirmed using a tube coagulase test (Remel, Lenexa, KS).

Sousa *et al.,* (2007), isolated 84 staphylococcal isolates obtained from cows, sheep, goats, and buffalo. Thirty isolates were identified as *S. aureus* by biochemical and molecular techniques and were comparatively characterized by phenotypic and genotypic methods.

2.2. Prevalence of pathogenic *Staphylococcus aureus* bacteria on retail raw chevon

Şanlıbaba, P. (2022), Analyzed total of 452 retail raw meat samples, including beef (n=200), sheep (n=125), and lamb (n=127) randomly purchased from various supermarkets and butchers in Ankara between July 2019 and November 2020, were tested for the prevalence of *S. aureus*. The overall prevalence of *S. aureus* among screened samples was 21.23%. Additionally, 65.62% of *S. aureus* strains contained SE gene regions.

Matubber *et al*., (2021) A total of 205 meat samples, including 70 chicken meat, 60 cattle meat, 50 buffalo meat, and 25 goat meat were aseptically collected and analyzed for the detection of antibiotic residues by thin layer chromatography, and the isolates obtained from these samples were subjected to antibiogram study against 16 commonly used antibiotics. The isolates found in this study were *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, and *Salmonella* spp. and their prevalence was 37.5% (77/205), 22.1% (48/205), 29.7% (61/205), 8.7% (19/205), respectively.

Saha (2021), In the years 2015–2016, a total of 120 raw randomly cut muscle pieces of Black Bengal goat chevon samples, 120 water samples were used for washing the carcasses and butchers' hands, and swab samples from cutting knives (80), butcher's hands (80), and chevon cutting wood surface (80) were collected from 80 retail shops in Tripura. Total viable count (TVC), total coliform count (TCC), total *Staphylococcus* count (TSC), isolation and identification of public health significance organisms, and antibiogram of the isolated bacteria were performed on the samples (480). The overall mean TVC, TCC, and TSC in chevon samples were 6.84±0.42, 4.97±0.23, and 3.67±.2710 log CFU/g, respectively. The chevon samples were predominantly contaminated with *Escherichia coli* (38.33%), followed by *Bacillus cereus* (35.83%), *S. aureus* (29.17%), and *Proteus spp*. (18.33%).

Mechesso *et al.,* (2021), analyze 481 *S. aureus* isolates (431 from the nasal cavity and 50 from carcass) that were recovered from 1146 carcasses and nasal swabs between July 2018 and January 2019. Approximately 82% and 72.6% of nasal and carcass isolate ware *S. aureus* positive.

Bantawa *et al.,* (2019), analyzed a total of 83 meat samples and found the prevalence of *S. aureus*, *Escherichia coli*, *Salmonella*, *Shigella,* and *Vibrio* were 68%, 53%, 35%, 6%, and 6% respectively.

Bantawa *et al.,* (2018), collected 50 meat samples from Dharan's local markets and transferred at 4°C to the microbiological laboratory. The meat samples were homogenized in a sterile glass homogenizer, and potential pathogens were isolated and identified using standard microbiological procedures. Coliforms were identified in 84% of the samples, *S. aureus* in 68% of the samples, *Salmonella spp*. in 34% of the samples*, Shigella spp*. in 6% of the samples, *Vibrio spp*. in only 3 samples, and *P. aeruginosa* in 40% of the samples.

Hasanpour *et al.,* (2017), tested 900 raw meat sample during various months of the year. Samples were cultured and those that were MRSA-positive were subjected to the disk diffusion method to study the antibiotic resistance pattern. One-hundred and sixty out of 900 raw meat samples (17.7%) were positive for MRSA. Raw sheep meat samples had the highest (24.0%), while raw camel meat samples had the lowest (10%) prevalence of MRSA. Samples which were collected in June, July, August, September and June months had the highest prevalence of MRSA. Bacterial strains were also resistant to ampicillin (100%), penicillin G (100%), gatifloxacin (96.8%), ceftriaxone (80%) and oxacillin (76.2%) antibiotics.

Ahmed *et al.,* (2013), Meat samples (n=140) of beef, mutton (sheep, goat), and chicken were gathered from abattoirs (n=60) and retail stores (n=80). Aerobic plate count (APC), *E. coli* count, *Staphylococcus aureus* count, and *Salmonella* detection were performed on all samples. *E. coli, S. aureus*, and *Salmonella* were found in 45%, 72%, and 26% of the samples, respectively.

Adesiji *at* al., (2011), investigate three hundred (300) samples of fresh raw chicken, beef, goat, and pork meat were screened for *Arcobacter* species by selective cultural procedures and for *Escherichia coli*, *Salmonella* species, and *S. aureus* enriched in peptone water and then streaked onto appropriate bacteriological agar. From the 300 samples analyzed, *S. aureus* 138 (46%) was the most frequently isolated organism, followed by *E. coli* 78 (26%), and *Salmonella* spp. 6(2%).

Gundogan *et al.,* (2005), collected 150 samples of raw calf/lamb meat samples (mince and chunks) and chicken parts (giblets, carcass) were analysed for the presence of *S. aureus*.

Eighty *S. aureus* strains were isolated and identified. Resistance of the strains to methicillin and other antibiotics was determined by the Kirby-Bauer disc diffusion test. The overall methicillin resistance rate for *S. aureus* was 67.5%. Of *S. aureus* strains, 87.5% were resistant to bacitracin. A high prevalence of penicillin G resistance was detected for *S. aureus* (53.8%). Few of the strains were resistant to erythromycin (7.5%). All strains were susceptible to vancomycin, sulbactam–ampicillin, ciprofloxacin and cefaperazone–sulbactam.

2.3. Antimicrobial sensitivity pattern of *Staphylococcus aureus* isolates

Şanlıbaba, P. (2022), Analyze total of 452 retail raw meat samples, including beef (n=200), sheep (n=125), and lamb (n=127) randomly purchased from various supermarkets and butchers in Ankara between July 2019 and November 2020. The phenotypic disc diffusion method was used to test antibiotic resistance in *S. aureus*. The overall prevalence of *S. aureus* was 21.23% among examined samples. A significant proportion of the isolates (40-100%) were found to be resistant to kanamycin, telithromycin, penicillin G, streptomycin, erythromycin, cloxacillin, ampicillin, pristinamycin, nalidixic acid, azithromycin, and ciprofloxacin. MDR (multi-drug resistance) was found in 96.87% of the *S. aureus* strains.

Matubber *et al*., (2021), A total of 205 meat samples, including 70 chicken meat, 60 cattle meat, 50 buffalo meat and 25 goat meat were aseptically collected and analysed for the detection of antibiotic residues by thin layer chromatography and the isolates obtained from these samples were subjected to antibiogram study against 16 commonly used antibiotics. The isolates found in this study were *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, and *Salmonella* spp. and their prevalence were 37.5% (77/205), 22.1% (48/205), 29.7% (61/205), 8.7% (19/205) respectively. The isolates showed different degrees of sensitivity to the antibiotics used in the study. The most resistant phenotype was against cefradine, amoxicillin, penicillin, oxytetracycline, erythromycin, and enrofloxacin. 18.5% (38/205) meat samples were found to be positive for antibiotic residues and the highest prevalence was observed in chicken meat compared to other meat types.

Hassan *et al.*, (2021), collected chevon sample of different body regions such as brisket, neck, and thigh, supplied at different places like Krishi market, Mohakhali kacha bazar, and Charulata market within Dhaka City Corporation area, were taken and the bacteria were isolated. The isolated bacteria were identified based on cultural, morphological, and biochemical characteristics. The isolated bacterial agents were Staphylococcus spp.,

Escherichia coli, Bacillus spp., Micrococcus spp., Streptococcus spp., Enterobacter spp., Proteus spp., Citrobacter spp., Salmonella spp., and *Pseudomonas spp*. Antibiotic susceptibility test using disc diffusion method revealed that most of the isolates were found to be resistant to tetracycline, ampicillin and erythromycin indicating that these may be a threat to consumer's health.

