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Isolation and identification the bacterial and fungal causes of otitis externa in dogs and humans in Diyala Province, Iraq

A Thesis
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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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Dedication

To Whom Who sent to us as a merciful prophet ((Mohammed)) peace be upon him. . . . . .

To that which was and is still the haven of national grief, my dear country ((Iraq)).

To those who light the way for science. . . . . .

To whom who taught me the letter ((My dear teacher)). . . . . .

To the candle that burned to lighten the way ((My beloved mother)). . . . . .

To whom who planted the seed of patience in myself and walked in my long way ((My darling father)). . . . . .

With my loyalty and commitment

Ibrahim

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Summary

Otitis externa is the most common disease of the ear canal in the dog and human with a multifactorial etiology, which includes bacteria, fungi, yeasts and ectoparasites. The study was conducted on (250) samples, represented (100) ear swabs collected from peoples, (50) from males and (50) swabs obtained from females, while (150) samples obtained from dogs (75) from males and (75) swabs collected from females. Samples collected from different places in Diyala Province, during the period from beging of 2020 the of 2021. Identification of isolates were depended on the morphology of the colony on agar used in the study (color and shape) in addition to their odor, and biochemical tests then conducted by VITEK 2 to identify the isolated bacterial and fungal species.

Clinically,skin scraping Scaly debris, discharge, ear wax, stenosed canal tragal tenderness, hyperaemic/edematous ear canal finding were recorded in patients with Otitis externa. While, erythema, dermatitis, loss of hair, crust hyperkeratinazion, lichenification of the ear epithelium; Increase of ceremonious with waxy material in ear and drooling of ear in some cases. On the other hand, Shaking of head, excessive scratching, hematoma, painful at palpated ear infected, itching in dogs Otitis externa.

Regarding to the patients and dogs gender, it was found that males had a tendency to get infection more than females when (98.7%) of dogs were males and (96 %) females. Moreover, the old age groups were most subjected to the infection of Otitis externa than other age group in dogs and patients.

Rsults showed hingh percent of bacterial spp (76%), while from dogs (90%). The results referred that the samples from human and dogs showed the bacterial isolates rate was *Staphylococcus spp*. 59/222 (26.6%) followed by *Pseudomonas spp*. 45/222 (20.3%), *Proteus spp*. 29/222 (13.1%), *E. coli* 26/222 (11.7%), *Streptococcus spp*. 25/222 (11.3%), and *Klebsiella spp*. 18/222 (8.1%), *Pasteurella spp.* 8/222 (3.6%), *Corynebacterium* 7/222 (3.2%) and *Enterobacter spp*. 5/222 (2.3 %).

In particular, from human samples, the highest isolates were *Staphylococcus spp* (26.3%), followed by *Pseudomonas spp.*

(21%), *Proteus spp.* (17%), *E. coli* (14.5%), *Streptococcus spp.*10/76 (13%), *Klebsiella spp*.7/76 (9.2%) and *Corynebacterium* (1.3%). While, from dogs samples, the highest one was *Staphylococcus spp*.39/135 (26.7%), followed by *Pseudomonas spp*. 29/135 (19.9%), *Proteus spp.* 16/135 (10.9%), *Streptococcus spp.* and *E. coli* each 15/135 (10.3%), *Klebsiella spp*. 11/135 (7.5 %), *Pasturella spp.* 8/135 (5.5%), *corynebacterium* 6/135 (4.1%) and *Enterobacter* 5/135 (3.4%) with significant differences $p \leq 0.05$.

On the other hand, the results referred that samples from human and dogs showed the highest fungal infection was *Candida spp.* 43/114 (37.7 %) followed by *Malassezia spp.* 35/114 (30.7 %), *Aspergillums spp.* 25/114 (21.9 %) and *Trichophyton* 11/114 (9.7 %) significant differences $p \leq 0.05$ between fungal isolates in dogs and human.

Antibiotic susceptibility of bacterial isolates against (10) of commonly used antibiotic was determined through disc-diffusion method. Results declared that, generally, all isolates were sensitive to Gentamycin; Ciprofloxacin; Tetracycline and Amikacin. While all isolates were resistant to Azithromycin ; Chloramphenicol and Ampicillin.

Genomic DNA was isolated from bacterial growth and universal primers were utilized to confirm the isolates. DNA was extracted from *Staphylococcus aureus, Pseudomonus aeruginosa* isolates and *E.coli.* Results showed that the recorded range of DNA concentrations were 36-97 ng/µl, 65-70 ng/µl, 34-50 respectively and the DNA purity was (1.5- 1.8).

New submission of local Iraqi Staphylococcus clinical isolated during the current study show successfully record of two isolate *Staphylococcus aureus* with GenBank accession number: OK560669.1 ,OK9560670.1; two isolate *E.coli* with

GenBank accession number: OK560673.1 and OK560674.1 and two isolate *Pseudomonus aeruginosa* with GenBank accession number: OK560672.1 and OK560671.1.

