Isolation, identification and antimicrobial sensitivity profiling for *Staphylococcus aureus* from captive small cats and deer

A clinical report submitted in partial satisfaction of the requirement for the degree of Doctor of Veterinary Medicine (DVM)

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December 2024

Table of Contents

List of Tables

List of Figures

Abstract

Staphylococcus aureus, an opportunistic pathogen commonly found on the skin and mucosal surfaces of mammals, is significant in both human and veterinary medicine. We investigated its presence and antibiotic resistance in captive felines and deer at Chattogram Zoo. A total of 18 samples were collected from 8 felines and one deer and cultured on Mannitol salt agar and Blood agar. *S. aureus* typically forms golden-yellow colonies on Mannitol salt agar due to mannitol fermentation and shows beta-hemolysis on Blood agar, characterized by a clear zone around the colonies. Molecular identification was performed using PCR targeting the *NUC* gene, specific to *S. aureus*. Ten samples showed selective bacterial growth, and four exhibited a band at 359 bp on a 1% agarose gel, confirming the presence of *S. aureus*. Antibiotic sensitivity testing for 11 antibiotics revealed varying resistance and susceptibility profiles. Ampicillin, Amoxicillin + Clavulanic Acid, and Oxacillin showed 100% resistance, indicating that these antibiotics were ineffective against the isolated strains. Conversely, Imipenem and Gentamicin were 100% effective, showing complete susceptibility. Resistance to Oxacillin by all isolates indicates multidrug resistant *S. aureus* which will further incorporate public health significance. These results are crucial for developing effective treatment strategies for infections in captive felines. This study provides evidence of *S. aureus* presence in captive felines at Chattogram Zoo and highlights their antibiotic resistance profiles. These findings lay the foundation for broader research on *S. aureus* colonization and antimicrobial resistance in captive animals in Bangladesh. Such research is essential for understanding the spread of resistant strains and devising strategies to ensure better health management in zoo animals, ultimately aiding in their treatment and preventing potential transmission to humans.

Key Words: *Staphylococcus aureus*, captive felines, antibiogram, MRSA

Introduction

Staphylococcus aureus is a gram-positive, facultative anaerobic bacterium that belongs to the genus *Staphylococcus* within the family Staphylococcaceae. The genus name *Staphylococcus* is derived from Greek, where "staphyle" means bunch of grapes, reflecting the characteristic cluster formation of the bacterial cells observed under a microscope. *S. aureus* is distinguished by its golden-yellow pigment, which gives colonies a distinctive appearance and is due to the production of staphyloxanthin, a carotenoid pigment that also acts as a virulence factor by providing resistance to reactive oxygen species (Kadariya et al., 2014; Tong et al., 2015). The taxonomy of *S. aureus* places it within the Firmicutes phylum, a major group of bacteria that includes other notable genera such as *Bacillus* and *Listeria* (Kadariya et al., 2014, Deurenberg & Stobberingh, 2008).

Isolation of *S. aureus* in the laboratory typically begins with the collection of clinical or environmental samples. Common sources include nasal swabs, wound exudates, and samples from infected sites. The samples are then cultured on selective media, such as mannitol salt agar (MSA), which contains a high concentration of salt (7.5-10%) to inhibit the growth of non-staphylococcal organisms. *S. aureus* ferments mannitol, resulting in a color change of the medium from red to yellow due to acid production, which aids in its identification. Blood agar plates are also used, where *S. aureus* produces beta-hemolysis, characterized by clear zones around colonies due to the lysis of red blood cells (Middleton & Fales, 2012, Price et al., 2012). Once isolated, further biochemical tests are conducted to confirm the identity of *S. aureus*. These include the coagulase test, which differentiates *S. aureus* (coagulase-positive) from other staphylococci (coagulase-negative). *S. aureus* produces coagulase, an enzyme that converts fibrinogen to fibrin, causing plasma to clot. Another confirmatory test is the catalase test, where the production of bubbles upon the addition of hydrogen peroxide indicates the presence of catalase, an enzyme that breaks down hydrogen peroxide into water and oxygen (Price et al., 2012, Tong et al., 2015). Additionally, *S. aureus* can be identified by its ability to ferment glucose anaerobically and its production of DNAse, an enzyme that degrades DNA (Rahman et al., 2020, Kadariya et al., 2014). Molecular identification of *S. aureus* has become increasingly important for precise characterization and epidemiological studies. Polymerase chain reaction (PCR) is commonly employed to detect specific genetic markers associated with *S. aureus*. One of the most widely used targets is the *NUC*

gene, which encodes a thermonuclease that is unique to *S. aureus*. Another key genetic marker is the mecA gene, which confers resistance to methicillin and other beta-lactam antibiotics by encoding an altered penicillin-binding protein (PBP2a). The presence of mecA is indicative of methicillin-resistant *Staphylococcus aureus* (MRSA), a major public health concern due to its resistance to multiple antibiotics (Stoesser et al., 2015). Whole-genome sequencing (WGS) has emerged as a powerful tool for the comprehensive analysis of *S. aureus* strains. WGS allows for the identification of virulence factors, resistance genes, and the overall genetic makeup of the bacterium. This approach provides detailed insights into the epidemiology, evolution, and transmission dynamics of *S. aureus*. For instance, WGS has been used to trace the spread of MRSA in hospital settings and to identify outbreaks linked to specific clonal lineages (Wardyn et al., 2018).