Mechesso *et al.,* (2021), show in the study, 481 *S. aureus* isolates (431 from the nasal cavity and 50 from carcass) were recovered from 1146 carcasses and nasal swabs between July 2018 and January 2019. Approximately 82% and 72.6% of nasal and carcass isolates, respectively, were resistant to at least one antimicrobial agent, with the highest rate of resistance to penicillin, followed by resistance to chloramphenicol and tetracycline. Relatively small proportions of the isolates were resistant to cefoxitin, clindamycin, and erythromycin. However, all *S. aureus* isolates were sensitive to linezolid, rifampin, and vancomycin.

Thakur *et al*., (2020), found total 26 *S. aureus* isolates recovered from 150 raw chevon samples were used in this study. These isolates were subjected to both the techniques for detection of Methicillin-Resistant *S. aureus.* In results, both the techniques could detect 2 (1.33%) isolates. The sensitivity (detection limit) of the Loop Mediated Isothermal Amplification assay was noted to be 10-fold higher than that of Polymerase Chain Reaction whereas the specificity of both was found to be similar (100%).

Bantawa *et al.,* (2019), analyzed a total of 83 meat samples and found the prevalence of *S. aureus*, *Escherichia coli*, *Salmonella*, *Shigella,* and *Vibrio* were 68%, 53%, 35%, 6%, and 6% respectively. The resistance of *Salmonella* was most frequently observed to amoxicillin (100%), tetracycline (24%), chloramphenicol (11%), and nalidixic acid (11%). *S. aureus* was resistant to amoxicillin (100%) followed by tetracycline (63%), nalidixic acid (17%), and cefotaxime (13%) respectively. *Vibrio* isolates resisted amoxicillin (100%), tetracycline (40%), and chloramphenicol (20%). *Shigella* expressed the highest resistance to amoxicillin (100%), followed by chloramphenicol (80%), tetracycline (60%), and nalidixic acid (20%). *E. coli* exhibited the highest resistance to amoxicillin (100%), followed by tetracycline (93%), nalidixic acid (25%), and cefotaxime (19%).

Wu *et al.,* (2018), The purpose of this study was to determine the prevalence and characteristics of *S. aureus* in 1,850 retail meat and meat products in China between July 2011 and June 2016. Only 11 isolates (1.26%) were susceptible to all antibiotics, whereas most isolates (821/868,

94.6%) showed resistance or intermediary resistance to more than three or more antibiotics. Of these strains, 104 (12.0%) were resistant to more than 10 antibiotics. However, the most frequent resistance was observed to ampicillin (85.4%), followed by penicillin (84.6%), erythromycin (52.7%), tetracycline (49.3%), kanamycin (45.3%), telithromycin (30.1%), clindamycin (29.6%), streptomycin (21.1%), norfloxacin (20.4%), gentamicin (19.4%), fusidic acid (18.4%), ciprofloxacin (16.9%), chloramphenicol (13.1%), amoxycillin/clavulanic acid (11.0%), and others (<10%). 7.4% of isolates (62/868) were confirmed as methicillin-resistance *S. aureus* (MRSA).

Dehkordi *et al.*, (2017), Nine-hundred raw meat samples were collected and cultured on a laboratory and isolated *S. aureus*. One-hundred and sixty out of 900 raw meat samples (17.7%) were *S. aureus* positive. Raw sheep meat samples had the highest (24.0%), while raw camel meat samples had the lowest (10%) prevalence of *S. aureus*. Samples that were collected in June, July, August, September, and June months had the highest prevalence of *S. aureus.* Bacterial strains were also resistant to ampicillin (100%), penicillin G (100%), gatifloxacin (96.8%), ceftriaxone (80%), and oxacillin (76.2%) antibiotics.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Study area

The present study was conducted during the period of January 2021 to June 2021. Samples were collected from the different retail shops and 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, Dhaka for further analysis.

3.1.2 Sample size

A total of 120 raw chevon (goat meat) samples were collected from six different markets in Dhaka city namely Krishi market, Geneva camp, Taltola bazaar, BNP Bazar, Agargaon Kacha Bazar, and Mohammadpur Town Hall Kacha Bazar. The samples were promptly stored in the icebox, and transferred to the laboratory under the Department of Sher-e-Bangla Agricultural University, Dhaka.

Table 1. Number of meat samples collected from different areas of Dhaka City

Sl. No.	Market Name	No. of Sample
1.	Krishi Market	20
2.	Geneva Camp	20
3.	Taltola Bazar	20
4.	BNP Bazar	20
5.	Agargaon Kacha Bazar	20
6.	Town Hall Kacha Bazar	20
	Total	**120**

3.1.3. Bacteriological Media

3.1.3.1. Liquid Media (Broth)

The liquid mediums used for this study were Nutrient broth (NB) and Peptone Broth.

3.1.3.2 Solid Culture Media

Nutrient Agar (NA), Blood agar (BA), and Mannitol salt agar (MS) were the media utilized for bacteriological examination. Mueller Hinton agar was used for the antibiotic sensitivity test.

3.1.4 Chemicals and Reagents

3.1.4.1 Phosphate buffer saline (PBS)

Phosphate buffer saline was used to prevent bacteria cells from rupturing or shriveling up. One tablet dissolved in 100ml of distilled water. Sterilize the PBS solution with autoclaving at 115°C for 15 mins.

3.1.4.2 Other chemicals and reagents

The chemicals and reagents used for this study are 0.1% Peptone water, reagents for Gram's staining (Crystal Violate, Gram's iodine, Safranin, Acetone alcohol), 3% Hydrogen peroxide, Phenol red, Methyl red, 10% Potassium hydroxide, Kovac's indole reagent (4-dimethylamino-benzaldehyde, concentrated HCL), Mineral oil, Normal saline, and other common laboratory chemicals and reagents.

3.1.5 Glass wares and other appliances

The following types of glassware and appliances utilized in the experiment:

Petridishes, conical flasks (100, 500, and 1000 ml), cotton, slides, and coverslips, Eppendorf tube, test tube (with or without Durham's fermentation tubes and stopper), test tube stand, pipette, micropipette, incubator, refrigerator, sterilizing instruments, thermometer, ice carrier, hand gloves, spirit lamp, match lighter, laminar air flow, hot air oven, centrifuge tubes, autoclave machine, electronic machine, glass spreader, inoculation loop, compound microscope, syringe and needle, tray, forceps, scalpel, scissors etc.

3.1.6 Materials required for antibiogram study

3.1.6.1 Muller Hinton Agar (MHA)

For the antibiogram study tests, Mueller Hinton agar plates were used (Hi media, India)

3.1.6.2 McFarland standards

The turbidity of bacterial suspensions is adjusted using McFarland standards to keep the number of bacteria within the specified range. McFarland standards are prepared by adding barium chloride to sulfuric acid to obtain a barium sulfate precipitate.

3.1.6.3 Antibiotic discs

The antibiotics susceptibility pattern was determined using commercially available antibiotic discs (OXOID Limited, Canada) presented on **Table 2**.

Table 2: Disc concentration of antimicrobial agents

Antimicrobial agents	Disc Concentration (µg)
Gentamycin (GM)	10
Tetracycline (TE)	30
Amoxycillin (AMX)	10
Ampicillin (AMP)	10
Ciprofloxacin (CIP)	5

3.2. Methods

3.2.1 Brief description of the experimental design

The entire experimental design was carried out in two stages. On the first step bacteria were isolated from chevon and then identified *S. aureus* based on cultural and morphological characteristics. Motility test with hanging drop preparation and carbohydrate fermentation tests were also done to confirm the organism as *S. aureus*. The second step included the study of response of the isolated bacteria were tested against commercially available antibiotic discs.

