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Original Research Article

Microbiological Study of *Staphylococcus aureus* Bacteria Collected from Equine Nasal Swab Sample

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Abstract: The process of collecting and identifying *Staphylococcus aureus* bacteria from nasal swab samples obtained from 20 horses at the Equestrian Club in Baghdad Governorate and the Horse Recreation Club in Babylon Governorate was conducted between October 2022 and March 2023. Identification of the isolates was carried out by observing colony morphology on various bacterial media, performing gram staining, and conducting biochemical tests, notably the catalase test. Antimicrobial susceptibility testing was also performed to determine the resistance profile of *S. aureus*. Out of the total nasal swab samples collected, *S. aureus* was detected in 12%, while other types of bacteria accounted for 8% of the samples. Confirmation of *Staphylococcus* samples was achieved through Gram staining and culturing on selective medium, such as Mannitol Salt Agar, which produced characteristic yellow colonies. Additionally, the catalase test yielded positive results, indicated by the production of bubbles, confirming the presence of the catalase enzyme. To assess the virulence factors and pathogenicity of the bacteria, they were cultured on Gonco Red Agar medium to evaluate biofilm formation, resulting in a positive outcome. Susceptibility testing revealed varying degrees of susceptibility among *S. aureus* strains to antimicrobial agents, with Ciprofloxacin (34%), Trimethoprim (20%), Amikacin (11%), and Tobramycin (18%) showing effectiveness. Conversely, *S. aureus* exhibited 100% resistance to Gentamicin and Ampicillin.

Keywords: *Staphylococcus aureus,* Antimicrobial susceptibility and resistance profile of *Staphylococcus.*

INTRODUCTION

Staphylococcus aureus is a zoonotic commensal and pathogen related to a number of potentially fatal infections in humans and other animals, including horses, with symptoms ranging from cosmetic to sever fatal disease [1].

S. aureus has a thick cell wall that shields it well from external environmental conditions. Therefore, one of the main targets of contemporary antimicrobial therapy is the cell wall. Peptidoglycan is the primary component of the cell wall. The global spread of methicillin-resistant *S. aureus* (MRSA) highlights *S. aureus'* mastery of antibiotic resistance [2].

Among of the most recognized pathogenic organisms is *S. aureus*, which can cause a wide variety of infections, from somewhat minor skin and soft tissue infections to potentially fatal conditions like endocarditis, pneumonia, and bacteremia. The invasive activities of *S. aureus* is frequently activated through injury or medical involvements, where the barrier function of the host cells or tissues is compromised. When host cells or tissues' ability to serve as a barrier is weakened, such as occurs after an injury or medical intervention, *S. aureus* often becomes penetrating. *S. aureus* generates a number of virulence factors that facilitate the bacterium's penetration of human tissue. It can adhere to the outermost layer of host cells and tissues, infiltrate host cells, or create biofilms on tissues and implant surfaces when it is unprotected by the epithelial or endothelial layers [3].

S. aureus is capable to successfully evade innate and adaptive immune defenses of the host, either through the secretion of particular virulence factors or the formation of biofilms [4]. Eventually, this may result in persistent or chronic infections [5].

Through secreting specific virulence factors or forming biofilms, *S. aureus* can effectively evade the host's innate and adaptive immune systems (4). This might later lead to chronic or recurrent infections [5].

Methicillin-resistant *S. aureus* (MRSA) generations have begun to appear in hospitals and the community, which is an important example of how difficult it has become to treat *S. aureus* infections in recent times due to the organism's acquisition of multiple antibiotic resistances [6].

Mostly unproductive bacteria from equines nasal passages and sinuses is aerobic in nature [7]. *S. aureus* is a main cause of surgical site infections, pneumonia, and other respiratory tract infections [8]. A potential risk for contracting *S. aureus* from animals is working exposure to meat-related products and animals [9]. The aimed of this study was to isolation and detection of some virulence factors of *S. aureus* that was collected from horse nasal swab samples.