Understanding the taxonomy, isolation, and molecular identification of *S. aureus* is crucial for effective clinical management and public health interventions. *S. aureus* is a versatile pathogen capable of causing a wide range of infections, from superficial skin infections to life-threatening conditions such as bacteremia, endocarditis, and pneumonia. Its ability to acquire and disseminate resistance genes poses significant challenges to treatment, necessitating ongoing surveillance and research to develop novel therapeutic strategies and to mitigate the impact of AMR (Laxminarayan et al., 2016, Deurenberg & Stobberingh, 2008). The study of *S. aureus* in wild felines adds another layer of complexity to our understanding of this pathogen. Wild felines, as part of the natural ecosystem, interact with various environmental reservoirs of *S. aureus*, potentially acquiring and spreading resistant strains. The isolation and molecular identification of *S. aureus* from these animals not only provides insights into the epidemiology of AMR but also highlights the interconnectedness of human, animal, and environmental health. This One Health perspective underscores the need for integrated approaches to combat AMR across different sectors (Royden et al., 2017, Rahman et al., 2020).

The study of antimicrobial sensitivity in wild felines affected by *S. aureus* is of paramount importance due to the escalating threat of antimicrobial resistance (AMR) globally. Wild felines, including big cats like tigers, lions, and leopards, play a significant role in the ecosystem as apex predators. However, they can also serve as reservoirs and vectors for AMR pathogens, posing a substantial risk to both animal and human health (Rahman et al., 2020). Understanding the

antimicrobial sensitivity patterns in these animals is crucial for developing effective treatment protocols, controlling the spread of resistant strains, and preserving biodiversity. AMR is a critical global health issue, with the World Health Organization (WHO) identifying it as one of the top 10 threats to global health (World Health Organization, 2020). The emergence of AMR in wildlife, particularly in wild felines, is an area of growing concern. Studies from various regions have reported the presence of resistant strains of *S. aureus* in wild felines, indicating that these animals could contribute to the environmental dissemination of AMR genes. For instance, research has shown that wild felines in North America harbor *S. aureus* strains resistant to multiple antibiotics, reflecting a potential zoonotic threat (Schaumburg et al., 2012). Recent studies in Bangladesh and surrounding regions have highlighted the presence of multidrug-resistant (MDR) *S. aureus* in both domestic and wild animals. A study conducted on wild felines in the Sundarbans, Bangladesh, found that a significant percentage of these animals tested positive for MDR *S. aureus*. The resistance was observed to commonly used antibiotics such as penicillin (85%), tetracycline (75%), and methicillin (50%) (Islam et al., 2019). Another study in Bangladesh reported similar findings, with wild felines showing high resistance to multiple antimicrobials, including erythromycin and ciprofloxacin (Hoque et al., 2020). These findings underscore the urgent need for continuous surveillance and effective antimicrobial stewardship in wildlife populations to mitigate the spread of AMR.

In captive settings such as zoos and safari parks, the risk of AMR transmission is exacerbated due to the close proximity of different animal species and frequent human-animal interactions. Studies have shown that captive wild felines are at a higher risk of acquiring and spreading resistant bacteria due to factors such as stress, diet, and medical interventions (Rwego et al., 2008). For example, a study in Portugal found that wild boars in captivity exhibited nasal carriage of *S. aureus* with resistance to methicillin (Silva et al., 2010). Similarly, research conducted in Spanish zoos revealed that captive wild mammals carried *S. aureus* strains harboring the *mecC* gene, indicative of methicillin resistance (Porrero et al., 2014). These environments can act as hotspots for the evolution and dissemination of AMR, making it crucial to implement robust biosecurity measures and responsible antimicrobial use policies. The transmission of AMR pathogens from wild felines to humans and other animals in zoos and safari parks represents a significant public health risk. Zoo workers, visitors, and other animals can become carriers of resistant bacteria, leading to potential outbreaks. The public health implications are profound, as

AMR can lead to treatment failures, increased healthcare costs, and higher mortality rates (Naz & Kumari, 2018). For instance, a study reported that *S. aureus* strains isolated from zoo animals in Germany were resistant to multiple antibiotics, posing a threat to zoo staff and visitors (Schaumburg et al., 2012). Similarly, a study in the United States found that *S. aureus* strains from wild mammals exhibited resistance to several antibiotics, highlighting the potential for zoonotic transmission (Wardyn et al., 2018).

Based on the comprehensive review of the literature and the identified gaps in current knowledge, this study was designed with the following objectives:

- To isolate and identify *Staphylococcus aureus* from oral and anal swabs of captive felines in Chattogram Zoo by conventional and molecular methods.
- To assess the antimicrobial sensitivity patterns of the isolated organism against a range of commonly used antimicrobials.

Materials And Methods

2.1. Samples

During the study, a total of 18 samples were collected from 9 animals Chattogram Zoo including 1 deer, 4 Leopard cat and 4 Fishing cat. The samples consist of 9 oral and 9 anal swabs. The ages of the animals were below 5 years and permission from zoo authorities was sought during sample collection for this study. All relevant data such as species, age, sex, health condition, body weight, feeding and housing etc. were collected by a standardized questionnaire for further analysis.