The following is a flow chart of representing design of the experiment

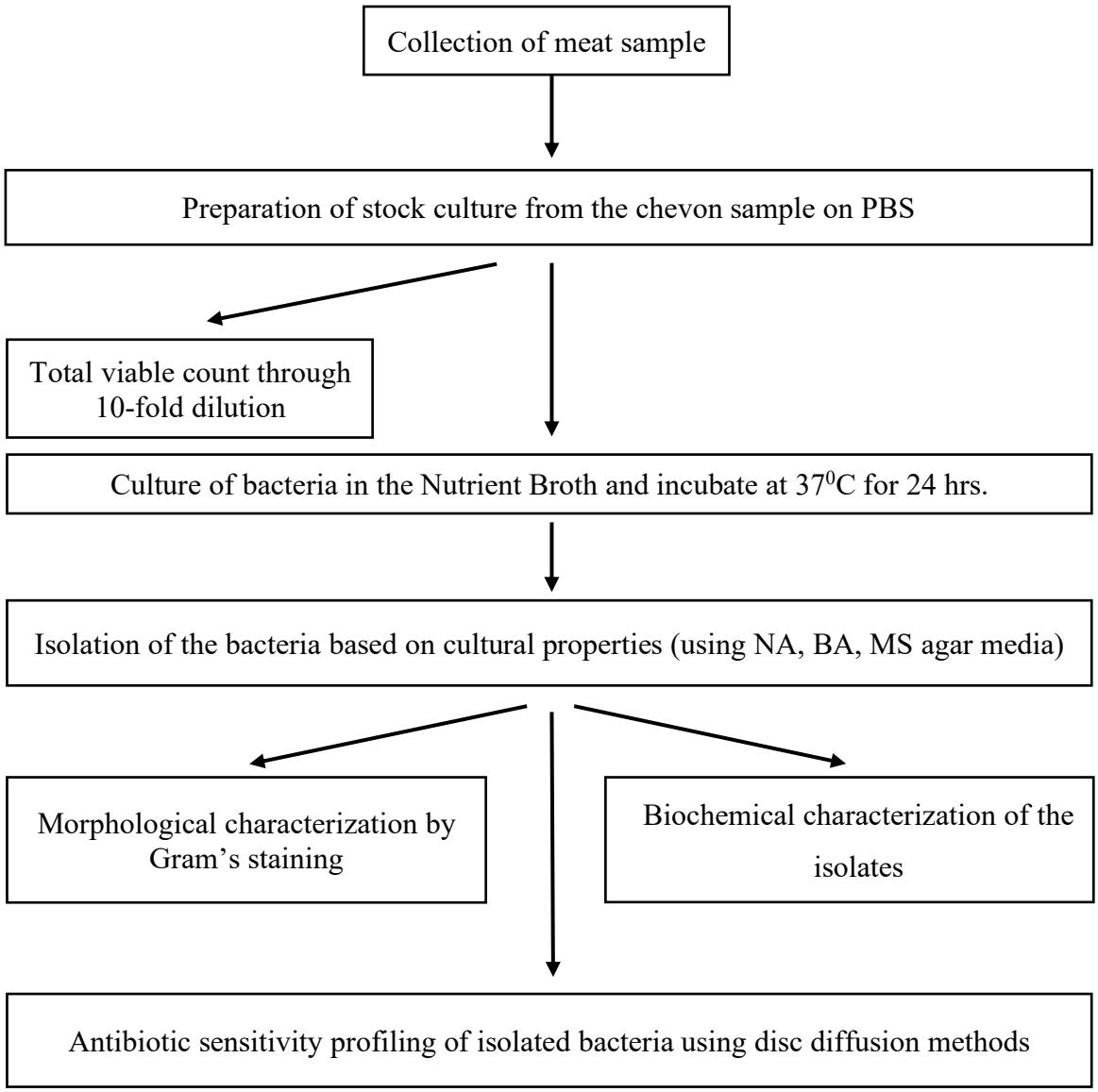

Figure 1: Schematic illustration of the experimental design

3.2.2 Preparation of different cultural media

3.2.2.1 Nutrient broth

The Nutrient Broth was prepared by dissolving 25 grams of NB base suspended in 1000 mL of distilled water. The heat was required to dissolve the media completely. Then it was sterilized by autoclaving at 15lbs. pressure (121°C) for 30 minutes. The broth was placed in test tubes and incubated at 37°C overnight to ensure sterility before being stored at 4°C until needed.

3.2.2.2 Nutrient agar

The agar was produced by following the manufacturer's guidelines. 28 grams of dehydrated nutrient agar base (Hi Media, India) were dissolved in 1000 ml of distilled water and autoclaved at 121^0C for 15 minutes at a pressure of 15 lbs./inch^2. The agar was then distributed into Petridis (90 mm and 100 mm) and incubated at 37°C overnight to ensure sterility before being stored at 4°C until required.

3.2.2.3 Blood Agar

This medium was used for the observation of haemolytic reactions and for encouraging the growth of *Staphylococcus* sp. *Bacillus* spp. *Pasteurella* spp. and antibiotic sensitivity tests. Forty grams of Blood agar (BA) base (Hi-media, India) powder was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely according to the manufacturer's guidelines. The medium was then sterilized by autoclaving. The autoclaved materials were allowed to cool to a temperature of 45^0C in a water bath. Defibrinated 5% sheep blood was then added to the medium aseptically and distributed to sterile Petri dishes and allowed to solidify. After solidification of the medium, the plates were allowed to incubate at 37^0C overnight to check their sterility and then stored in a refrigerator for future use.

3.2.2.4 Mannitol Salt Agar

According to the manufacturer's guideline, 111 grams of dehydrated Mannitol Salt medium (Hi-media, India) were suspended in 1000 mL of cold distilled water and heated to completely dissolve the medium. The solution was then autoclaved to sterilize it. The autoclaved materials were cooled in a water bath to 45°C before being dispersed to sterile Petri dishes. Petri dishes were placed in an incubator after solidification for sterility testing.

3.2.2.5 Mueller Hinton agar

Mueller Hinton agar was prepared according to manufacturer's guideline. Thirty-eight grams of agar base were suspended in 1000 ml distilled water and heated to boiling to completely dissolve the medium. After sterilization, autoclave for 15 minutes at 15 lbs. pressures at a temperature of 121°C. The temperature was lowered to 45-50°C. Then pour the Petri dishes

into that medium. After solidification of the medium in the Petri dishes, incubate at 37ºC for overnight to check their sterility and then stored it at 4ºC in a refrigerator for future use.

3.2.3. Preparation of different reagents

3.2.3.1. Methyl Red and Voges-Proskauer (MR-VP) broth

A total of 3.4 gm of MR-VP medium was diluted in 250 ml of distilled water and dispensed in 2 ml portions in each test tube before autoclaving at 121°C for 15 minutes at a pressure of 15 lbs./inch^2 according to the manufacturer's guideline. After autoclaving, the medium-filled tubes were incubated overnight at 37°C to ensure sterility before being kept in the refrigerator for future use.

3.2.3.2. Kovac's reagent

This reagent was made by dissolving 0.1 gram of Bacto methyl-red in 300 mL of 95 % alcohol, then diluting it to 500 mL with distilled water (Cheesbrough, 2006).

3.2.3.3 Preparation of sugar solution

The sugar solutions were made following the manufacturer's guidelines. The medium was made up of 1% peptone water with fermentable sugars added to it. Peptone water was made by combining 1 gram of Bacto peptone (Difco, USA) and 0.5-gram sodium chloride in 100 mL distilled water, boiling for 5 minutes, adjusting the pH to 7.6 with phenol red (0.02%), cooling, and filtering through filter paper. The solutions were then poured into cotton-plugged test tubes containing inverted Durham's fermentation tubes in 5 ml increments. The sugars used for fermentation are dextrose (MERCK, India), maltose (s.d. fiNE-CHEM Ltd.), lactose (BDH, England), sucrose (MERCK, India), and mannitol (Peterstol Tenbeg), were made separately as 10% solutions in distilled water (10 grams sugar was dissolved in 100 ml of distilled water). To dissolve the sugar, a little heat was required. After that, they were autoclaved for 15 minutes to sanitize them. For three days, the sugar solutions were sterilized in Arnold's steam sterilizer at 100ºC for 30 minutes. In each culture tube containing sterile peptone water, 0.5 mL of the sterile sugar solution was added aseptically. To ensure sterility, the sugar solutions were incubated at 37°C for 24 hours. Biochemical tests were conducted using these solutions.

