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CULTURAL AND BIOCHEMICAL IDENTIFICATION OF ANTIBIOTIC-RESISTANT BACTERIA IN THE EARS OF DOGS AND PATIENTS WITH OTITIS EXTERNA

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Abstract

Otitis externa is a common ear condition in humans and dogs caused by bacteria, fungus, yeasts, and ectoparasites. The aim of the study was molecular identification and sequencing of isolated antibiotic-resistant bacteria. The study consisted of 250 swab samples, with 100 samples from human patients and 150 from dogs. The samples were collected from October 2023 to January 2024 in Diyala Province. The isolates were identified using Vitek 2 depending on colony's color, form, and odor on study agar. Biochemical tests were performed to identify the bacteria. DNA extraction and PCR were performed to confirm the identity of the bacterial isolates identified by the VITEK system. While VITEK provides preliminary identification based on biochemical tests, while molecular techniques such as PCR allow for more definitive confirmation of the species, particularly for identifying antibiotic-resistant strains. The study revealed that Staphylococcus spp. was the most common infection in humans, affecting 26.3% of cases, followed by *Pseudomonas, Proteus, Escherichia coli, Streptococcus, Klebsiella,* and *Corynebacterium*. Dog samples exhibited the highest infection rate of 26.7%, predominantly caused by Staphylococcus spp., followed by *Streptococcus, Pseudomonas, Escherichia coli, Proteus, Klebsiella, Pasteurella* species, and *Corynebacterium* respectively. Eight antibiotics were tested against these bacterial isolates using the disc-diffusion method, with all isolates

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showing susceptibility to Gentamycin, Ciprofloxacin, Tetracycline, and Amikacin. The investigation found two *Staphylococcus aureus* isolates OK 560669,1 and OK 9560670,1. Two GenBank entry numbers OK 560673,10 and OK 560674,1 were *Escherichia coli* isolates. Two *Pseudomonas aeruginosa* isolates with GenBank entries OK 560672,1 and OK 560671,1, were detected.

Key words: Bacterial isolates, Otitis externa, Dogs and humans, Molecular and phylogenic, Antibiotic susceptibility testing

BIOHEMIJSKA IDENTIFIKACIJA BAKTERIJA REZISTENTNIH NA ANTIBIOTIKE U UHU PASA I KOD PACIJENATA SA ZAPALJENJEM SPOLJAŠNJEG UHA (OTITIS EXTERNA)

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Kratak sadržaj

Otitis externa je često oboljenje uha kod ljudi i pasa, izazvano bakterijama, gljivicama, kvascima i ektoparazitima. Cilj studije je bila molekularna identifikacija i sekvenciranje izolovanih bakterija otpornih na antibiotike. Analizirano je 250 uzoraka briseva, od kojih je 100 uzoraka bilo od ljudi, a 150 od pasa. Uzorci su prikupljeni od oktobra 2023. do januara 2024. godine u provinciji Dijala. Izolati su identifikovani pomoću Vitek 2, u zavisnosti od boje, oblika i mirisa kolonija na nutritivnom agaru. Biohemijski testovi su izvršeni radi identifikacije bakterija. Ekstrakcija DNK i PCR su sprovedeni kako bi se potvrdio identitet bakterijskih izolata identifikovanih VITEK sistemom. Dok VITEK pruža preliminarnu identifikaciju na osnovu biohemijskih testova, molekularne tehnike poput PCR-a omogućavaju precizniju potvrdu vrste, posebno u identifikaciji sojeva otpornih na antibiotike. Studija je pokazala da je *Staphylococcus* spp. bio

najčešći uzročnik infekcije kod ljudi, prisutan u 26,3% slučajeva, a zatim su sledili *Pseudomonas*, *Proteus*, *Escherichia coli*, *Streptococcus*, *Klebsiella* и *Corynebacterium*. Uzorci pasa pokazali su najvišu stopu infekcije od 26,7%, pri čemu je *Staphylococcus* spp. bio najzastupljeniji, nakon čega su sledili *Streptococcus*, *Pseudomonas*, *Escherichia coli*, *Proteus*, *Klebsiella*, *Pasteurella* spp. i *Corynebacterium*. Osam antibiotika je testirano protiv ovih bakterijskih izolata metodom difuzije diska, pri čemu su svi izolati pokazali osetljivost na gentamicin, ciprofloksacin, tetraciklin i amikacin. Istraživanje je otkrilo dva izolata *Staphylococcus aureus*, OK 560669,1 i OK 9560670,1. Dva GenBank broja unosa, OK 560673,10 i OK 560674,1, odnosila su se na *Escherichia coli* izolate. Otkrivena su i dva izolata *Pseudomonas aeruginosa* sa GenBank brojevima OK 560672,1 i OK 560671,1.