2.2. Study area and period

The samples were collected from the captive animals of Chattogram Zoo from August to September 2024. The experiment was carried out at Poultry Research and Training Center (PRTC), Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram. The study period was from August to November 2024.

2.3. Collection of samples

The oral and anal samples were collected directly from the oral cavity and rectum of the animals respectively by sterile cotton swab and immediately transferred into autoclaved cryovial filled with Stuart Transport Media. The containers were labeled immediately and carried to the laboratory in a cool box and preserved at −4°C temperature.

2.4. Isolation of *Staphylococcus aureus*

To prepare the Mannitol Salt Agar (MSA) medium, 300 mL of distilled water was measured into a clean conical flask, and 33.3 grams of MSA powder were gradually added. The flask was then placed on a hot plate, and the solution was gently heated until the powder was completely dissolved. Once dissolved, the MSA solution was autoclaved at 121°C for 15 minutes to ensure sterility. After autoclaving, the medium was allowed to cool and then poured into sterile petri dishes at a volume of 20 mL per plate. The plates were left to solidify at room temperature before being stored at 4°C. For culturing bacteria on MSA, an inoculating loop was first sterilized by flaming it in a Bunsen burner until red hot, and then it was allowed to cool. A loopful of the bacterial sample was then taken and streaked onto the MSA plates using the four-quadrant streak method. The inoculated plates were incubated at 37°C for 24 hours to allow bacterial growth.

To prepare the blood agar medium, 8.4 grams of nutrient agar powder were accurately weighed and mixed with 300 mL of distilled water in a clean conical flask. The mixture was gently heated on a hot plate until the powder was completely dissolved, taking care to avoid boiling. The medium was then sterilized by autoclaving at 121°C for 15 minutes. Once the medium cooled to 45-50°C, 15-30 mL of defibrinated sheep blood was added and mixed gently to avoid creating bubbles. The prepared blood agar was poured into sterile petri dishes at a volume of 20 mL per plate and allowed to cool and solidify at room temperature. After the agar plates were prepared, bacteria were cultured on them following the same technique as previously described: an inoculating loop was sterilized by flaming, allowed to cool, then used to streak the bacterial sample onto the plates using the four-quadrant streak method. The inoculated plates were incubated at 37°C for 24 to 48 hours.

2.5. Molecular identification of *S. aureus*

2.5.1. Genomic DNA extraction

Total genomic DNA was extracted from the bacterial samples using the Double "Boiling Method" for *Staphylococcus aureus*. The procedure began by preparing a bacterial suspension, where 100 µL of nuclear-free water (NFW) was added to an Eppendorf tube, followed by the addition of 2 to 3 bacterial colonies, which were mixed thoroughly. The Eppendorf tubes were then subjected to a heat shock step by placing them in a heating block at 99°C for 12 minutes, facilitating the lysis of bacterial cells and the release of DNA. Following this, the tubes were immediately transferred to a freezer at -20°C for 5 minutes, allowing the released DNA to stabilize and halting enzymatic activity. After the cold shock, the tubes were returned to the heating block at 99°C for a brief period to ensure complete lysis of the cells. The samples were then centrifuged at 1000 rpm for 5 minutes to pellet any remaining cell debris. Finally, the supernatant containing the DNA was carefully transferred to a new sterile tube and stored at -80°C for future use.

2.5.2. Polymerase Chain Reaction

PCR for the amplification of the *NUC* gene of *Staphylococcus aureus* was performed using DNA samples extracted through the boiling method. A total of 10 samples were analyzed. The PCR reaction mix consisted of 20 µL per reaction, prepared in a PCR tube with the following reagents: 10 µL of master mix, 1 µL of forward primer, 1 µL of reverse primer, 4 µL of nuclear-free water (NFW), and 4 µL of extracted DNA. A positive control (known DNA) and a negative control (PCR

water) were included in each reaction. The reagents were mixed gently by tapping the Eppendorf tube or brief centrifugation. The thermal cycler program included an initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was carried out at 72°C for 2 minutes to ensure complete DNA synthesis. The PCR products were then held at 4°C indefinitely to maintain stability until further use. The amplification products were visualized on a 1% agarose gel stained with SYBR Green dye.

Table 1: Components used and their volume to make 20 µL PCR mix

Component	Volume (µL) per reaction
Master mix	10
Forward primer	1
Reverse primer	1
NFW (Nuclear-free water)	2
gDNA/template	6

2.5.3. Agarose gel electrophoresis

Agarose gel electrophoresis was performed to visualize the PCR product of the NUC gene. The gel was prepared using 1% agarose in Tris-Acetate-EDTA (TAE) buffer. SYBR Green dye, which strongly binds to DNA by interacting with its base pairs and fluoresces under UV light, was used to stain the gel. First, the 1% agarose gel was prepared, and then 5 µL of the PCR product was mixed with 1 µL of loading dye. This mixture was loaded into the wells of the agarose gel. A DNA ladder was also included to estimate the size of the NUC gene product. The gel was then subjected to electrophoresis at 110 V for 30-40 minutes. After electrophoresis, the gel was stained with SYBR Safe dye, and the PCR product was visualized under UV light to confirm the amplification of the desired NUC gene fragment.