3.2.3.4 Preparation of 50% Buffered Glycerol Saline

In 700mL distilled water, 8.3 grams of Buffered Glycerol Saline Base were suspended. After that, 300mL glycerol was added. The heat was used to completely dissolve the medium, which was then well mixed and delivered in screw-capped tubes or other appropriate containers. Autoclaving at 15 lbs./inch^2 of pressure at the temperature of 121°C for 15 minutes was used to sterilize the items.

3.2.4. Isolation and identification of *Staphylococcus aureus*

For the isolation and identification of bacterial flora, the procedure suggested by Carter (1986) was followed throughout the experiment. The morphological and cultural features of *S. aureus* were used to isolate it. On NA/BA, Staphylococci colonies are round, glistening, convex, smooth, and opaque. They are Gram-positive cocci clustered together. Most strains on BA produced beta (β) hemolysis. The coagulase test was used to distinguish pathogenic *S. aureus* from non-pathogenic *S. aureus*.

3.2.4.1 Serial dilution of bacterial culture (10-fold dilution)

To reduce the bacterial count for the total viable count (TVC), the stock sample was serially diluted suggested by Ben-David and Davidson (2014). It was performed by filling 10 (1-10) Eppendorf tubes with 900 µl of PBS. From the stock tube (2ml), 100 µl of the stock sample was transferred to the Eppendorf tube adjacent to the stock tube. Then, from the first Eppendorf tube to the next, 100 µl of diluted material is passed. To the last tube, make successive dilutions in the same manner, and discard 100 µl of diluted material from the last tube. From the 2nd and 6th tube, 100 µl of the liquid sample were transferred to the nutrient agar plate to determine the Total Viable Count (TVC).

3.2.4.2 Primary culture of microorganisms

The primary growth of all kinds of bacteria present in the collected samples was performed in nutrient broth. The samples were inoculated in nutrient broth and incubated at 37°C overnight to allow the organisms to proliferate.

3.2.4.3 Isolation in culture media

After primary culture of the organism, a tiny amount of inoculums from Nutrient broth were streaked through inoculating loop on Blood agar and Mannitol salt agar to observe the colony morphology. The organisms had distinct colony shape, showing that *S. aureus* was chosen for culturing on selective mediums such as Blood agar and mannitol salt agar. The morphological characteristics (shape, size, surface texture, edge and elevation, color, opacity, and so on) of potential colonies on various agar mediums that grew during 18 to 24 hours of incubation were meticulously documented.

3.2.4.4 Morphological identification of bacteria using Gram's staining methods

Merchant and Packer (1976) recommended using Gram's staining to identify the size, shape, and arrangement of bacteria. The steps were as follows: An inoculation loop was used to pick up a small colony, which was then spread on a glass slide and gently heated to fix it. The smear was then stained with crystal violet solution for 2 minutes before being cleansed under running tap water. After that, Gram's iodine was applied as a mordant for one minute before being rinsed with running water again. Then, acetone alcohol was added as a decolorizer. After rinsing with water, safranine was used as a counter stain and left to set for 2 minutes. The slide was then cleaned with water, blotted, and air-dried before being inspected under a microscope using immersion oil and a high-power objective (100X).

3.2.4.5 Motility test

The motility test was carried out according to Cowan and Steel's (1985) method to distinguish motile bacteria from non-motile bacteria. A pure culture of the test organism was allowed to develop in nutrient broth before the test. To make hanging drop preparation, one drop of cultured broth was placed on the cover slip and inverted over the concave depression of the hanging drop slide. To avoid air movement and evaporation of the fluid, Vaseline was applied to the concave depression of the hanging drop slide for better adhesion of the cover slip. The hanging drop slide was then inspected using a compound microscope with a 100X objective and immersion oil. Observing motility in contrast to bacterial to and from movement allowed the motile and non-motile organisms to be distinguished.

3.2.4.6 Haemolytic activity

Bacteria isolated from collected samples were inoculated into blood agar (BA) media and cultured for 24 hours at 37^0C to test their haemolytic ability. The colony that grew on the BA was tested for several types of haemolysis. The bacteria's haemolytic pattern was classified according to the forms of haemolysis generated on BA, following Winn and Konema (2006) guidelines, and is described below:

I. Alpha (α) hemolysis: a zone of greenish discoloration around the colony manifested by partial hemolysis.
II. Beta (β) hemolysis: complete clear zone of hemolysis around the colony .
III. Gamma (γ) hemolysis: no detectable hemolysis.

3.2.5. Biochemical tests to identify isolated *Staphylococcus aureus*

Staphylococcus aureus isolates were confirmed using a variety of biochemical tests.

I. Indole test
II. Methyl Red test
III. Voges-Proskauer test
IV. Catalase test
V. Coagulase test
VI. Sugar fermentation test

3.2.5.1 Indole test

The test organisms were cultivated for 48 hours at 37°C in test tubes containing 3 ml of peptone water containing tryptophan. Then 1 ml diethyl ether was added, shaken thoroughly, and set aside until the ether rose to the top. Then, 0.5 ml of Kovac's reagent was carefully poured down the side of the test tube, forming a ring between the medium and the ether layer, and the colour of the ring was noted. Indole production was suggested by the development of a brilliant red coloured ring. There is no development of red colour in the negative scenario (Cheesbrough, 2006).

3.2.5.2 Methyl Red test

A single colony from a pure culture of the test organism was inoculated in 5 mL of sterile MR-VP broth for the test. After a 5-day incubation period at 37°C, 5 drops of methyl red solution were added, and colour development was seen. The development of red colour was positive, indicating an acid pH of 4.5-6 due to glucose fermentation. The appearance of a yellow colour indicated a negative outcome (Cheesbrough, 2006).

3.2.5.3 Voges-Proskauer test

The test *S. aureus* organisms were cultured for 48 hours at 37°C in 3 mL of sterile MR-VP broth. Then, per ml of a broth culture of the test organism, 0.6 ml of 5% alpha-naphthol and 0.2 ml of 40% potassium hydroxide containing 0.3% creatine were added. Then give it a good shake and let it sit for 5-10 minutes to see how the color develops. The appearance of a bright copper-red color signified a positive case. There was no development of copper color in the negative cases (Cheesbrough, 2006).

3.2.5.4 Catalase test

This test was used to distinguish bacteria that produce the enzyme catalase, such as Staphylococci, from those that do not, such as Streptococci. A tiny colony of good growth pure culture of test organism was smeared on a slide to perform this test. The smear was then treated with one drop of catalase reagent (% H_2O_2). The production of bubbles on the slide was observed. The presence of a bubble within a few seconds signified a positive test, but the absence of a bubble indicated a negative result according to Winn and Koneman (2006).

3.2.5.5 Coagulase test

Human plasma was utilized in the coagulase test. Undiluted 0.5 ml human plasma was combined separately in two test tubes containing an equal volume of 24-hour old staphylococcal cultured broth and incubated at 37^0C for 6 hours. After 3-6 hours of mixing cultured broth, the tubes were inspected for the presence of plasma clots and the results were recorded using the usual procedure outlined by Harley (2005). The negative tubes were left at room temperature overnight before being checked again. A basic slide coagulase test Harley, (2005) was performed on a slide with an equivalent volume of freshly cultivated broth of a

specific organism and evaluated under a microscope for the presence of any coagulation. Pathogenic *S. aureus* is distinguished by coagulase positivity.

3.2.5.6 Sugar fermentation test

In the sugar fermentation test, a loop of BA culture of the organisms was inoculated into each tube containing five basic sugars (e.g., dextrose, sucrose, lactose, maltose, and mannitol) individually and incubated for 24 hours at 37^0C. The formation of gas bubbles in the inverted Durham tube indicated acid generation, which was shown by a colour change from reddish to yellow in the liquid (Cheesbrough, 2006).