MATERIALS AND METHODS

Study Area:

The research was conducted at the Equestrian Club in Baghdad Governorate, focusing on horses used for racing, and at the Horse Entertainment Club in Babylon Governorate, where horses are utilized for entertainment purposes. The study took place between October 2022 and March 2023.

Sample Collection:

Twenty horses were selected for the study, with each horse providing a single nasal swab sample. Nasal swabs were taken by gently inserting a cotton-tipped swab into a single nasal passageway, ensuring contact with the nasal mucosa. Samples were promptly transported with ice packs to the Department of Veterinary Microbiology Research Laboratory at the University of Al-Qasim Green for further processing.

Bacterial Isolation and Identification: Preparation of Culture Media:

Culture media were prepared as per manufacturer instructions, sterilized by autoclaving at 121°C for 15 minutes under 1 bar pressure, and refrigerated for 24 hours at 37°C to check for contamination.

Culture Method:

Standard culture techniques were employed for bacterial growth. Samples were initially inoculated onto nutrient agar medium for bacterial growth. Differential and selective media available in the laboratory were used, and diagnostic confirmation was achieved through ram staining and biochemical testing.

Biochemical Test (Catalase Test):

A small portion of bacterial culture was obtained from nutrient agar and placed on a slide with a few drops of 3% hydrogen peroxide (H2O2). The immediate release of gas bubbles indicated a positive result, following the method described by [10].

Biofilm Formation by Congo Red Agar Method:

Congo Red Agar medium was utilized to assess biofilm formation capability. The agar was prepared by mixing sucrose and Congo red with Brain Heart Infusion Broth, autoclaved, and combined with agar and sucrose. Bacterial strains were inoculated onto the agar and incubated at 37°C for 24 to 48 hours.

Biofilm producer isolates manifested as black colonies with a dry crystalline appearance, while non-biofilm producer isolates displayed red colonies, following the method outlined by [11].

Antimicrobial Resistance Profile:

Antimicrobial susceptibility testing for *S. aureus* isolates was conducted using the disc diffusion method with various antibiotics, following protocols outlined by the [12].

RESULT AND DISCUSSION

Occurrences

Out of 20 horses sampled from total of 40 nasal swab samples collected from the horses, 12 (60%) harbored *S. aureus* and 8 (40%) other different types of bacteria were recovered as show in figure (1).

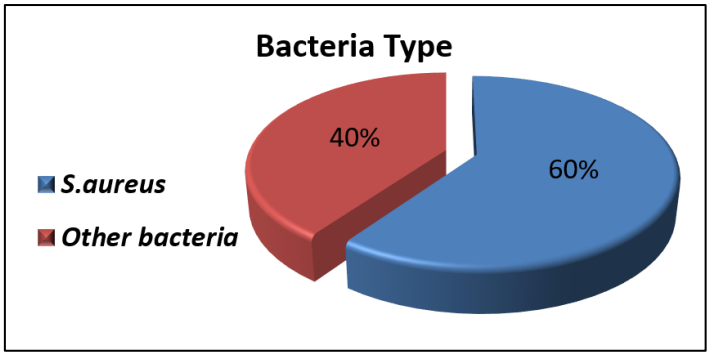

Figure 1: Percentage of *Staphylococcus* and other bacteria isolated from nasal mucosa of horse

Gram Staining

Gram staining was performed for all samples, *S. aureus* is a Gram-positive commensal bacterium and opportunistic pathogen.

S. aureus showed the shape of grape clusters under the microscope after staining (Gram Positive Stain) as show in figure (2).