2.6. Antimicrobial sensitivity profiling

For antimicrobial sensitivity testing, Muller Hinton agar was prepared using the following materials: Muller Hinton agar powder, distilled water, measuring equipment (beaker, graduated cylinder), an electronic balance, autoclave, petri dishes, and a stirring rod. The preparation began

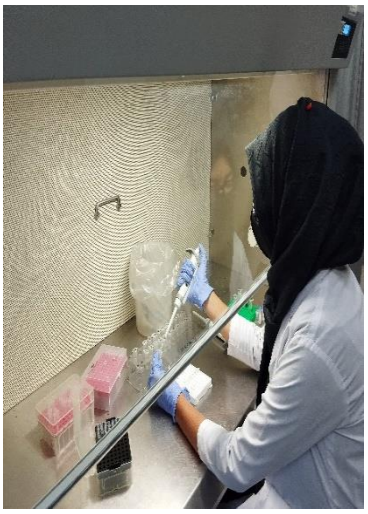
by weighing the required amount of Muller Hinton agar powder (38 g/L) and dissolving it in an appropriate volume of distilled water. The mixture was then heated on a hot plate with continuous stirring to ensure complete dissolution of the agar. Once dissolved, the medium was sterilized by autoclaving at 121°C for 15 minutes to eliminate any microbial contamination. After sterilization, the agar medium was allowed to cool to 45-50°C, at which point it was poured into sterile petri dishes to a uniform depth of 4 mm, taking care to avoid bubble formation. The prepared plates were then left to solidify at room temperature before being stored in a refrigerator at 2-8°C for future use in antimicrobial sensitivity testing.

The antimicrobial sensitivity testing was performed using the disk diffusion method, also known as the Kirby-Bauer method, following the 2023 Clinical and Laboratory Standards Institute (CLSI) guidelines. Muller Hinton agar plates were inoculated with a bacterial lawn of *Staphylococcus aureus* after adjusting the inoculum to the 0.5 McFarland turbidity standard. Antibiotic-impregnated paper disks, including Ampicillin (10 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Imipenem (10 µg), Gentamicin (10 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Amoxicillin + Clavulanic Acid (20/10 µg), Oxacillin (1 µg), Sulfamethoxazole + Trimethoprim (23.75/1.25 µg), and Streptomycin (10 µg), were placed onto the inoculated agar plates. The plates were then incubated at 37°C for 18 hours. After incubation, the diameters of the zones of inhibition were measured and interpreted according to the CLSI guidelines. The zones were classified as susceptible, intermediate, or resistant based on the standard breakpoints for *Staphylococcus aureus*, with specific measurements for each antimicrobial agent. These results were used to determine the antimicrobial susceptibility profile of the isolated strain.

Table 2: List of panels of antimicrobials used and interpretation according to Clinical and Laboratory Standard Institute (CLSI) M100 document of 2023 in this study

Antimicrobial group	Antimicrobials	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Penicillin	Ampicillin (10 µg)	≥17	14-16	≤13
	Amoxicillin + Clavulanic Acid (20/10 µg)	≥18	14-17	≤13
	Oxacillin (1 µg)	≥15	12-14	≤11
Cephalosporins	Ceftriaxone (30 µg)	≥23	18-22	≤17
	Cefepime (30 µg)	≥22	17-21	≤16
Carbapenems	Imipenem (10 µg)	≥19	15-18	≤14
Aminoglycosides	Gentamicin (10 µg)	≥16	12-15	≤11
	Streptomycin (10 µg)	≥18	14-17	≤13
Tetracyclines	Tetracycline (30 µg)	≥19	14-18	≤13
Fluoroquinolones	Ciprofloxacin (5 µg	≥21	16-20	≤15
Sulfonamides	Sulfamethoxazole + Trimethoprim (23.75/1.25 µg)	≥17	12-16	≤11

Culturing of bacteria



Performing PCR

Performing CS test

Figure 1: Pictorial presentation of activities in the PRTC lab

Results

3.1. Sample demographics

The study comprises nine samples, encompassing one deer, four leopard cats, and three fishing cats, with detailed records of age, sex, and body weight. The lone deer, a 2-year-old female, has a body weight of 20 kg. Among the leopard cats, there are two males aged 3 and 4.5 years, weighing 3.5 kg and 3 kg respectively, and three females aged 3, 3.5, and 3.5 years, weighing 3.5 kg, 4 kg, and 3.5 kg respectively. The fishing cats, all males, are aged 4 and 4.5 years, with body weights of 11 kg, 12 kg, and 14 kg (Table 3).