3.2.6 Maintenance of stock culture

The stock culture was made by mixing 1 ml of sterilized glycerol with 1 ml of pure culture in nutrient broth and storing it at -20^0C.

3.3 Antibiotic sensitivity test

By disc diffusion or the Kirby-Bauer method Bauer *et al.,* (1966), three isolates from five genera were tested for antimicrobial drug susceptibility against five regularly used antibiotics. Selection of 3 to 5 isolated colonies from the MSA and BA agar plate. Using a sterile cotton bar, 0.5 MacFarland suspension of colonies is uniformly scattered onto Mueller-Hinton agar plates. The antibiotic disc was then placed on Mueller-Hinton agar and incubated for 24 hours at 37°C. Examined the plates and used a meter ruler to measure the diameter of inhibitory zones in millimeters from the edge of the disc to the edge of the zone.

3.3.1 Antimicrobial discs

Table 2. shows a list of commercially available antibacterial discs (Oxoid, UK) and their concentrations. The discs are put to the plates as soon as possible after inoculation, but no later than 15 minutes. The discs were individually placed on the agar with sterile forceps and gently pressed down. On a 100-mm plate, no more than 5 discs were placed.

3.3.2 Recording and interpreting results

After placing the discs on the plate, the plates were inverted and incubated at 37ºC for 16-18 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's (2007) zone diameter interpretation standards, antimicrobial testing findings were classified as sensitive, intermediate, or resistant on **Table 3.**

Table 3: Drugs with their disc concentration for the Enterobacteriaceae family

Name of Antibiotic	**Disc Conc.**	**Zone Diameter Interpretive Standard (mm)**		
	(µg /disc)	**Resistant**	**Intermediate**	**Susceptible**
Gentamycin (GM)	10	≤13	14 -17	≥18
Tetracycline (TET)	30	≤14	15 -18	≥19
Amoxicillin (AMX)	10	≤13	14 -17	≥18
Ampicillin (AMP)	10	≤13	14 -16	≥17
Ciprofloxacin (CIP)	5	≤15	16 -20	≥21

3.4 Statistical Analysis

Data were entered into the Microsoft Office Excel 2021 spreadsheet. To explain the outcome of the prevalence rate, self-evaluation model and descriptive statistics were utilized. The prevalence was given as a percentage. Results of total viable count (TVC) and antibiotic sensitivity test of different antibiotics on tests meat type were analysed by Statistix 10 uses single factor CRD with $P < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

The results presented below demonstrated the isolation and identification of bacteria isolates from retail raw chevon samples around Dhaka city. The results also indicated the prevalence and antibiotic sensitivity resistant pattern of the isolates to different antibiotics.

4.1. Results

4.1.1 Determination of TVC (Total Viable Count)

Table 4: Total Viable Count (TVC) of chevon sample sold at different wet markets in Dhaka

Name of the markets	TVC log cfu/ml (Mean ± SD)	P value
Krishi Market	9.06 ± 0.02	**0.00 ***
Geneva Camp	9.23 ± 0.01	**0.00 ***
Taltola Bazar	9.12 ± 0.02	**0.00 ***
BNP Bazar	9.18 ± 0.01	**0.00 ***
Agargaon Kacha Bazar	9.13 ± 0.02	**0.00 ***
Town Hall Kacha Bazar	9.02 ± 0.03	**0.00 ***

* =Significant at 5% level of probability ($P < 0.05$)

The assessment of total viable bacterial counts using Aerobic plate count is shown in **Table 4.** According to Mukhopadhya *et al.,* 2009, the presence of a high number of microorganisms (APC $>10^7$cfu/cm^2) accelerates the deterioration of meat. 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 BNP Bazar ($\log_{10}9.18 \pm 0.01$ CFU/gm), Agargaon Kacha Bazar ($\log_{10}9.13 \pm 0.02$ CFU/gm), Taltola Bazar ($\log_{10}9.12 \pm 0.02$ CFU/gm), and Krishi Market ($\log_{10}9.06 \pm 0.02$ CFU/gm). Town Hall Kacha Bazar had the lowest TVC ($\log_{10}9.02 \pm 0.03$ CFU/gm). In a TVC column were found to be statistically significant (P=0.00).

In another study Haque *et al.,* (2008) 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. Parvin *et al.,* (2017) found different TVC values in different time intervals in another study. She observed TVC values of 5.17±0.28 log CFU/gm (0 hrs), 6.64±0.05 log CFU/gm (2 hrs), and 8.47±1.27 log CFU/gm (5hrs) respectively. In Tripura of India, another study was conducted by Shah (2021), who found a TVC of 6.84±0.42 log CFU/gm, which was significantly lower than the current study. This discrepancy could be attributed to differences in season or environmental variation or differences in study methods of different study areas.

4.1.2 *Staphylococcus aureus* bacteria identification based on cultural and biochemical properties

4.1.2.1 Properties on Nutrient broth

After 24 hours of aerobic incubation at 37^0C, the Nutrient broth revealed the growth of bacteria as indicated by the turbidity. **Fig. 2 (A).**

In this current study, *S. aureus* produced turbidity in nutrient broth, which is supported by McLandsborough (2005), Cheesbrough (2006), and John (1990).

4.1.2.2 Properties on Nutrient agar

After 24 hours of aerobic incubation at 37^0C, the NA plates revealed the growth of bacteria as indicated by the growth of Gray to whitish colonies. **Fig. 2 (B)**.

In this current study, *S. aureus* produced Gray to whitish round, convex, smooth colonies on the nutrient agar plate, which is supported by McLandsborough (2005), Cheesbrough (2006), and John (1990).

4.1.2.3 Properties on Blood agar

The BA plates were streaked separately with the organism and aerobically incubated at 37^0C for 24 hours shown in **Fig. 2 (C)**. All 57 isolated *S. aureus* produced large, creamy white, beta-hemolytic colonies, which are typical of *Staphylococcus aureus* on blood agar.

In this current study, *S. aureus* produced large, creamy white, beta-hemolytic colonies on blood agar, which is supported by McLandsborough (2005), Cheesbrough (2006), and John (1990).