Ključne reči: Izolati bakterija, *Otitis externa*, ljudi i životinje, molekularna i filogenska, testiranje osetljivosti na antibiotik

INTRODUCTION

Inflammation of the external ear, known as otitis externa, is a common inflammatory condition in dogs which often occurs due to yeast and bacterial infections (Bradley et al., 2020). The severity of external otitis ranges between acute or chronic inflammation, and it can affect the inner ear and lead to the development of an inflammatory condition, causing a range of signs and symptoms. Researchers have conducted many studies to better understand how otitis externa occurs in dogs (O'Neill et al., 2021; Griffin, 2020). A pneumatic otoscope is the most accurate and reliable tool, offering higher specificity for diagnosis compared to a regular otoscope. However, a tympanometer and other methods can assist in the diagnostic process when an otoscope is unavailable (Griffin, 2020). Both will yield decreased TM mobility on tympanometry or pneumatic otoscopy (Minnat et al., 2021; Lilenbaum, et al., 2000). According to Sood et al. (2002) and Daneshrad et al. (2002), between 3% and 5% of the population might be affected. Ninety percent of individuals experience their initial attack on one side, typically occurring seven years after the onset of ear illness, and it is more common in those with a prior history of the condition. Middle ear infections can be viral, bacterial, fungal, or a result of coinfection. The most common bacterial organisms causing otitis media in humans are *Streptococcus pneumoniae*, followed by non-typeable hemophilic influenza (NTHi) and Moraxella catarrhalis (Hu et al.,2021). Following

the introduction of the conjugate pneumococcal vaccines, the pneumococcal organisms have evolved to non-vaccine serotypes. There are more common viral pathogens that cause otitis media, including respiratory syncytial virus (RSV), coronavirus, influenza virus, adenovirus, human metapneumovirus, and picornavirus (Bulut et al., 2007). Otitis media is diagnosed clinically by observing the signs presented by the body after a visual physical examination. An otoscope is then used, along with an assessment of the patient's medical history and the apparent symptoms, to make a thorough diagnosis. There are many diagnostic tools, including the pneumatic endoscope, tympanometer, and acoustic reflectometer, which help doctors to diagnose a case of middle ear infection (Shand and Campe, 2016). Otitis media begins as an inflammatory process after infection of the upper respiratory tract with a viral disease that affects the nasal mucosa, pharynx, middle ear mucosa, and Eustachian tube. From an anatomical point of view, the middle ear passages are narrow (Atila et al., 2021). The edema resulting from the inflammatory process closes the narrow part of the Eustachian tube, and this leads to reduced ventilation. As a result, a chain of consecutive events causes an increase in negative pressure in the middle ear, which in turn leads to an increase in secretions from the inflamed mucous membrane. This buildup of mucous secretions creates an environment that promotes the growth of bacterial colonies and viral infections in the middle ear (Dilesh, 2017). The proliferation of these microbes in the middle ear leads to suppuration, eventually resulting in noticeable purulence within the middle ear space. Tu et al., (2014) clinically demonstrated a bulging or erythematous tympanic membrane along with purulent middle ear fluid. Tang et al. (2022) described chronic serous otitis media (CSOM), which is characterized by thick, amber-colored fluid in the middle ear space and a retracted tympanic membrane upon otoscopic examination. In addition to heightened sensitivity to loud sounds, an acute otitis externa patient's symptom may worsen with exposure to warm water, taking a bath, wearing hearing protection, or experiencing any form of trauma (Beers and Abramo, 2004). Overall, this study aims to contribute to our understanding of antibiotic resistance in otitis externa and provide insights into the prevalence, distribution, and genetic basis of antibiotic resistance genes in these bacterial isolates. The samples from dogs and humans are used together in the study as both groups are investigated for the presence of antibiotic-resistant bacteria causing otitis externa (Tang et al., 2022). The research highlights the prevalence of these bacteria in both populations, indicating a shared concern regarding antibiotic resistance in veterinary and human medicine. This connection is relevant and significant, as it underscores the importance of understanding antibiotic resistance across species, which can have implications for treatment and public

health (Dilesh, 2017). The aim of the study is molecular identification and sequencing of isolated antibiotic-resistant bacteria from dogs and human.