Table 3: Demographic data of captive animals sampled in this study

Sample ID	Species	Age (year)	Sex	Body weight(Kg)
1	Deer	2	Female	20
2	Leopard cat	3	Male	3.5
3	Leopard cat	4.5	Male	3
4	Fishing cat	4.5	Male	14
5	Fishing cat	4	Male	12
6	Fishing cat	4	Male	11
7	Leopard cat	3	Female	3.5
8	Leopard cat	3.5	Female	4
9	Leopard cat	3.5	female	3.5

3.2. Prevalence of *Staphylococcus aureus*

The dataset includes 18 samples tested for bacterial growth on Mannitol Salt Agar, Blood Agar (Beta hemolysis), and Polymerase Chain Reaction (PCR). On Mannitol Salt Agar, 10 samples (O1, O2, O4, O5, O7, O8, O9, A3, A4, A8) showed positive growth, while 8 samples (O3, O6, A1, A2, A5, A6, A7, A9) were negative. Blood Agar (Beta hemolysis) revealed that the same 10 samples (O1, O2, O4, O5, O7, O8, O9, A3, A4, A8) displayed beta hemolysis, indicating positive results, whereas the remaining 8 samples were negative. PCR results showed that only 2 samples (O5 and O6) were positive, while 16 samples were negative (Table 4).

Table 4: Growth and Detection of Bacterial Samples on Mannitol Salt Agar, Blood Agar (Beta Hemolysis), and PCR Results

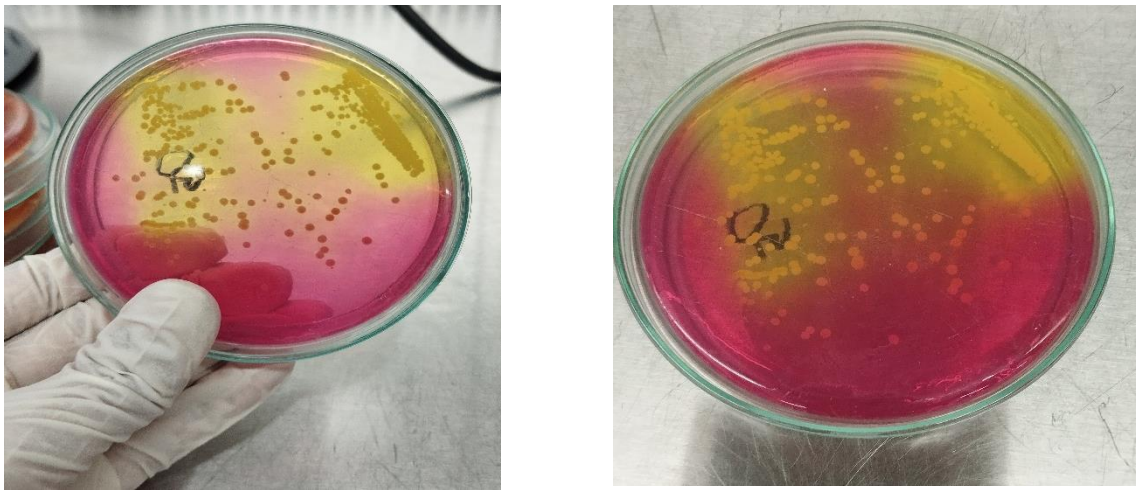
Sample ID	Mannitol Salt Agar (+/-)	Blood Agar (Beta hemolysis) (+/-)	PCR (+/-)
O1	+	+	-
O2	+	+	-
O3	-	-	-
O4	+	+	-
O5	+	+	+
O6	-	-	+
O7	+	+	-
O8	+	+	-
O9	+	+	-
A1	-	-	-
A2	-	-	-
A3	+	+	-
A4	+	+	-
A5	-	-	-
A6	-	-	-
A7	-	-	-
A8	+	+	-
A9	-	-	-

Here, +: Positive, -: Negative

The samples were tested for the presence of *Staphylococcus aureus* using Mannitol Salt Agar (MSA), Blood Agar, and PCR. Mannitol Salt Agar results indicated the presence of yellow colonies in 10 samples (O1, O2, O4, O5, O7, O8, O9, A3, A4, A8), suggesting mannitol fermentation and acid production by *Staphylococcus aureus*, which turns the phenol red indicator yellow. Blood Agar results showed white to golden colonies surrounded by a zone of beta-hemolysis in the same 10 samples, indicating complete lysis of red blood cells, a characteristic

feature of *Staphylococcus aureus*. PCR results confirmed the presence of *Staphylococcus aureus* in 2 samples (O5 and O6) (Figure 2).

Among the 18 samples, comprising 9 oral and 9 anal samples, 10 samples showed positive results on Mannitol Salt Agar, 10 samples exhibited beta-hemolytic colonies on Blood Agar, and 2 samples tested positive by PCR (Table 4). The consistent appearance of yellow colonies on MSA and beta-hemolytic colonies on Blood Agar in these samples confirms the growth of *Staphylococcus aureus*.



***S. aureus* colonies on Mannitol salt agar**

***S. aureus* colonies on blood agar**

Figure 2: Colony characteristics of *S. aureus* in different selective medium

3.3. Antimicrobial susceptibility pattern

CS test was performed for 10 positive samples of *S. aureus* for 11 commonly used antibiotics. The antimicrobial susceptibility testing revealed that all samples were resistant to ampicillin, amoxicillin + clavulanic acid, and oxacillin (Figure 3).