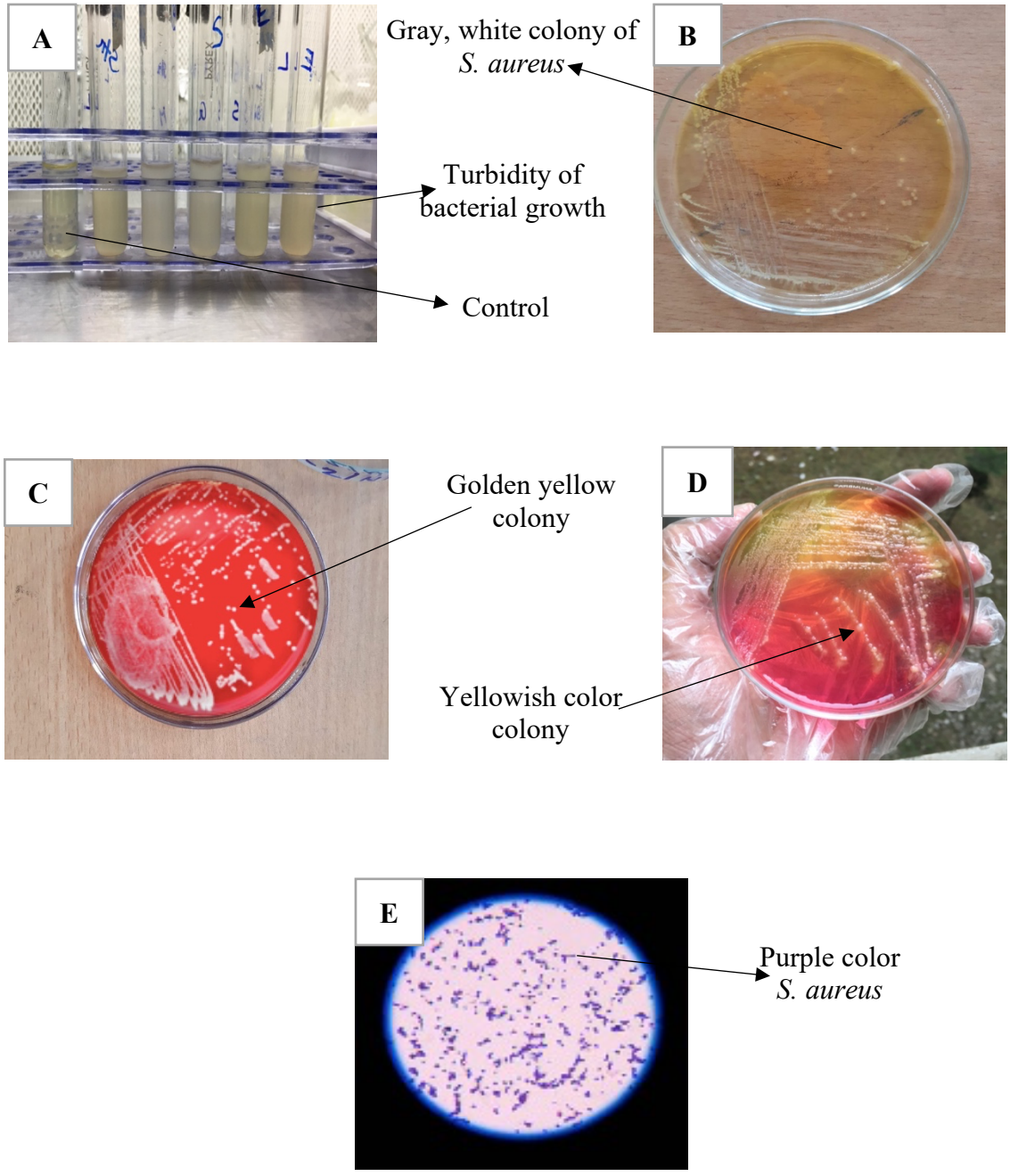

Figure 2: **A**. Nutrient broth (NB); **B**. Nutrient agar (NA); **C**. Blood agar (BA); **D**. Mannitol salt agar (MSA); **E**. Gram's staining

4.1.2.4 Properties on Mannitol salt agar

The MSA plates were streaked separately with the organism and incubated at 37^{o}C for 24 hrs.. The growth revealed golden yellow color colony shown in **Fig. 2 (D)**.

The colonies of *S. aureus* on mannitol salt agar showed colonies that fermented mannitol and appeared golden yellow, which is previously supported by McLandsborough (2005), Cheesbrough (2006), and John (1990).

4.1.2.5 Identification of *Staphylococcus aureus* by Gram's staining

Gram's stained smears from NA, BA, MC, and MSA were examined microscopically which revealed Gram-positive, cocci arranged in grapes-like clusters. **Fig. 2 (E).**

The morphology of the *S. aureus* in Gram's staining exhibited Gram-positive cocci arranged in grapes-like clusters which were previously supported by McLandsborough (2005), Cheesbrough (2006), and John (1990).

Table 5: Morphological and cultural properties of *S. aureus* isolated from chevon

Feature	Appearance
Nutrient agar	Gray, white, or yellowish colony formed
Blood agar	White to golden yellow colony were found
Mannitol salt agar	Yellow colour colony were observed
Catalase test	Formation of bubbles on the slide
Staining property	Gram positive, cocci arranged in grapes like clusters observed

4.1.2.6 Identification of *Staphylococcus aureus* by biochemical test

Staphylococcus aureus ferments all the five basic sugars and produces only acid shown in **Fig. 3 (C)**. and **Table 7**. It showed a positive reaction in the cases of catalase & coagulase tests **Fig. 3 (B & D)**, and MR-VP tested positive but negative results were found in the case of the Indole reaction shown in **Fig. 3 (A).**

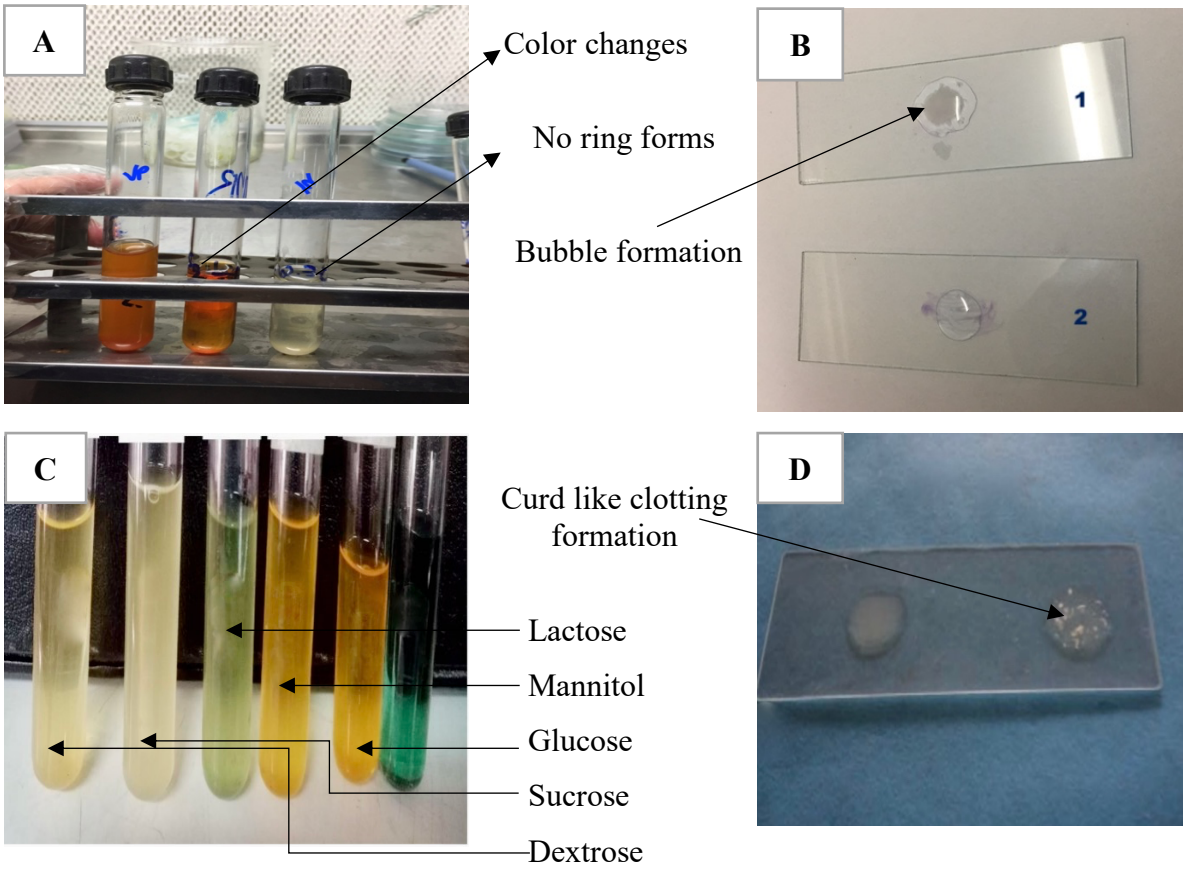

Figure 3: **A.** Indole, MR-VP test; **B.** Catalase test; **C.** Sugar fermentation test. **D.** Coagulase test

Table 6: Biochemical reaction patterns 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.

Isolates of *S. aureus* showed a complete fermentation of 5 basic sugars and the production of acid in this current study, which was supported by Cheesbrough (2006), OIE Manual (2000), and Freeman (1979). The isolate also revealed a positive reaction in the catalase and MR-VP

test but a negative reaction in the Indole test which was supported by Cheesbrough (2006), OIE Manual (2000), and Freeman (1979). The coagulase test is the most accurate method for identifying *S. aureus* Koneman, (1997). The isolates of *S. aureus* revealed a positive reaction in the coagulase test, which was supported by Cheesbrough (2006), OIE Manual (2000), John (1990), and Freeman (1979).

