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Isolation and molecular identification of *Staphylococcus lentus* from ear infections in dogs and cats

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Abstract

This study was designed to investigate *Staphylococcus lentus* as an etiological agent of ear infection in dogs and cats in Baghdad during the period between 1 May 2025 till 1 August 2025. Biochemical and molecular study revealed presence of 8 strains of this bacteria 5 out of 50 in dogs and 3 out of 50 in cats. Antibiotic sensitivity test was done using ordinary antibiotics and this shows clear disparity between antibiotics used that extends from 100% resistant to penicillins and 100% sensitive to gentamicin and this was in disagreement with a lot of studies even that done in Iraq. We conclude that *Staphylococcus lentus* which isolated from dog and cat otitis, measured as a pathogenic bacteria and it has the capability to cause illness as well as multi antibiotic resistance.

Keywords: *Staphylococcus lentus*, otitis, dogs, cats

Introduction

The bacterium *S. lentus* is a gram-positive which belongs to *Staphylococci*, a commensal infections [1]. Madhaiyan *et al.*, (2020) [2]. Reclassified *S. lentus* to a new genus picture of *Mammalicoccus lentus*. The earlier studies provide genome sequences of 64 category strains in place of 50 species of *Staphylococcus* that have grade in the taxonomy [3]. *Staphylococcus lentus* is a commensally bacterium colonize on the skin of numerous animals. It has usually been isolated from animal products, *S. lentus* has been connected with subclinical mastitis [4]. It has been linked with infections in animals [5]. This bacterium is a part of the *Staph. Sciuri*. It is a widespread bacterium in the surroundings. These bacteria are habitually well thought-out animal pathogens and have been isolated from a broad array of pets [6]. Otitis is an inflammation of the hearing canal normally observed in dogs and cats in small animal veterinary practice. Signs consist of irritation, pain/tenderness, malodor, release of exudate, extreme scratch, and head trembling. Chronic cases show extended clinical picture or development to end of the hearing canal, leading to auditory polyps, or burst of the tympanic covering membrane, all of these signs lead to chronic hurt and deafness [7]. The normal-habitant bacterium of external ear is fairly slim, like to flora of the conjunctiva excluding pneumococci, *Staphy. aureus* and *Enterobacteriaceae*, *Pseud. aeruginosa* and non-*Candida albicans* [8]. In cats, *Staphylococcus* spp. considered as most common cause, followed by *Proteus* spp., and *Pseudomonas* spp. [9]. While, in canine *Staphy. spp.*, *Pseudomonas*, *Proteus*, *Enterococcus*, *strept.*, and *Coryne.* are the common [10].

Materials and Methods

Ethical approval

This experiment was conducted in compliance with the institutional rules of the University of Baghdad's College of Veterinary Medicine's Animal Care and Use Committee, Iraq. Project Number. (P.G./2691 in 26/10/2025). The herd's owners gave their verbal agreement before samples were taken.

Collection of samples

Swabs were collected from ears of 50 dogs, 50 from cats aged between 1-5 years in the period between 1 May 2025 to 1 August 2025.

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All these animals show respiratory infection and itching ears. Collected samples then inserted into Cary-Blair transportation media tubes and immediately send to the laboratory in a cold pack for isolation.

Isolation and identification

Each sample inoculated on to brain heart infusion broth and transport to the laboratory in zoonotic disease research unit. Samples were cultured on, and when growth appeared, cultured on Mannitol salt medium at 37 °C for 24 hours. Biochemical tests for isolation and identification by catalase and coagulase tests was done [11]. All samples were submitted to standard methods and biochemical tests via viable kits (GP-VITEK2 colorimetric identification kit) for *Staphylococcus* bacteria (BioMerieux, France).

Antibiotic Sensitivity Test (AST)

Antibiotic resistance test was done via "Vitek 2 system compact method".

Molecular detection

Genomic DNA-extraction of *Staph. lentus* performed by extraction of DNA from bacteria according to the procedure of Genaid DNA removal magnification of a fragment of *S. lentus* 23S rRNA by PCR by means of primers. Forward (5'-TCGGAATCTGGGAGGACCAT-3'); Reverse (5'-AATCGTAAGTCGGTTCGGTCC-3').

Results

Some isolates had the capability to mannitol fermentation and have appearance of huge yellow colonies enclosed by large gold color zones and twisted the medium from cherry to golden color, others which were non mannitol-fermenting bacteria showed tiny white colonies and no color alter was occurred on the medium Figure (1A). The end results clarified that the positive results for *Staphylococcus lentus* show dogs (5) isolates and cats (3) isolates. Other samples consider being negative results. *Staphylococcus lentus* show convex, mucoid, yellow color on mannitol salt agar, figure (1 B). While on blood agar grey to white colonies and show B hemolysis, figure (1 C).