MATERIAL AND METHODS

Sample collection

This study involved the collection of 250 swab samples, which were divided into three groups: 100 ear swabs from individuals (50 from males and 50 from females), and 150 swab samples from the ears of dogs. The control group included 150 swab samples, divided into 75 female swab samples and 75 male samples. The samples were collected in accordance with ethical guidelines and verbal approval from the Ethics Committee of the Technical Institute. They were collected in Baqubah city, Diyala Governate, Iraq, for the period from October 2023 to January 2024. Furthermore, the patient's ear condition appeared to worsen in warm water, after bathing, while wearing hearing protection, or following an injury. Additionally, the patient's ear condition made them more sensitive to loud noises, as noted by (Beers and Abramo, 2004). The samples were grown on the blood Agar and MacConkey Agar, and chemical tests were performed to isolate and preliminary identify bacteria (Riedel et al., 2018). In addition, biochemical tests were conducted using the (VITEK 2) device to determine the bacterial species. The bacterial culture was transferred to Mueller Hinton Agar for drug sensitivity testing.

DNA extraction from the genome

The procedures were carried out according to the protocol required of the producer (Anatolia/Turkey). The Deoxyribonucleic acid (DNA) that was acquired was stored at a temperature of -20 °C to ensure its preservation and facilitate its future utility and processing. Following the instructions in the PrestoTM Mini gDNA Bacteria Kit, genomic DNA was extracted from bacterial growth. A Nanodrop spectrophotometer was used to examine the extracted DNA, measuring DNA content (ng/L) and DNA purity by reading the absorbance at (260/280 nm) (Lee, et al., 2010; Mohammad et al., 2024).

Primer selection and 16S rRNA amplification preparation

The sequences of molecular duplication primers and the size of amplified fragments related to three different genes were determined in *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. In *Staphylococcus au-*

reus, the Arc primer was used with the sequence TTGATTCACCAGCGCGTATT for the forward primer and AGGTATCTGCTTCAATCAGCG for the reverse primer, yielding an amplified fragment 570 base pairs long. For *Pseudomonas aeruginosa*, the AroE primer was applied with the sequence ATGTCACCGTGCCGTTCAAG for the forward primer and TGAAGCAGTCGGTTCCTTG for the reverse primer, generating an amplified fragment of 1,500 base pairs in size. Finally, in *Escherichia coli*, the ArcA primer with the sequences GAAGACGAGTTGGTAACACG and CTTCCAGATCACCGCAGAAGC were used for the forward and reverse primers, respectively, resulting in a duplication of a similar size of 1,500 base pairs (Nee, 2018). The selection of these primers was driven by their ability to amplify specific genes associated with pathogenicity and antibiotic resistance in *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Understanding these genes is crucial for developing effective diagnostic tools and treatment strategies for otitis externa infections, especially in light of the increasing issue of antibiotic resistance (Lee, et al., 2010).

PCR amplification

To obtain the best results in PCR amplification, a specific set of chemicals was used in a total volume of 25 µL: these include 12.5 µL of Go Taq® basic mix from Promega, USA, plus 2 µL of primers, 2 µL of template DNA, 8.5 µL of nuclease-free water. The amplification protocol was carried out using a Mastercycler (Eppendorf) and included the following steps: the first step involved preheating the specimens at 94 °C for one minute. The first step involves heating for thirty-five cycles at the same temperature, followed by a temperature reduction to 63 °C for annealing. The process then proceeds with an extension at 72 °C for the same duration. The final step consists of a single extension cycle at 72 °C for ten minutes.

Agarose gel electrophoresis

The PCR amplified products were tested by electrolysis on a 1.5% agarose gel, the gel was stained with Safe Red, and the products were photographed using ultraviolet light with the help of the Imagemaster VDS device produced by the American Pharmacia Biotech. 25 µL of PCR positive products were used for sequence analysis, with the BLAST algorithm from the National Center for Biotechnology Information (NCBI) used to evaluate the results. The improved protocol for PCR and gel electrophoresis provides valuable information about genetic composition (Ligozzi et al., 2010; Sami Awayid and Qassim Mohammad, 2022).

Ethical approval

Ethical approval was obtained from the Research Ethics Committee at the Southern Technical University, Baqubah Technical Institute.