4.1.3 Occurrence of isolated bacteria

The *S. aureus* (**Table 7**) was isolated from the chevon sample based on their morphological, cultural properties, and biochemical characteristics with standard reference organisms.

Table 7: Isolated *S. aureus* bacteria of meat samples from different markets in Dhaka

Market Name	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 kacha bazar	20	8	40
Total	**120**	**57**	**47.5**

Occurrence of *S. aureus* found in this current study of goat meat from different markets was (47.5%), but the BNP Bazar having the greatest prevalence rate (65%) and the Krishi market followed by Taltola Bazar having the lowest (35%) prevalence. In a previous study Hassan *et al.,* (2021) found a 98% prevalence rate of *S. aureus* in goat meat in Dhaka city wet market. On the other hand, Das *et al.,* (2015) found a 43.33% prevalence rate in goat meat in Southern Assam, which was slightly lower than the current study. In Southern Assam, the prevalence was slightly low probably due to water conditions or environmental variation.

4.1.4 Antibiotic sensitivity profiling of *Staphylococcus aureus*

A total of 57 isolates of *S. aureus* isolated from chevon samples were further used to determine the antibiotic sensitivity pattern. Out of 57 *S. aureus* positive isolates all 57-sample ware resistant to amoxicillin, 52 ampicillin, 33 tetracycline, 31 ciprofloxacin, and 36 gentamycin. The antibiotic sensitivity profile of *S. aureus* isolates presented in **Table 8**. The antibiotic sensitivity profile of *S. aureus* isolates presented in **Fig. 4.** All isolated bacteria showed 100% resistant to amoxicillin, followed by ampicillin (92.98%), gentamycin (63.15%), tetracycline (57.9%), and ciprofloxacin (56.14%) presented on **Fig. 5.**

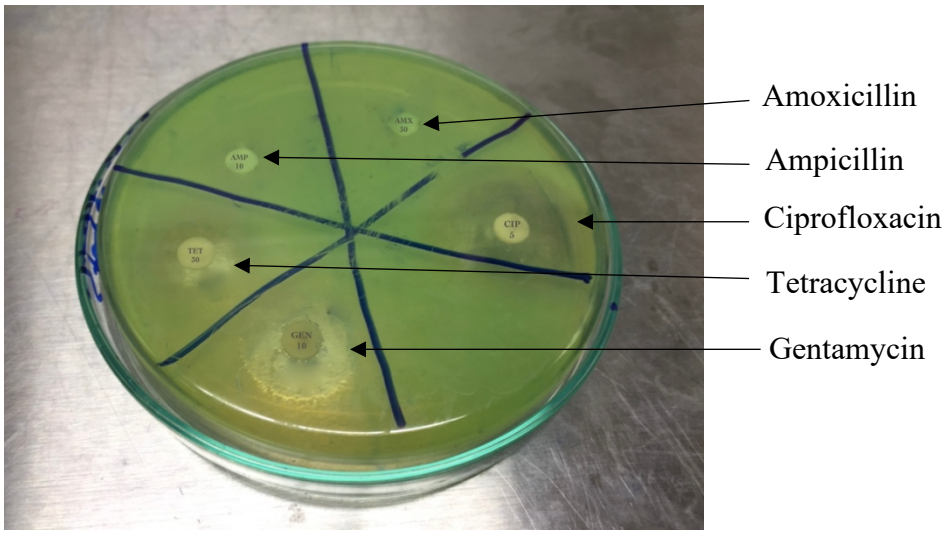

Figure 4: Antibiotic sensitivity test of *Staphylococcus aureus*

Table 8: Antibiotic sensitivity pattern of *Staphylococcus aureus*

SN.	Antibiotics Used	Drug Conc. (Mcg/disc)	Resistant	Intermediate	Susceptible
1	Amoxicillin	30	57 (100)	-	-
2	Ampicillin	10	53 (92.98)	4 (7.02)	-
3	Gentamicin	10	7 (12.28)	17 (29.82)	33 (57.89)
4	Tetracycline	30	36 (63.15)	12 (21.05)	9 (15.8)
5	Ciprofloxacin	5	6 (10.53)	19 (33.33)	32 (56.14)

Note: Figure in parenthesis indicates the percentages of *S. aureus* response to antibiotics

Table 9: Antibiotic susceptibility patterns of *S. aureus* in chevon using single factors complete randomized design

Antibiotic	Mean Zone Diameter (mm)
Amoxicillin	5.057 e
Ampicillin	11.275 d
Gentamicin	17.525 b
Tetracycline	13.800 c
Ciprofloxacin	20.725 a
CV (%)	21.89
$LSD_{(0.05)}$	1.32

Note: Mean diameter zone of inhibition presented in alphabetical order, which is significantly different at the 5% level of significance.

Table 9. shows the mean value of the antibiotic sensitivity pattern of different antibiotics used in this test. Where Ciprofloxacin has the greatest (20.725 mm) mean value followed by Gentamycin (17.525 mm), Tetracycline (13.800 mm), Ampicillin (11.275 mm), and Amoxicillin (5.057 mm).

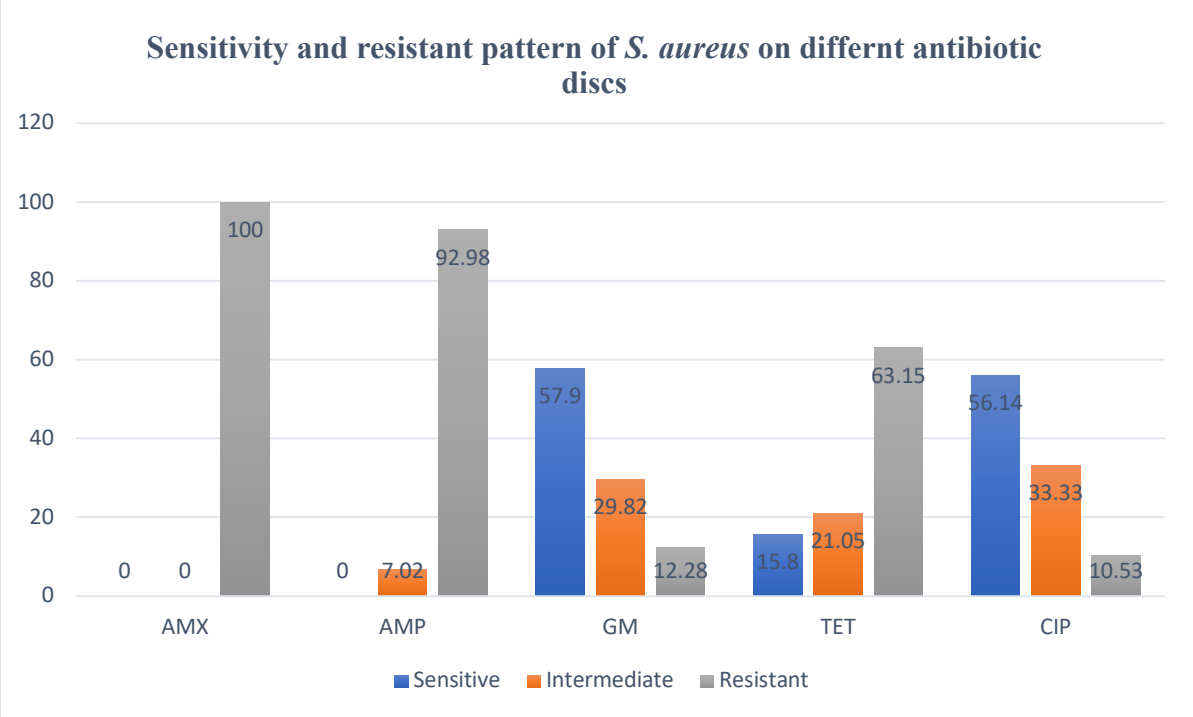

Figure 4: Sensitivity pattern of *Staphylococcus aureus*

The *S. aureus* was found to be 100% resistant to amoxicillin and 92.98% resistant to ampicillin followed by 63.15% of tetracycline. Gentamycin shows 57.9% sensitivity with 29.82% intermediate sensitivity followed by ciprofloxacin sensitivity to 56.14% with 33.33% intermediate sensitivity.