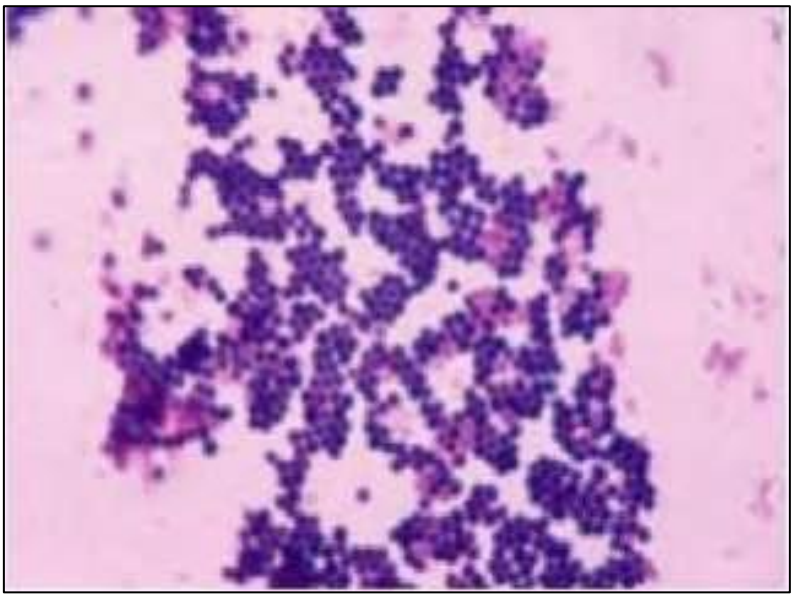

Figure 2: Gram positive stain of *S. aureus*

Culturing on Selective Media

The diagnosis of the bacteria was confirmed by culturing it on selective media (Mannitol Salt Agar) and it gave a positive result by showing yellow colonies as show in figure (3).

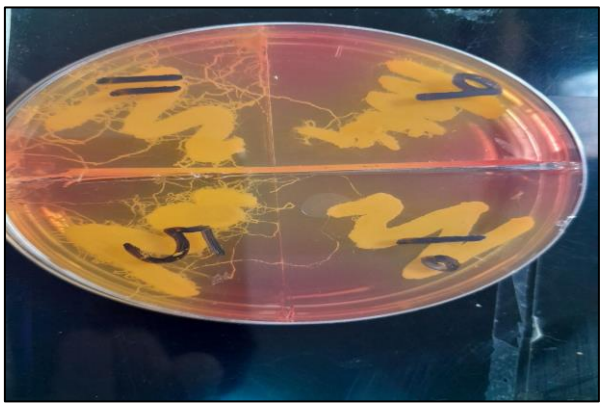

Figure 3: *S. aureus* on Mannilol Salt Agar media

Catalase Production Test

S. aureus is showing a catalase test positive by presentation a bubbles indicating the bacteria's production of the catalase enzyme as show in figure (4).

Figure 4: Production catalase by of *S. aureus*

Result of Biofilm: *S. aureus* is stick to surfaces and contribute in biofilm production as show in figure (5).

Figure 5: Biofilm of *S. aureus* (CRA)

Result of Antimicrobial Susceptibility/ Résistance

In this study, Ciprofloxacin, Trimethoprim, Amikacin, Tobramycin, Gentamicin and Ampicilin were determined as the antibiotics to which the agents were most susceptible to *S. aureus* as show in table (1).

Table 1: *S. aureus* antimicrobial susceptibility/ résistance

Antimicrobial	*S. aureus percentage*	Susceptible/Resistance
Ciprofloxacin	34%	Susceptible
Trimethoprim	20%	Susceptible
Amikacin	11%	Susceptible
Tobramycin	18%	Susceptible
Gentamicin	100%	Resistance
Ampicilin	100%	Resistance

Figure 6: Antimicrobial susceptibility/ resistance to isolated *S. aureus*

DISCUSSION

In this study the percentage of *S. aureus* was 12% from total collected sample.

S. aureus are predominantly exciting bacteria, this bacteria is part of the common equine nasal microganism [13].

The incidence of *S. aureus* in horse nasal mucosa is assessed to be nearly 10% [14].