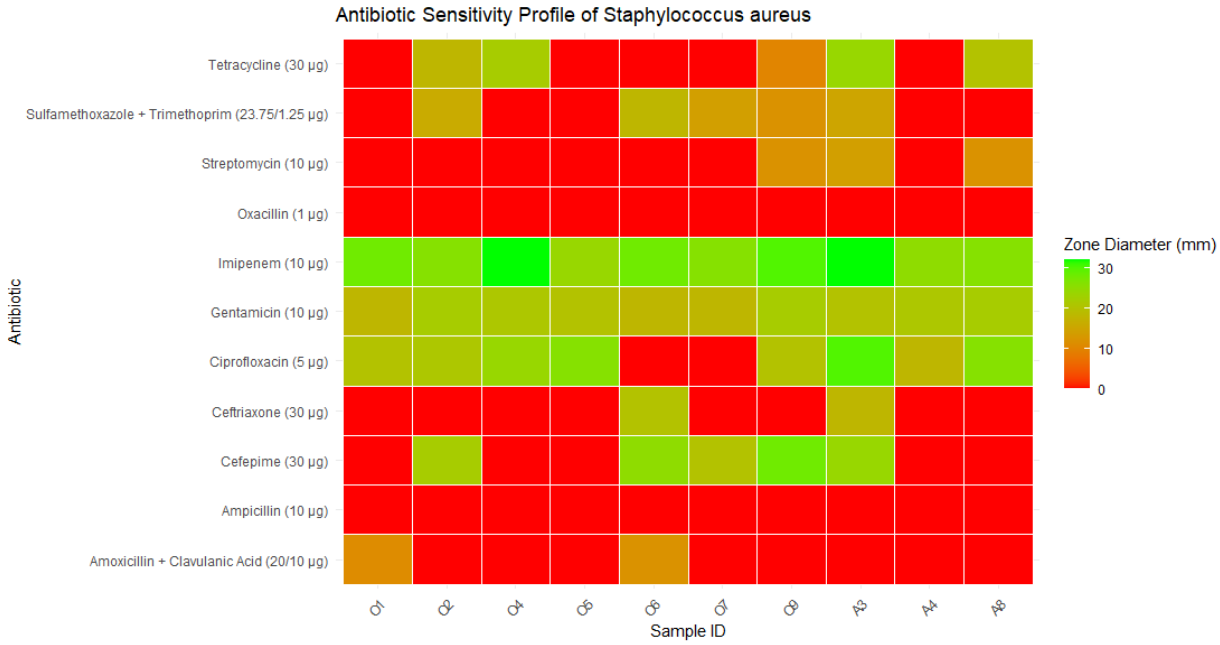


Figure 3: Antibiogram of *S. aureus* isolated from oral and anal swabs of captive felines and deer

High resistance rates were also observed for ceftriaxone (80%), tetracycline (60%), sulfamethoxazole + trimethoprim (60%), and streptomycin (90%). However, all samples were susceptible to imipenem and gentamicin, indicating these antibiotics' effectiveness. Moderate susceptibility was observed for cefepime (40%) and ciprofloxacin (50%), with varying intermediate resistance levels (Table 5).

Table 5: Antimicrobial Susceptibility rate of isolates to used antimicrobials

Antibiotics	Number of Samples	Susceptible	Intermediate	Resistance
Ampicillin (10 µg)	10	-	-	100%
Ceftriaxone (30 µg)	10	-	20%	80%
Cefepime (30 µg)	10	40%	10%	50%
Imipenem (10 µg)	10	100%	-	-
Gentamicin (10 µg)	10	100%	-	-
Tetracycline (30 µg)	10	30%	10%	60%
Ciprofloxacin (5 µg	10	50%	30%	20%
Amoxicillin + Clavulanic Acid (20/10 µg)	10	-	-	100%
Oxacillin (1 µg)	10	-	-	100%
Sulfamethoxazole + Trimethoprim (23.75/1.25 µg)	10	10%	30%	60%
Streptomycin (10 µg)	10	-	10%	90%

