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Isolation of some pathogenic *Staphylococci* from cat otitis and diagnosed by Vitek-2 system

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Abstract

This study was designed to investigate *Staphylococci* isolates from clinical otitis without consideration that it was otitis externa, media and interna. We recorded 28 positive *Staphylococcus* species out of 50 clinical cases of otitis. The major isolates were *Staphylococcus lentus* (14%) then *Staphylococcus xylosus* and *Staphylococcus felis* (10%). The lowest isolates were *Staphylococcus hominis ssp hominis*, *Staphylococcus schleiferi* and *Staphylococcus vitulinus* (6%, 6%, and 4%) respectively. All isolates were coagulase negative except *Staphylococcus schleiferi* which behave with variable coagulase. This study concluded that *Staphylococcus* species were the major bacterial cause of otitis in cats with a clear evident that coagulase negative *Staphylococci* in the most important.

Keywords*: Staphylococcus*, Otitis, *S. lentus, S. schleiferi, S. xylosus*

Introduction

By definition, otitis is an inflammation of the pinna or canal of ear (Kennis, 2013). Otitis externa resembles infections of the skin and soft tissue in other regions. There are two types of external ear infection: acute and chronic; acute external ear infection can be either localized or diffuse (Hayyawi, 2012). Otitis media is an inflammation of the mucoperiosteal lining of the middle ear, either partially or completely (Al-Obaydi, 2011; Al-Hamadany, 2013), there are three classifications of otitis media: acute otitis media, otitis media with effusion, and chronic suppurative otitis media (Schilder *et al.*, 2016). Otitis interna indicates impairment of the auditory system; neurological manifestations and hearing loss are typically observed (Kennis, 2013; Wiegand *et al.*, 2019).

Otitis is a condition frequently associated with unclean cat ears, leading to the accumulation of earwax and fluid. Otitis results from the inflammation of the ear canal epithelium and other structures, including the external auditory meatus and pinna (Usrina *et al.*, 2023). Acute otitis occurs due to a cold, allergies, or upper respiratory infection affecting the ear, nose, sinuses, larynx, or throat. The presence of germs or viruses results in the formation of pus, inflammation, and mucus behind the eardrum, obstructing the Eustachian tube (Al-khafaji, 2013). The presence of middle ear fluid, or middle ear effusion (MEE), along with acute infection symptoms, is characteristics of AOM (Lieberthal *et al.*, 2013).

Otitis media with effusion (OME) is the collection of fluid in the middle ear chambers, absent any indications of acute infection (Rosenfeld *et al.*, 2016). This condition can result in loss of hearing, which may hinder a child's language and behavioral development (Vanneste and Page, 2019; Rahim, 2019). Chronic suppurative otitis media (CSOM) is a disorder characterized by persistent inflammation of the middle ear mucosa and mastoid cavity. It persists for over two months, culminating in a perforation of the eardrum and causing continuous leakage from the ear canal. This chronic condition can lead to severe health consequences, including cerebral problems and considerable morbidity in affected individuals (Morris, 2012; Khairkar *et al.*, 2023; Bhutta *et al.*, 2024). Otitis interna, or labyrinthitis, refers to the inflammation of the labyrinth, a complex of fluid-filled passages within the inner ear (Rosenfeld, 2003; Wipperman, 2014). The flora of the external ear is typically very sparse, and it is qualitatively similar to that of the conjunctival sac (Hayyawi, 2012). The intimate relationship between felines and humans promotes the dissemination of many microorganisms, including critical pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA), which is linked to multiple challenging human diseases (Muniz *et al.*, 2013; Ibraheim *et al*., 2023a, b).

With the exception of the Scottish Fold, domestic cats exhibit erect pinnae, which enhance air circulation. This makes them less vulnerable to environmental factors that contribute to otitis, such as humidity and the effects of bathing or swimming. Despite the structural variation in the Scottish Fold, an increase in otitis has not been observed in this breed (Brame and Cain, 2021).

Staphylococci can induce several fairly severe skin and soft tissue infections, as well as severe and frequently deadly conditions such as sepsis, osteomyelitis, endocarditis, and pneumonia (Cheung and Otto, 2023). Approximately fifty species within the *Staphylococcus* species are non-pathogenic members of the normal flora present on the skin and mucous membranes of mammals (Becker *et al.*, 2014). Species inhabiting the same environment may exchange genetic material, particularly plasmids, which can impart genes that confer antimicrobial resistance to pathogenic species like *S. aureus* (Rossi *et al.*, 2016). Consequently,

organisms that have been traditionally misidentified as innocuous may pose a threat to human health by serving as reservoirs for genes associated with closely related illnesses (Otto, 2013; Rossi *et al.*, 2017).

The principal etiological factors of external otitis affecting the ear such as foreign bodies, parasites, fungi (e.g., *Aspergillus*, *Sporothrix*, and *Stephanoascus*), allergies, autoimmune and immune-mediated disorders, endocrine and glandular dysfunctions, viruses, and additional variables (Mittal *et al.*, 2014; Mascarenhas *et al.*, 2019). Secondary causes of external ear infection affect unusual ear conditions and include bacteria, fungi, bad treatment reactions, excessive cleaning, and overgrowth of yeast (Kittl *et al.*, 2018).