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samples from dogs and patients with Otitis externa

Abbreviation	Meaning
OE	otitis externa
CT	computerized tomography
rRNA	Ribosomal ribonucleic acid

List of abbreviations

bp	base pair
US	United state
DNA	Deoxyribo nucleic acid
IV	Intravenous
H2S	hydrogen sulfide
KOH	Potassium hydroxide
Lab	Laboratory
CTAB	Cetyl trimethylammonium bromide
E.M.B	Eosin methylene blue
PCR	Polymerase Chain Reaction
pH	power of Hydrogen
DW	Distal water
MR-VP	Methyl red / voges-proskauer
GD	Genomic DNA
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
rpm	rounds per minute
RT	Room temperature
TSI	Triple Sugar Iron
SDA	Sabouraud Dextrose Agar
Sig	significant
spp.	species
SPSS	Statistical Package for Social Sciences
µ	Micro

1.1. Introduction

Canine otitis externa (OE) is a common inflammatory disease that is frequently complicated by secondary bacterial and/or yeast infections (Bradley *et al*., 2020). Otitis externa cases present across a wide clinical spectrum, ranging from acute inflammatory or inflammatory/infectious to chronic cases, with or without middle ear involvement, and may also be associated with hyperplastic or neoplastic changes (O'Neill *et al*., 2021; Griffin, 2020 and Saridomichelakis, 2007).

Breed-associated ear carriage conformation is important predisposing factors for canine otitis externa. Greater awareness of these associations for both predisposed and protected breeds could support veterinary practitioners to promote cautious and low-harm approaches in their clinical advice on preventive care for otitis externa, especially in predisposed breeds (O'Neill *et al*., 2021).

The most common pathogenic agents patient associated with acute otitis externa are *Pseudomonas aeruginosa, Staphylococcus epidermides, Staphylococcus aureus,* and *Streptococcus pyogenes* (Roland and Stroman 2002). Coagulase-negative species as a group constitute a major component of the normal micro flora of human beings and dogs are considered as important opportunistic pathogens in those species.Nevertheless, in contrast with humans, the presence of these staphylococcal species in animals have received little attention to date (Lilenbaum *et al*., 2000).

Pseudomonas spp. and *Proteus phaireticuentes bactium* are the most common isolates in acute disease, followed by *Staphylococcus spp.* and *Streptococcus spp., Enterococcus spp.,* and *Corynebacteria,* and *Proteuspora bacitirrenium*, with *Escheria coli*; *Pseudomonas spreadthregs*; Pseudohremonasimons (Paterson 2020).

Fungi and yeast are usually found in patients with chronic otitis externa or those who are immunocompromised (Malayeri *et al*., 2010). Most opportunistic yeast is the fast-multiplication opportunistic yeast like *Malassezia* seems to thrive when the climate is suitable (Chiavassa *et al*. 2014). Anatomical causes leading to stenosis, cerumen buildup, elevated secretions, and bronchial obstructions and aniamdsieties, those three things, respectively, all predispose the patient to *Malassezia fungiformis* (Stenosing Parasitic Complex) (Chiavassa *et al*. 2014).

Manege production usually causes a thick discharge in the ear canal. Hyperplasia of the ceruminous glands, in those that last more than a few months (Paterson 2013). The presence of M. *pachydermatis* as the most prevalent species in the skin and external ear canal of healthy and diseased dogs. Also, playing a causative role in the etiology of canine otitis externa (Minnat, *et al*., 2021).

Signs can include head shaking, pain, malodor, erythema, erosion, ulceration, swelling, and/or ceruminous gland inflammation and lichenification and drooling of ear (Minnat *et al.*, 2015). Chronic inflammation causes desquamate the auditory cartilage to form a sclerosis which also obstructs the ear canal, which leads to chronic ostia. Additionally, embedded secretions cause chronic infection of the auditory bone lesions, and calcification of the bones, which results in par Ear Pinhole syndrome can only be treated by surgically. Calcified cartilage is a fixed condition, and the only solution is surgery (Hnilica and Patterson 2017).

There are about 3% to 5% of the population is likely to be affected (Sood *et al.*, 2002; Daneshrad *et al.*, 2002). Around 90% of people get a first attack on one side; the first attack seems to happen within seven years of onset of ear disease; it is more frequent among the ear-diseased people. An acute Otitis Externa patients' often increases with warming of the water, being in a bath, hearing

protector, or has some injury; on top of that, his ear condition is more sensitive to loud noises (Beers and Abramo, 2004).

Diagnosis is based on otoscopic examination, cytology, and culture. Treatment depends on the specific diagnosis. The inciting cause must be addressed to prevent recurrence (Woodward, 2020). The analysis of 16S rRNA gene sequences has been the technique generally used to study and confirm the identification and taxonomy of *Staphylococci sp., E. coli and Pseudomonas sp*, However, bacterial species cannot always be distinguished from each other using cultural methods. The 16S rRNA gene is used as the standard for classification and identification of microbes, because it is present in most microbes and shows proper changes. Type strains of 16S rRNA gene sequences for most bacteria and archaea are available on public databases such as NCBI. (Tang and Stratton,2006**;** Trieber and Taylor, 2012).

1.2. Aims of study to:

1. Isolate and Identified bacterial and fungal of zoonotic importance from ear of dogs and human.
2. Molecular identification and sequences of some bacterial isolates resistant to antibiotic.