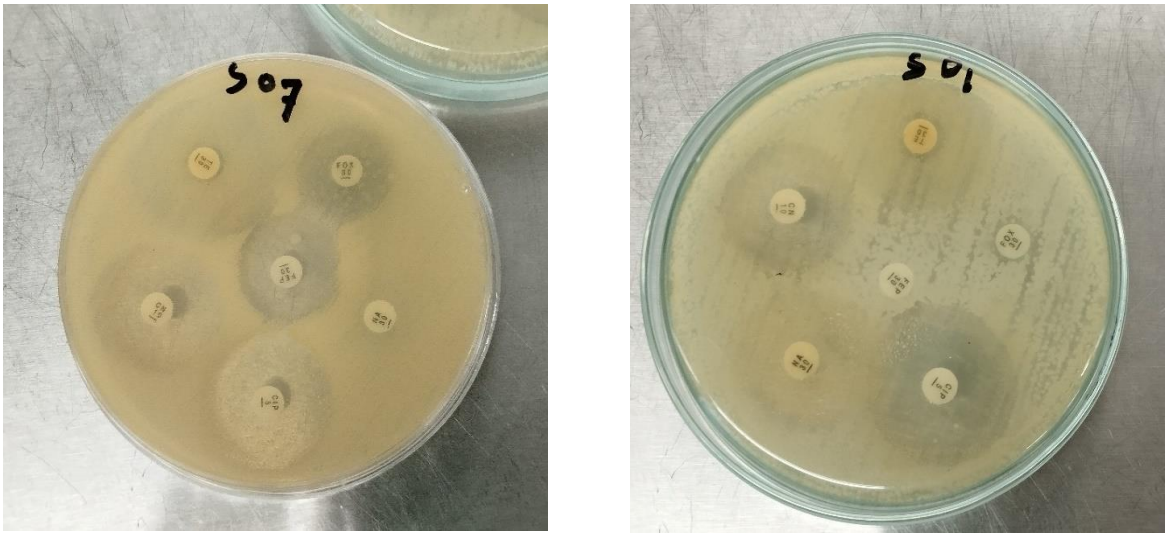


Figure 4: Zone of inhibition on Muller Hinton Agar

3.4. Result of PCR

For molecular identification of *S. aureus* by PCR based approach, total DNA was extracted from the samples. Known positive standards were used during each PCR run. PCR was performed following standard protocol, NUC gene was amplified, and bands were found at approximately 350bp. The bands were visualized by gel electrophoresis using 1% agarose gel and 100bp ladder. Among the 10 samples, 4 showed amplification at approximate 359bp which confirms that the samples contained *S. aureus*.

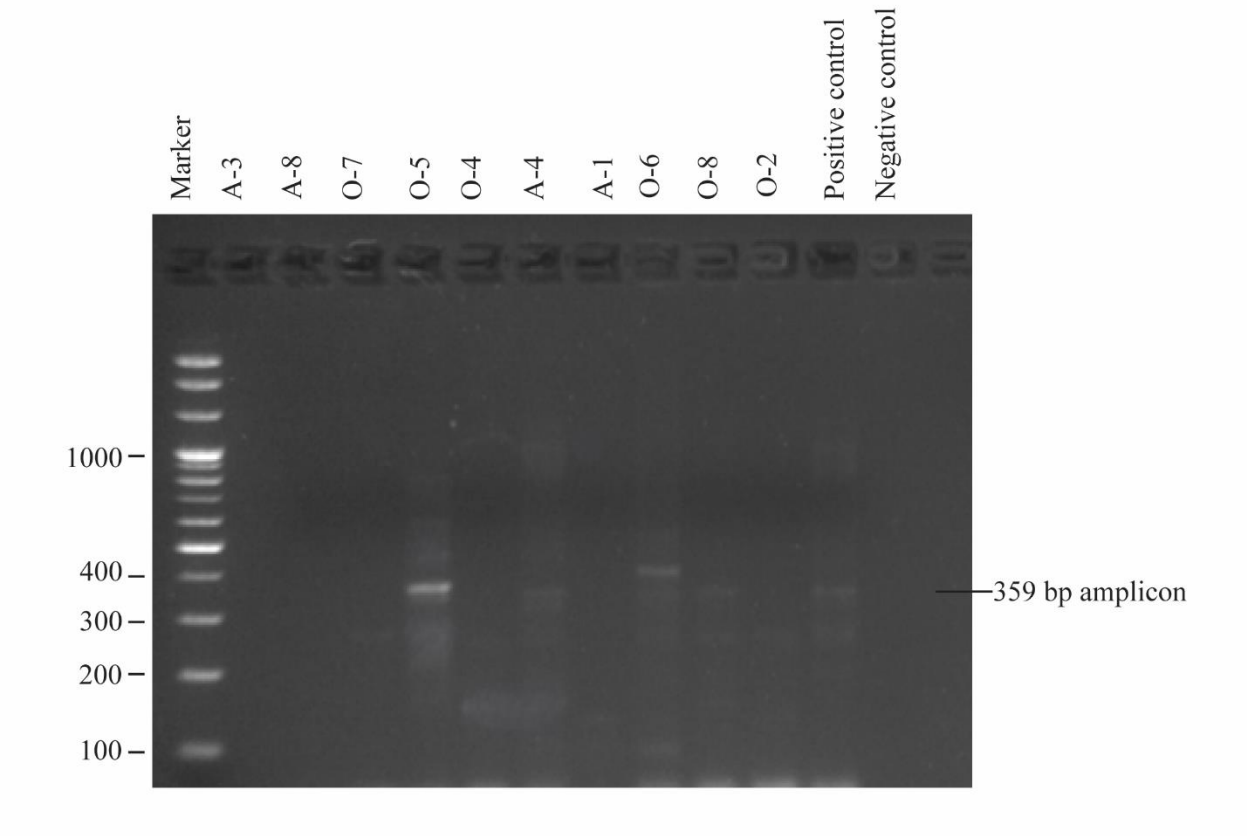


Figure 5: PCR Gel Electrophoresis Results of PCR amplicons of NUC gene of *S. aureus* isolates

Discussion

The current study provides a comprehensive analysis of antimicrobial susceptibility profiles for bacterial isolates from wildlife species, including deer, leopard cats, and fishing cats. One of the primary strengths of this study is the systematic approach employed to evaluate the bacterial growth and antimicrobial resistance patterns using Mannitol Salt Agar (MSA), Blood Agar, and Polymerase Chain Reaction (PCR). By incorporating these methods, we were able to confirm the presence of *Staphylococcus aureus* through both phenotypic and genotypic means. The study's robust design, which includes multiple isolates and a range of antibiotics, allows for a detailed understanding of the antimicrobial resistance landscape within these wildlife populations.