Earlier observations by Hassan *et al.,* (2021) found Tetracycline 100% & Ampicillin 90% resistant to *S. aureus* and 100% sensitive to ciprofloxacin. Ayama *et al.,* (2021) found 97.22% to ampicillin in Ghana, and Bantawa *et al.,* (2019) in Nepal found 100% amoxicillin resistant. Isolates from raw chevon were sensitive to gentamycin (63.15%) followed by ciprofloxacin (56.14%). The results strengthen the earlier observations of Zehra *et al.,* (2019) who found 54.86% to gentamycin 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, water conditions, environmental variation, or study methods.

CHAPTER 5

SUMMARY AND CONCLUSION

The current study was conducted from January 2021 to June 2021 to determine the bacteriological state, prevalence, and antibiotic sensitivity pattern of zoonotic *S. aureus* bacteria on raw chevon from different wet markets in Dhaka, Bangladesh. For this purpose, a total of 120 samples were collected from 6 different wet market to suggest public health importance based on present hygienic condition, prevalence, and antibiotic sensitivity pattern of *S. aureus*.

In the present study, 120 samples were collected from 6 wet markets named Krishi Market, Geneva Camp, BNP Bazar, Taltola Kacha Bazar, Agargaon Kacha Bazar, and Town Hall Kacha Bazar at Dhaka North City Corporation. Using standard bacteriological technique 57 samples were positive among 120 samples. And the prevalence of *S. aureus* bacteria was 47.5%. The highest TVC ($\log_{10}$ 9.22±0.01 CFU/ml) on Geneva camp and the lowest TVC was found in Town Hall Kacha Bazar ($\log_{10}$ 9.02±0.03 CFU/ml).

The isolates of *S. aureus* were tested for antibiogram against five antibiotics from different groups that are often used in fields and found on the market. *S. aureus* shows moderate sensitivity against antibiotics for ciprofloxacin (56.14%) and gentamycin (57.9%). All the isolated bacteria were resistant to ampicillin and amoxicillin.

Based on the outcomes of this study, it could be concluded that:

- The *S. aureus* was isolated and confirmed by different cultural properties and biochemical tests.
- The prevalent in BNP Bazar was higher compared to other market.
- Most of the isolates showed multi-drug resistance, but sensitive to ciprofloxacin (56.14%) and gentamycin (57.9%), resistant to amoxicillin (100%) and ampicillin (92.98%).

Further research on the following topics may be conducted in relation to the current study:

- Antibiogram analysis using a specific antibiotic resistance gene and a microdilution approach with specific antibiotics.
- Molecular characterization of *S. aureus.*
- In vivo study for pathogenic effects of *S. aureus.*
- Notice to higher authorities, policymakers, and consumers the importance of public health and zoonoses.

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APPENDIX I

COMPOSITION OF DIFFERENT MEDIA

1. Nutrient broth

peptic digest of animal tissue	5.0 gm
Sodium chloride	5.0 gm
Beef extract	1.5 gm
Yeast extract	1.5 gm
Distilled water	1000 ml
Final pH (at 25ºC)	7.4 ± 0.2

2. Nutrient Agar

Peptone	5.00 gm
Sodium chloride	5.00 gm
HM peptone B#	1.50 gm
Yeast extract	1.50 gm
Agar	15.00 gm
Final pH (at 25°C)	7.4 ± 0.2

3. Mannitol Salt Agar

Proteose peptone	10.0gm
Beef extract	1.00 gm
Sodium chloride	75.00 gm
D- mannitol	10.00 gm
Phenol red	0.025 gm
Agar	15.00 gm
Final pH (at 25°C)	7.4 ± 0.2 gm

4. MacConkey Agar

Peptones (meat and casein)	3.00 gm
Pancreatic digest of gelatin	17.00 gm
Lactose monohydrate	10.00 gm
Bile salts	1.50 gm
Sodium chloride	5.00 gm
Crystal violet	0.001 gm
Neutral red	0.03 gm
Agar	13.50 gm
pH after sterilization (at 25°C)	7.1 ± 0.2

5. Blood Agar

Blood agar	60 gm
Distilled water	1000ml
Bovine Blood	5.00 ml

6. Mueller Hinton Agar

HM infusion B from	300.00 gm
Acicase	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Final pH (at 25°C)	7.4 ± 0.1

7. Kovac's reagent

P-dimethyl aminobenzal dehyde	5.0gm
Amylalcohol	75.0gm
Conc. HCl	25ml

8. Methyl Red Indicator

Methyl red	0.20 gm
Ethyl alcohol	60.00 ml
Distilled water	40.00 ml

9. Voges–Proskauer (MR-VP) broth

Buffered peptone	7.00 ml
Dextrose	5.00 gm
Dipotassium phosphate	5.00 gm
Final pH (at 25°C)	6.9 ± 0.2

10. Phosphate Buffer Saline

Sodium chloride	8.00 gm
Disodium hydrogen phosphate	2.80 gm
Potassium chloride	0.20 gm
Potassium hydrogen phosphate	0.20 gm
Distilled water to make	1000 ml

APPENDIX II

Completely Randomized AOV for TVC

Statistics 10.0 (30-day Trial) 3/23/2022, 11:45:26 PM

Completely Randomized AOV for TVC

Source	DF	SS	MS	F	P
Treatment	5	49251.2	9850.24	228.49	0.0000
Error	114	4914.5	43.11		
Total	119	54165.7			

Grand Mean 134.55 CV 4.88

Homogeneity of Variances	F	P
Levine's Test	2.50	0.0349
O'Brien's Test	2.36	0.0443
Brown and Forsythe Test	2.33	0.0471

Welch's Test for Mean Differences

Source	DF	F	P
Treatment	5.0	239.28	0.0000
Error	52.9		

Component of variance between groups 490.357
Effective cell size 20.0

Treatment	Mean
1	114.85
2	169.10
3	128.50
4	149.75
5	135.30
6	109.80

Observations per Mean 20
Standard Error of a Mean 1.4682
Std Error (Diff of 2 Means) 2.0763

LSD All-Pairwise Comparisons Test of TVC by Treatment

Treatment	Mean	Homogeneous Groups
2	169.10	A
4	149.75	B
5	135.30	C
3	128.50	D
1	114.85	E
6	109.80	F

Alpha 0.05 Standard Error for Comparison 2.0763
Critical T Value 1.981 Critical Value for Comparison 4.1131
All 6 means are significantly different from one another.

APPENDIX III

Completely Randomized AOV for Antibiotics

Statistix 10.0 (30-day Trial) 3/23/2022, 2:31:05 PM

Completely Randomized AOV for Antibiotics

Source	DF	SS	MS	F	P
Treatment	4	5770.42	1442.61	160.83	0.0000
Error	195	1749.10	8.97		
Total	199	7519.52			

Grand Mean 13.680 CV 21.89

Homogeneity of Variances	F	P
Levene's Test	20.04	0.0000
O'Brien's Test	19.52	0.0000
Brown and Forsythe Test	20.18	0.0000

Welch's Test for Mean Differences

Source	DF	F	P
Treatment	4.0	318.12	0.0000
Error	93.8		

Component of variance between groups 35.8409
Effective cell size 40.0

Treatment	Mean
1	5.075
2	11.275
3	17.525
4	13.800
5	20.725

Observations per Mean 40
Standard Error of a Mean 0.4735
Std Error (Diff of 2 Means) 0.6697

LSD All-Pairwise Comparisons Test of Antibiotics by Treatment

Treatment	Mean	Homogeneous Groups
5	20.725	A
3	17.525	B
4	13.800	C
2	11.275	D
1	5.0750	E

Alpha 0.05 Standard Error for Comparison 0.6697
Critical T Value 1.972 Critical Value for Comparison 1.3208
All 5 means are significantly different from one another.