Materials and Methods

Collection of samples

Ear swabs were obtained from 50 animals, comprising both foreign and indigenous cats. Of the investigated animals, 20 had respiratory disease, 15 had ear mites, and 15 were experiencing ear pruritus. Collected swabs were thereafter placed into Cary-Blair transport media tubes and dispatched to the laboratory within two hours of collection in a cooler box for bacterial isolation.

Preparation of cultural Media

All media were prepared following the manufacturer's guidelines and autoclaved for 15 min at 121°C and 15 psi to ensure sterility. The media were subsequently allocated into sterile Petri dishes. The media were incubated at 37°C for a whole day to ensure they were devoid of contaminants. Specific additives were required for particular media (Al-Mashhadani, 2022).

Isolation and identification of Staphylococci

Each specimen was infected immediately into brain heart infusion broth (Safana and ASMAA, 2002), and transported to the laboratory in the zoonotic disease research section. Each sample was inoculated onto non-selected blood agar, and upon the appearance of growth, it was then cultivated on selective Mannitol salt agar plates in the laboratory. Incubated Petri dishes at 37°C for 24 hours (Al-Mathkhury and Rhumaid, 2012; Al-Hasnawi, 2017). Biochemical assays for isolated bacteria were determined using catalase and coagulase tests (Wed and Huda, 2024). All specimens were diagnosed microscopically (Gram stain), morphologically, and biochemically according to standard techniques and biochemical tests utilizing commercial kits (GP-VITEK2 Gram positive colorimetric identification kit) for *Staphylococcus* bacteria (BioMerieux, France) (Abu-Raghif, 2016).

Statistical analysis

One-Way ANOVA in the GraphPad Prism Software was used to estimate significant differences between the obtained values at $p<0.05$ (Gharban, 2024).

Results

Our results showed isolation of many strains of *Staphylococcus*. We isolate 28 of different species of genus *Staphylococcus*. The major isolates were *Staphylococcus lentus* then *Staphylococcus xylosus* and *Staphylococcus felis*. No significant differences recorded between male and female used in this study. These were clearly obtained in table (1).

Table (1): Isolation of *Staphylococcus* species from otitis cats according to sex

Bacterial spp.	Cats (Total 50 Samples)				Percentage %
	Male	Female	P-value	Total	
S. lentus	4 (57.14%)	3 (42.86%)	0.705 NS	7	14%
S. xylosus	3 (60.00%)	2 (40.00%)	0.654 NS	5	10%
S. hominis ssp hominis	0 (0.00%)	3 (100%)	0.049 *	3	6%
S. schleiferi	1 (33.33%)	2 (66.67%)	0.593 NS	3	6%
S. vitulinus	2 (100%)	0 (0.00%)	0.167 NS	2	4%
S. felis	2 (40.00%)	3 (60.00%)	0.654 NS	5	10%
S. simulans	0 (0.00%)	3 (100%)	0.049 *	3	6%
Total isolates	12 (42.86%)	16 (57.14%)	0.449 NS	28	56%
Chi-square–χ^2 (P-value)	3.977 * 0.0463	3.869 * 0.0498	--	--	1.917 0.495 NS
* (P≤0.05), NS: Non-Significant					

Culture media

Some isolates of *Staphylococcus lentus* exhibited the capacity to ferment mannitol, resulting in the formation of large golden colonies encircled by extensive yellow zones, which altered the medium's colour from pink to yellow. Conversely, other isolates did not ferment mannitol, appearing as small white colonies without any observable colour change in the medium (Al-Salamy *et al.,* 2017). Figure 1A. *Staphylococcus schleiferi* are smooth, round, white in hue and do not ferment mannitol. Figure 1B. *Staphylococcus xylosus* exhibited yellowish, mucoid, convex, smooth colonies that did not ferment mannitol. Figure 1C.