Antimicrobial susceptibility testing, or antibiograms, plays a crucial role in identifying the most effective antibiotics for treating infections, thereby guiding appropriate therapeutic interventions. This is particularly important in veterinary medicine and wildlife conservation, where misuse or overuse of antibiotics can lead to the development and spread of resistant bacterial strains. Studies have shown that wildlife can act as reservoirs for antimicrobial-resistant bacteria, which can be transferred to humans and domestic animals (Guenther et al., 2011; Arnold et al., 2016). Therefore, continuous monitoring and detailed antimicrobial susceptibility profiling are essential for maintaining both animal and public health.

The results of this study have significant implications for public health and wildlife conservation. Wildlife species, such as those investigated in this study, can harbor bacteria that are resistant to multiple antibiotics, posing a potential threat to human health through zoonotic transmission. For instance, the presence of multidrug-resistant *Staphylococcus aureus* in wildlife can lead to infections that are difficult to treat in both humans and animals (Schwarz et al., 2010). The high resistance rates observed for antibiotics such as ampicillin, amoxicillin + clavulanic acid, and oxacillin in this study underscore the necessity for prudent antibiotic use in veterinary practice. Additionally, the 100% susceptibility of the isolates to imipenem and gentamicin suggests that these antibiotics remain effective treatment options. However, the reliance on a limited number of effective antibiotics could accelerate the development of resistance if not managed carefully (Ventola, 2015).

In terms of wildlife conservation, understanding the antimicrobial resistance patterns in wildlife can help inform strategies for managing health risks in wildlife populations. For example, conservation programs can use this information to design better health monitoring and treatment protocols, ensuring that the use of antibiotics in wildlife rehabilitation centers is based on susceptibility data rather than empirical treatment. This targeted approach can help preserve the efficacy of critical antibiotics and reduce the risk of developing resistant bacterial strains in wildlife populations (Smith et al., 2014).

Comparing our results with existing data reveals both similarities and differences in antimicrobial resistance patterns. Previous studies have documented the presence of antibiotic-resistant bacteria in various wildlife species, often attributing the resistance to environmental contamination and human-wildlife interactions. For instance, a study by Silva et al. (2020) found high levels of resistance to tetracycline and sulfonamides in bacterial isolates from wild birds, which is consistent with our findings of high resistance rates to tetracycline (60%) and sulfamethoxazole + trimethoprim (60%). Other studies have reported varying resistance patterns depending on the geographic location and the specific wildlife species involved. For example, a study on European wild boars by Wasyl et al. (2014) observed high resistance rates to beta-lactam antibiotics, similar to our findings of 100% resistance to ampicillin and oxacillin. However, the same study noted lower resistance rates to tetracycline compared to our results, highlighting the potential influence of regional factors on resistance patterns. Our findings of 100% susceptibility to imipenem and gentamicin align with studies that suggest these antibiotics remain effective against a broad range of bacterial pathogens in both humans and animals (Livermore et al., 2007; Chikwendu et al., 2018). The continued effectiveness of these antibiotics is crucial for treating serious infections, particularly those caused by multidrug-resistant organisms.

Strength and Limitations

The strength of this study lies in its comprehensive approach, utilizing both phenotypic and genotypic methods to confirm the presence of *Staphylococcus aureus* and assess antimicrobial susceptibility. This dual approach enhances the reliability of the results and provides a more complete picture of the resistance patterns present in the bacterial isolates.

However, the study also has limitations that should be acknowledged. The sample size is relatively small, consisting of only 18 isolates, which may limit the generalizability of the findings. Additionally, the study focuses on a specific geographic area and a limited number of wildlife species, which may not fully represent the broader wildlife population. Future studies should aim to include a larger and more diverse set of samples to provide a more comprehensive assessment of antimicrobial resistance in wildlife.

Conclusion

This study highlights the critical role of antimicrobial susceptibility testing in identifying effective antibiotics for treating bacterial infections in wildlife. The high resistance rates observed for several commonly used antibiotics underscore the need for careful antibiotic stewardship to prevent the further spread of resistant strains. The results also emphasize the importance of monitoring antimicrobial resistance in wildlife as part of broader efforts to protect public health and ensure the success of wildlife conservation programs. By comparing our findings with existing data, we can better understand the factors influencing resistance patterns and develop more targeted strategies for managing antibiotic use in both human and veterinary medicine. Continued research and surveillance are essential to stay ahead of the evolving threat of antimicrobial resistance and to safeguard the health of both wildlife and human populations.

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Acknowledgement

The author would like to express profound gratitude and all praises to the Creator, whose blessings have enabled the completion of this report.

She is deeply grateful to her supervisor, Dr. Tofazzal Md. Rakib, Associate Professor, Department of Pathology and Parasitology, CVASU, for his sympathetic supervision, inspiration, constructive criticism, valuable suggestions, and providing essential information throughout the work.

Special thanks are extended to Dr. MD. Tanvir Ahmed Nizami, Lecturer, Department of Microbiology and Veterinary Public Health, CVASU, for his invaluable advice and suggestions during the study.

She also extends her sincere thanks to Dr. Md. Sarwar Uddin, Veterinary Surgeon at Chattogram Zoo, for his kind assistance during sample collection.

Finally, heartfelt thanks to all the lab technicians and support staff of PRTC for their help in lab work.

The Author