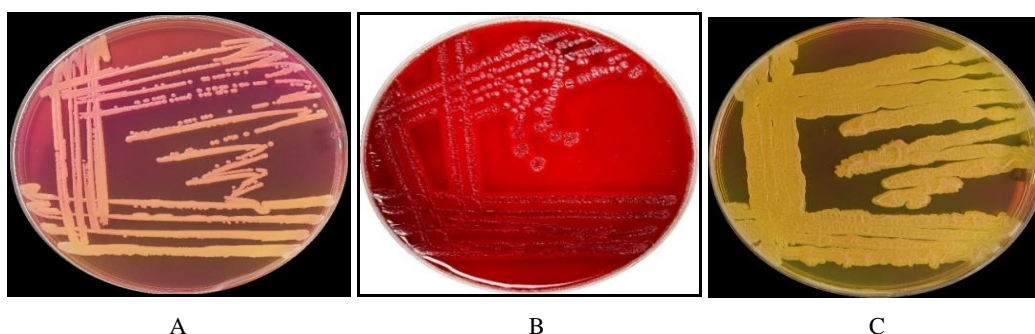


Fig 1: *Staphylococcus lentus* on A: Mannitol Salt Agar show pink to yellow colony, B: Blood agar, C: Mannitol Salt Agar

Biochemical tests

Biochemical tests revealed that all isolates were positive results for catalase figure (2), and negative results for

coagulase, figure (3, 4). These all results were also mentioned also by [12].



Fig 2: Catalase test of *Staphylococcus lentus* show positive result



Fig 3: Coagulase test of *Staphylococcus lentus* show negative result: Coagulase tube method



Fig 4: Coagulase test of *Staphylococcus lentus* show negative result: A: Coagulase tube method (Coagulase slide method).

Antibiotic Sensitivity Test

Antibiotic sensitivity test for *S. lentus* isolates showed elevated resistance to almost well-known antibiotics. This called multidrug resistant (MDR). Thus, they exhibited highly resistance (100%) to Benzylpenicillin, Piperacillin/Tazobactam and Oxacillin. While sensitive 100% to Gent., Tob., Levo., Mox., Lin., Vanc., Tetr., Tigec., Rifam and Trimeth. Sulfameth. Resistant to Clindamycin (66%), and sensitive to Fusidic-Acid (66%), Erythr. (66%), Teicoplanin (66%).

Molecular tests

Molecular test of positive cases that confirmed the infection was five cases of dogs and three cases of cats by amplification of the 23s rRNA gene of *S. lentus*.

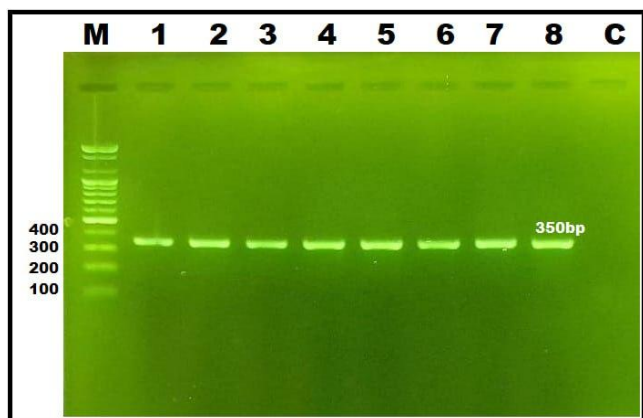


Fig 5: Gel electrophoresis (1.2%) 80 mA, 100 vol. with red safe stain show the result of amplification of 350 bp of *Staphylococcus lentus*. Line M: DNA ladder, Line 1-8: Positive cases, Line C: Negative control.

Discussion

Staphylococcus lentus. Although it is commonly regarded as part of the normal skin, ears and mucosal micro biota of animals, recent research has shown that it can possess clinical and epidemiological importance, particularly as an opportunistic pathogen and a reservoir for antimicrobial resistance genes [13]. Biochemical results concerning isolation and identification of this bacteria typically agree with standard bacteriological procedures and results designed for *Staphylococcus* species [14]. *Staphylococcus lentus* represents an important isolate among staphylococcus family in dog and cat otitis in the current study (10%) in dogs and (6%) in cats otitis, and this was supported by many studies that considered this bacteria a commensal organism that can be isolated from animals [4]. The study also agrees with [6], who considered the bacteria the major isolates of pets. This results relatively have a high agreement with [15] who record a high level of resistance toward members of penicillin family, but disagreement with their results in clindamycin which record 66% resistant in our study and 34.2% resistant in their study. Other studies record 100% resistant to erythromycin, [16], which highly differ from our results which record 66% resistant. Generally, almost the resistance of penicillin was recorded by most researchers [17, 18, 19], but the current findings with regard to oxacillin show high disagreement with [20] who record 40% resistant and [21] who detect 29.5%, while gentamicin in present study show 100% activity and all isolates (100%) were sensitive, this was in disagreement with [22, 23, 24] who recorded 39.1%,

27.35%, and 59%-80% respectively sensitive to gentamicin. The overall researchers concerning antibiotics sensitivity may have a broad different aspects and this may due to many factors such as environmental differences and health rules of countries and random use of antibiotics. In molecular study, we identified *Staphylococcus* gene by using the references gene 23S rRNA and all selected isolates show positive result that shown in figure 4 which indicate the positive results of amplification with high purity on 350 bp. We considered the molecular result as the documented for number of *Staphylococcus lentus* due to the closely related biochemical behaviors of members of staphylococci [25].

Conclusions

The appearance of a novel type of bacteria *S. lentus* which isolated from hospitals formerly pets otitis, measured as a pathogenic bacterium and have the capability to induce illness as well as multiple drug resistance. *Staphylococcus lentus* should no longer be overlooked as a mere commensally organism. Its ability to harbor resistance determinants and occasionally cause infections makes it an important bacterium for veterinary and zoonotic research. Continuous monitoring, molecular characterization, and prudent antibiotic use in animals are essential to control its potential impact on both animal and human health.

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Conflict of Interest

Authors declare, there is no conflict of interest.

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