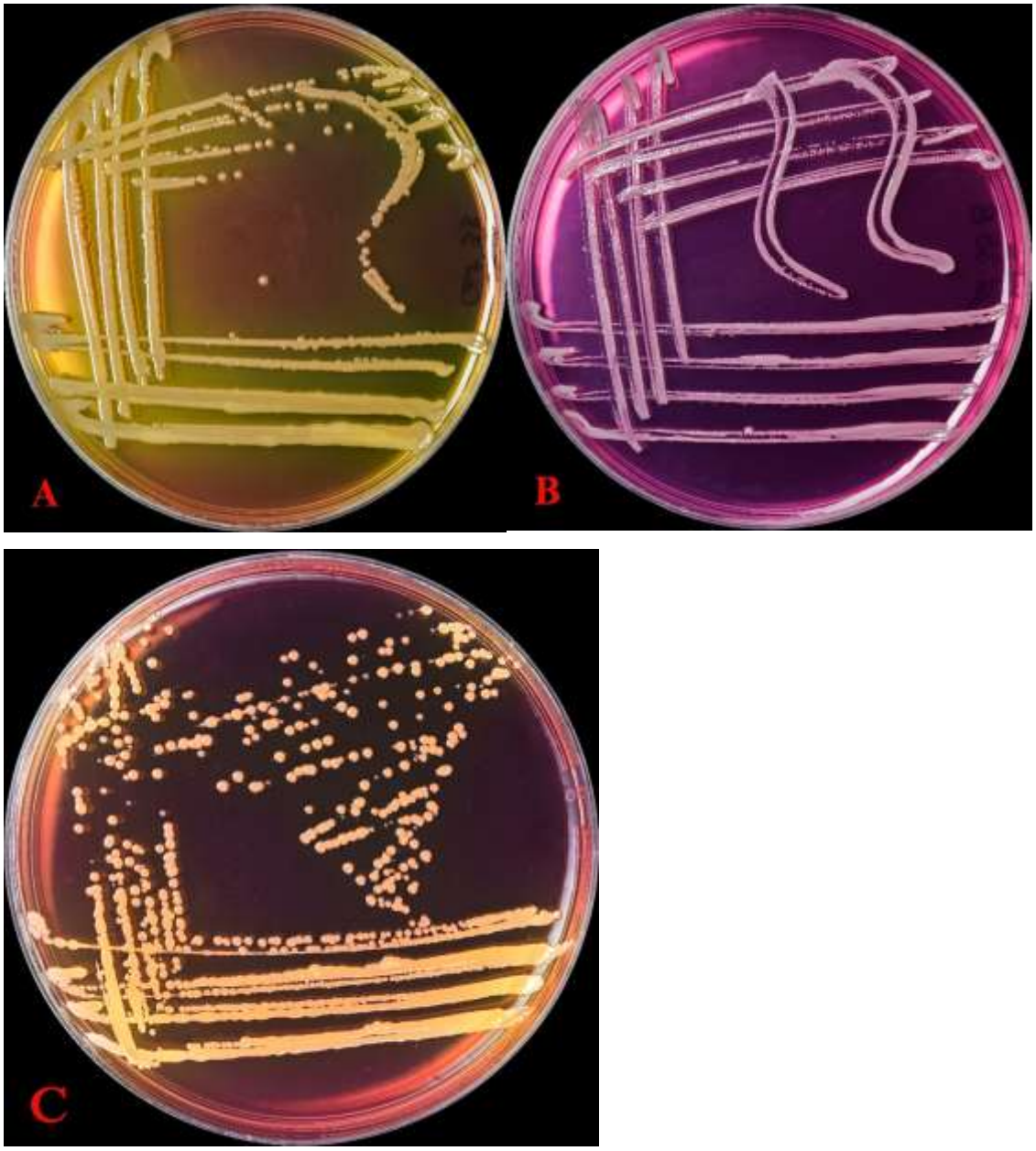

Figure (1): *Staphylococcus* species on mannitol salt agar. A) *S. lentus*; B) *S. Schleiferi*; C) *S. xylosus*

Discussion

Staphylococcus lentus was the major isolates in the current study and this was supported with many studies that considered this bacteria as a commensal organism that can isolated from a human and animals (Schwendener & Perreten, 2012; Choudhury *et al.*, 2023). We agree also the results with Hay and Sherris (2020) who considered the bacteria as the major isolates of pets. *Staphylococcus xylosus* was also isolated in this study with high percentage. This bacterium has a special important due to its strong ability to form biofilm. So it isolated from human and small mammals (Nagase *et al.*, 2002), and it considered as an opportunistic bacteria causing infection in human and animals (Gozalo *et al.*, 2010).

Staphylococcus schleiferi was also isolated in high percentage in this study. This was in agree with Abouelkhair and Kania (2024) who mention that this bacteria have the ability to cause infection in human and animals. Our study was in agree with Cain *et al*. (2011) who isolated *S. schleiferi* from dogs and cats.

Our study also support many previous results done by many researchers and conclude a fact that coagulase negative *Staphylococci* increasingly associated with animal diseases (Somerville, 2016; Morris *et al.,* 2017). Other researchers such as David and Elliott (2015); Siciliano *et al*. (2018); Couffin *et al*. (2018) had isolated these bacteria from severe cases of endocarditis and meningitis in animals but they didn't mentioned about otitis. Hiblu *et al.,* (2020) considered Staphylococci as a major and most prevalent among bacterial causes of otitis and this was highly agreed with our study. Other isolates in the current study which are *Staphylococcus hominis ssp hominis*, *Staphylococcus vitulinus* and *Staphylococcus felis* which are coagulase negative didn't highlighted in studies previously but they were isolated in a considered percentages in the cuurent study. So, they must be high attention in further studies because the isolated from a clinical severe otitis. As a general view, our results of isolation agree with many studies different parts of the word. Many researchers recorded the same isolates in different proportions (Lal *et al.,* 2018; Far *et al.,* 2021; Sips *et al.,* 2023).

Conclusions

This study concluded that *Staphylococcus* species were the major bacterial cause of otitis in cats with a clear evident that coagulase negative *Staphylococci* in the most important.

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