Statistical analysis

Patients' demographics and cross-tabulations were calculated using the Statistical Package for the Social Sciences for Windows, version 2017 (SPSS, Armonk, NY: IBM Corp). Pearson's chi-square test and Pearson's correlation coefficient were used to assess the association between variables from the two tests. Statistical significance was set at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

Table 1. Primer selection and 16S rRNA amplification

Bacteria	Genes	Primer sequence	Size (bp)	Reference
Staphylococcus aureus	Arc	F: TTGATTCACCAGCGCGTATT R: AGGTATCTGCTTCAATCAGCG	570bp	Sastalla et al., 2017
Psedomonas aeruginosa	AroE	F: ATGTCACCGTGCCGTTCAAG R: TGAAGGCAGTCGGTTCCTTG	1500bp	Embaby et al., 2014
Escherichia coli	ArcA	F: GAAGACGAGTTGGTAACACG R: CTTCCAGATCACCGCAGAAGC	1500bp	Beutin et al., 2005

Table 2. The research provides data on the numerical distribution and proportional representation of bacterial samples.

The objective is to isolate	People	Dog	Sum
Staphylococcus spp.	20(26.3 %)	39(26.7%)	59(26.6%)
Streptococcus spp.	8(10.5 %)	17(11.6%)	25(11.3%)
Pseudomonas spp.	16(21.1 %)	29(19.9 %)	45(20.3%)
Escherichia coli	11(14.5 %)	15(10.3 %)	26(11.7%)
Proteus spp.	13(17.1 %)	16(10.9%)	29(13.1%)
Klebsiella spp.	7(9.2 %)	11(7.5 %)	18(8.10%)
Pasturella spp.	0(0%)	8(5.5%)	8(3.6%)

The objective is to isolate	People	Dog	Sum
Corynebacterium spp.	1(1.3%)	6(4.1%)	7(3.2%)
Enterobacter spp.	0(0%)	5(3.4%)	5(2.3 %)
Total	76(34.2%)	146(65.8%)	222(100%)

Table 3. Sensitivity of isolates Bacteria to antibiotics

Antibiotic		*Klebsiella* spp.	*Staphylococcus* spp.	*Escherichia coli*	*Proteus* spp.	*Pseudomonas* spp.
Gentamycin	(GEN10µg)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Amoxicillin	(AML25µg)	Resistance	Resistance	Resistance	Resistance	Resistance
Ampicillin	(AMP10µg)	Resistance	Resistance	Resistance	Resistance	Resistance
Azithromycin	(AZ15µg)	Resistance	Resistance	Resistance	Resistance	Resistance
Chloramphenicol	(C30µg)	Resistance	Resistance	Resistance	Resistance	Resistance
Lincomycin	(L2µg)	Resistance	Resistance	Resistance	Resistance	Resistance
Doxycycline	(DO30µg)	Resistance	Resistance	Resistance	Resistance	Resistance
Amikacin	(AK30µg)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Tetracycline	(TET30µg)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Ciprofloxacin	(CIP5µg)	Sensitive	Sensitive	Sensitive	sensitive	Sensitive

Table 4. Single (conventional) PCR detection results of clinical samples from dogs and patients with otitis externa.

Samples	Pathogens	Proportion of positive samples (%)	Concordance rate (%)
Dogs ear swabs 9 (3 of each pathogen)	*E. coli*	3/3 (100)	100
	P. aeruginosa	3/3 (100)	100
	S. aureus	3/3 (100)	100
Patient ear swabs 6 (2 of each pathogen)	*E. coli*	2/2 (100)	100
	P. aeruginosa	2/2 (100)	100
	S. aureus	2/2 (100)	100

Distribution of bacterial isolates based on sample origin

The research provides data on the numerical and relative distribution of bacteria samples, with the aim of isolating bacterial species in humans. As shown in (Table 2.), The results show the isolation of 20 samples (26.3%) of *Staphylococcus* species, 8 samples (10.5%) of *Streptococcus* species, 16 samples (21.1%) of *Pseudomonas* species, and 11 samples (14.5%) of *Escherichia coli*, 13 samples (17.1%) of *Proteus* species, 7 samples (9.2%) of *Klebsiella* species, and one sample (1.3%) of *Corynebacterium auris*. The samples isolated from dogs included 39 samples (26.7%) of *Staphylococcus* species, 17 samples (11.6%) of Streptococci, 29 samples (19.9%) of *Pseudomonas*, 15 samples (10.3%) of *Escherichia coli*, and 16 samples (10.9%) of) of *Proteus*, 11 samples (7.5%) of *Klebsiella*, 8 samples (5.5%) of *Pasteurella* species, and 6 samples (4.1%) of *Corynebacterium*. As for *Enterobacter* species, no samples were isolated from people, but 5 samples (3.4%) were isolated from dogs. These results show the distribution and relative representation of bacterial samples out of a total of 222 samples. Of these, 76 samples (34.2%) were taken from people, while a higher number, 146 samples, were collected from dogs (65.8%). These numbers highlight the diversity of bacterial species isolated and their importance in the natural environment for both people and animals.