S. aureus incidence in this study is higher than the 2.0%, 7.9%, 6.8% nasal swab *S. aureus* incidence in healthy horses in the Iran [15], Canada [16], Germany [17]. On the other hand, it is lower than 40%, 20.3% and 19.5%, *S. aureus* incidence among horses in Belgium [13], USA [18] and South Korea [19].

Variations in *S. aureus* incidence across studies can be attributed to variations in the methods used to identify (phenotypic or molecular) *S. aureus* strains, animal health, equine farming applies, environmental features, and *S. aureus* contamination level.

Facultatively anaerobic, Gram-positive, non-motile *S. aureus* typically has a diameter of one millimeter. *Staphyle*, which means bunch of grapes in *Greek*, is an ir-regular cluster designed when cells division on multiple planes [20].

The term "*Aureus*" (golden) comes from the appearance of *S. aureus* colonies, which often exhibition a golden-yellow pigmentation on a variety of media, mostly after prolonged incubation [20].

Among the staphylococci, *S. aureus* is by far the most important pathogen and is catalase positive. It releases enzymes that are thought to be virulence determinants, such catalase. This enzyme makes bacteria more resistant to hydrogen peroxide's ability to kill them both within and outside of cells [21].

According to [22], the ability to produce catalase, is a defining characteristic of the species within the genus *Staphylococcus*. Among them, only two species *Saccharolyticus saccharolyticus* and *S. aureus subsp. anaerobius* cannot release catalase.

In our study the susceptibility of *S. aureus* to antimicrobial agents Ciprofloxacin (34%), Trimethoprim (20%), Amikacin (11%), Tobramycin (18%), and the resistance of *S. aureus* to antimicrobial agents Gentamicin and Ampicilin were (100%).

Unsuitable usage of antimicrobial agents has directed to the increase the resistance *S. aureus*. All over the world, it is possible to use antimicrobial drugs as preventatives and therapeutics for severe equine infections such endotoxaemia, sepsis, and *Rhodococcus equi* infection without initial susceptibility testing [23].

Researches on active and correct use of antibiotics in horses with *S. aureus* infection of is not sufficient. Prospective studies is a need for that monitor horses with and without antibiotic therapy. Isolate your agent and perform an antimicrobial test, it will be more suitable to prevent these negative results.

A variety causes of antibiotic resistance in *S. aureus* can occur. Once clinicians prescribe antibiotics unnecessarily can cause *S. aureus* to become hardy to destroyed. When owner misuse antibiotics and don't finish the entire course prescribed, for instance, when animals receive the correct medicinal products, the owner might provide only a portion of the course and not the entire quantity, which could leave bacteria untreated. Antibiotic resistance will rise as a consequence of this [24].

Misuse of antibiotic will cause undesirably affects and prolong the prognosis of infection in horses and causes low performance rate. By collecting a nasal swab samples, quick identification of causative agent can be done without causing pain to the horse and without the requirement for tranquilizer.

Training equipment’s, stable environments and horses sent from cold weather conditions to warm weather conditions, contaminated hands of peoples, flies that colonize horses act as vectors, and/or contaminated grass/ herbs in contact with horses may serve as routes of transmitted of *S. aureus* to horse.

CONCLUSIONS

There was a greater infection of horse by *S. aureus* than other bacteria. Additionally, there had been varying degrees of susceptibility among *S. aureus* strains to antimicrobial agents. Horses with improper or inadequate antibiotic

administration have a worse prognosis for infections and have lower performance. Therefore, perfect recognition of the *S. aureus* responsible for the infection in horses is crucial.

Ethical Reflection

All ethical consideration, including novelty of arrangement, plagiarism, and multiple submissions, was considered by the authors.

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A Conflict of Interest: The authors have not declared any conflicts of interest.

Authors' Contribution: Each author participated in the similar way.

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