Antimicrobial susceptibility testing of bacteria

The study shows bacterial sensitivity to a group of antibiotics among laboratory isolates of different bacterial species, including *Klebsiella* spp.*, Staphylococcus* spp.*, Escherichia coli*, *Proteus* spp., and *Pseudomonas* spp.. The results indicate that all tested isolates were sensitive to gentamycin (GEN 10μg), with *Klebsiella, Staphylococcus, Escherichia coli, Proteus,* and *Pseudomonas* demonstrating a sensitive response. On the other hand, complete resistance to amoxicillin (AML 25 μg), ampicillin (AMP 10 μg), azithromycin (AZ 15 μg), chloramphenicol (C 30 μg), lincomycin (L 2 μg), and doxycycline (DO 30 μg), without exception, was recorded for all bacteria tested. Furthermore, isolates show sensitivity to both amikacin (AK 30 μg) and tetracycline (TET 30 μg) across all bacterial species tested. In addition, the data show that the antibiotic ciprofloxacin (CIP 5 μg) was effective against all types of bacteria. All bacterial isolates tested in the study were found to be susceptible to amikacin (AK 30 μg), Tetracycline (TET 30 μg), and Ciprofloxacin (CIP 5 μg). Therefore, there were no bacteria that exhibited a high level of resistance to these antibiotics.

Isolate bacterial deoxyribonucleic acid (DNA) and extract its genetic material

For each type of a swab, the proportion of positive samples and the concordance rate were determined for three pathogens: *E. coli*, *P. aeruginosa*, and *S. aureus*. The 9 dog ear swabs analyzed (3 for each of the three etiologies) all yielded 100% positive results for each of the three etiologies, demonstrating a concordance rate of 100%. As for the patients' ear swabs, which numbered 6 (2 for each of the three causes), the percentage of positive samples was 100% for each of the causes, and the concordance rate was also 100%. These data confirm the effectiveness of detection and the strength of the association between the samples and the three causes in both groups.

16S rRNA gene amplification

The study included five *E. coli* isolates, three from dogs and two from human patients. The use of specific PCR primers resulted in efficient amplification of the 16S rRNA gene of *E. coli*, as indicated by the results shown in Figure 1, which showed that this gene has a molecular weight of 1500 base pairs.

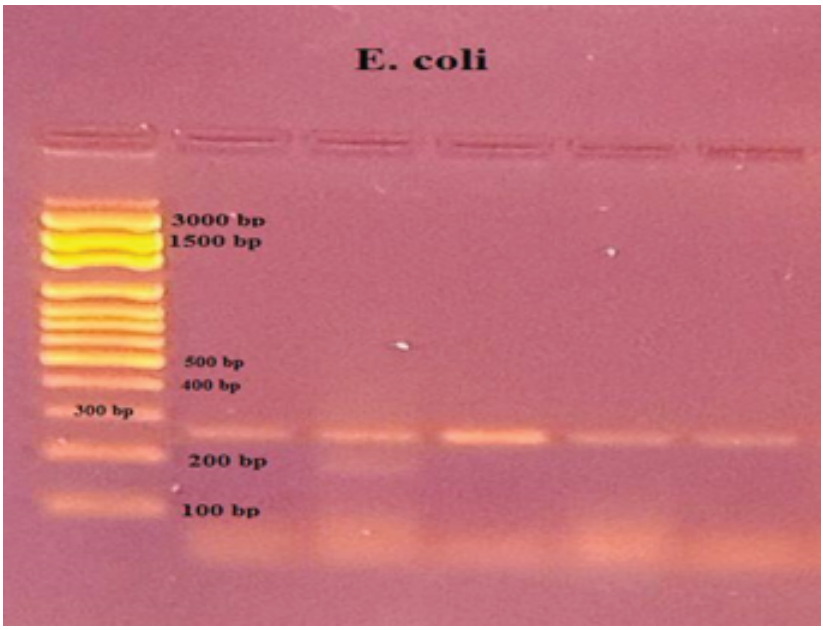

Figure 1. PRISMA flow diagram according to Renald et al. (2023)

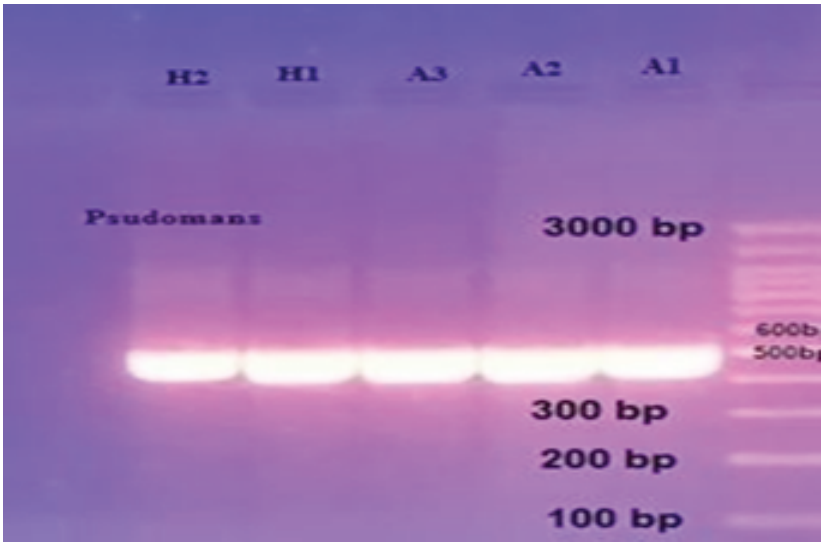

Figure 2. The DNA genome of *Pseudomonas aeruginosa* isolates

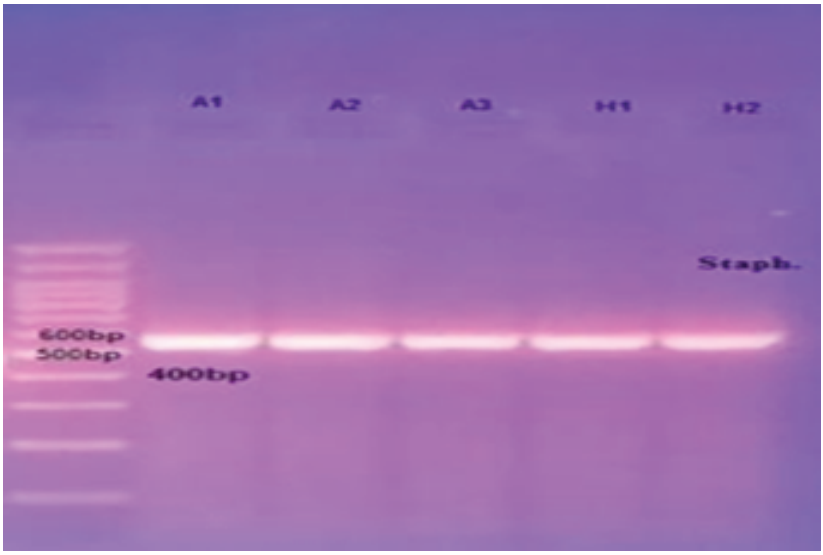

Figure 3. The DNA genome of *Staphylococcus aureus* isolate

Phylogenetic tree analysis

The present study employed phylogenetic tree analysis using partial ribosomal RNA (16S) gene sequences to identify and classify local *Staphylococcus* spp. strains. This study presents a recent submission of locally isolated Iramogre *Staphylococcus* spp. clinical specimens. The findings demonstrate the successful identification of two *S. aureus* isolates, each assigned unique Gen-Bank entry numbers: OK 560669,1 and OK 9560670,1 (Figure 4). Our findings include the successful identification of 2 isolated *E. coli* strains, each assigned a unique Gen-Bank entry number: OK 560673,1 and OK 560674,1 (Figure 5). In contrast, it is important to highlight a recent submission of indigenous Iraqi Pseudomonas clinical isolates, which has been documented during the course of the present investigation. This submission has yielded 2 distinct *Pseudomonas aeruginosa* isolates, each assigned with unique Gen-Bank entry numbers: OK 560672,1 and OK 560671,1 (Figure 6).

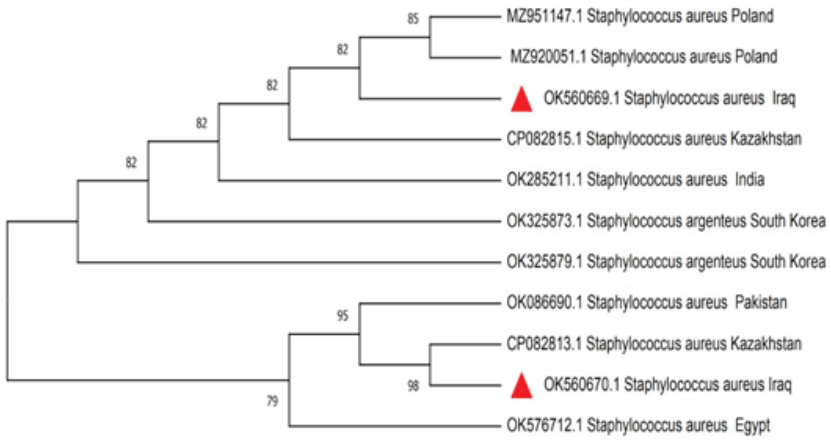

Figure 4. The recognition of local *Staphylococcus* spp. isolates were performed by phylogenetic-tree analysis utilizing partial sequences of the 16-S ribosomal Ribonucleic Acid (RNA) genes.

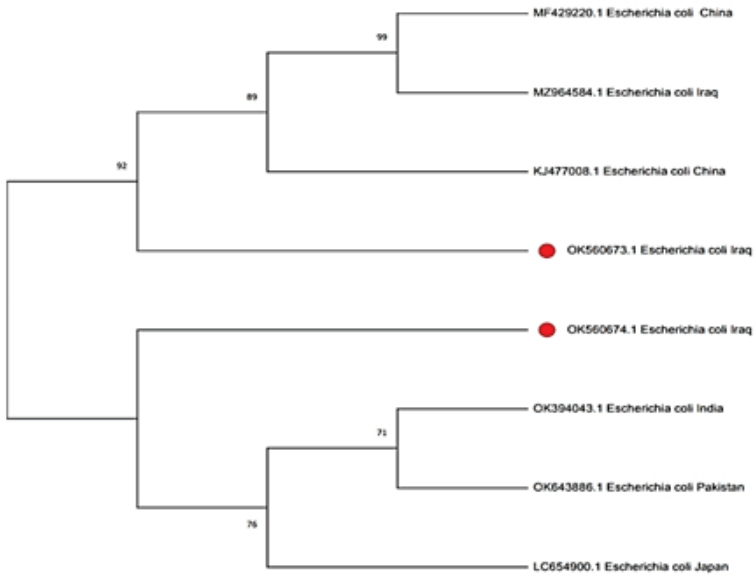

Figure 5. The identification of local *E. coli* strains was performed by phylogenetic-tree analysis utilizing the partial sequence of the 16-S ribosomal ribonucleic acid (RNA) genes.

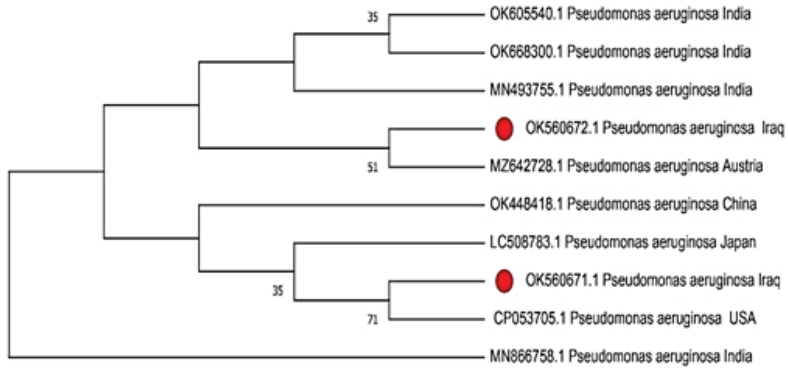

Figure 6. The identification of local *Pseudomonas* strains (*Pseudomonas aeruginosa*) was performed by phylogenetic-tree analysis utilizing the partial sequence of the 16-S ribosomal ribonucleic acid (RNA) genes.

DISCUSSION

The study revealed a high overall infection rate of 90% in dogs, while in patients, the infection rate was slightly lower at 68%. Among the patient cases, 68% were associated with *acute otitis externa*, highlighting the prevalence of this condition. This finding is consistent with the studies by Turner et al. (2023) and Salim et al. (2023). In the auditory canal, various bacteria were identified, including both Coagulase-positive and -negative strains. *Staphylococci* and *Streptococci* species were frequently found, while *Corynebacterium* strains and coliform germs were less commonly detected. On the other hand, the presence of other types of bacteria, *Pseudomonas* spp. and *Proteus* spp., was detected infrequently in the specific area of the outer ear. It is worth noting that the mere presence of microorganisms is not conclusive evidence of *otitis externa*, as indicated by (Deneva et al., 2020 and Szewczuk et al., 2020). The results confirmed that bacterial infection is complicated in the case of ear infection, and therefore it is necessary to make an accurate diagnosis and appropriate treatment for the condition (Rawson et al., 2023). The presence of specific bacterial species in the auditory canal indicates knowledge of the species associated with ear infections, which helps in finding therapeutic methods that target the specific type of bacteria, which facilitates the treatment of the disease, and this is consistent with the findings of Chen et al. 2024. These differences in prevalence were found to be statistically significant, with a p-value of ≤ 0.05 that is compatible with (Petrov et al. 2013). The observed outcomes might potentially be associated with the production of biofilm by *Staphylococcus* spp. and *Pseudomonas* spp. bacteria, leading to the persistence of infection even when appropriate treatment measures are implemented. For any antimicrobial medication to be effective in treating the infection, it must be able to destroy the biofilm. The study has provided evidence that *Pseudomonas aeruginosa* and *S. aureus* are frequently acknowledged as the predominant causative agents responsible for the development of *acute otitis externa* (Ghapanvari et al., 2022). This study aligns with our findings, where *Staphylococcus* spp. was frequently isolated from the auditory canal of dogs with otitis (Pirashanna and Rajapaksha, 2021). In another study conducted on dogs with ear infections, it was noted that bacterial infection often occurs as a secondary infection in about 50% of cases, given that the main infection is caused by pathogenic fungi. The prevalence of *Staphylococcus* spp. among the analyzed dog specimens was determined to be 26.7% (39 out of 146 samples), while Pseudomonas species had a prevalence of 19.9% (29 out of 146 samples). Among the dog samples analyzed, *Staphylococcus* spp. showed a very high prevalence of 26.7% (39

out of 146 samples), followed by *Pseudomonas* species at 19.9% (29 out of 146 samples). *Proteus* species accounted for 10.9% (16 out of 146 samples), while both *Streptococcus* species and *Escherichia coli* were found in 10.3% (15 out of 146 samples) each. *Klebsiella* species were present in 7.5% (11 out of 146 samples), while *Pasteurella* species were detected in 5.5% (8 out of 146 samples). *Corynebacterium* species and intestinal bacteria species were less prevalent, reaching 4.1% (6 out of 146 samples) and 3.4% (5 out of 146 samples), respectively (here it is explained which bacteria had a higher percentage during diagnosis. and insulation) (Paterson, 2013). This study is consistent with Bajwa (2019), which identifies prevalent perpetuating factors such as bacteria like *Staphylococcus* and *Pseudomonas*, as well as *Malassezia* yeast. The presence of this infection in the middle ear may also act as a perpetuating factor, causing repeated external ear infections if it spreads to the tympanic bulla. In dogs with recurrent *otitis externa*, the primary cause of therapy failure is often perpetuating factors. To authenticate the identity of the *Staphylococcus* spp., *Pseudomonas* spp., and the *E. coli* strain that were isolated during the current investigation, it was imperative to conduct a sequencing analysis of the 16srRNA gene (Karnad et al., 2020). This alignment has also been successfully documented for the two *Staphylococci* isolates. Sequencing using the UPGMA tree in MEGA version 10.0 revealed that the *S. aureus* strain, with accession numbers OK9560670.1 and OK560669.1, shows 98% identity with the two *S. aureus* strains CP086690.1 and CP082813.1, which were isolated in Pakistan, Kazakhstan, and local Iraqi clinical isolates of *Staphylococci* obtained during the current study. This sequence arrangement also successfully documented the two *Staphylococci* isolates. Likewise, the application of the UPGMA tree in MEGA version 10.0 revealed that an *E. coli* strain with accession numbers OK560673.1 and OK560674.1 showed 98% concordance with closely related strains identified through NCBI-Blast, specifically *E. coli* strain OK560673.1 from China and OK394043, isolated in Pakistan, respectively. Thus, this analysis provides a noteworthy record of two *E. coli* isolates, each bearing GenBank accession numbers OK 560673.1 and OK 560674.1. In contrast, the current study found two successful cases of two strains of *Pseudomonas aeruginosa*, OK560672.1 and OK560671.1, which were isolated from local Iraqi medical samples (Rudra et al., 2022). Utilizing the UPGMA-tree method in the MEGA 10.0 versions, sequence alignment analysis revealed the genetic relatedness of these (Pseudomonas aeruginosa) strains, as indicated by their respective accession numbers: OK560672.1 and OK 98% of the variations between Iraqi bacterial isolates and isolates from other countries may be attributed to environmental factors and varying levels of isolation (Shehab et al., 2020).

CONCLUSION

S. aureus, *P. aeruginosa*, and *E. coli* are the most prevalent bacterial pathogens in humans and dogs, posing a significant health threat. Prevention measures, responsible antibiotic use, accurate diagnosis, effective treatment, and clear infection prevention guidelines are crucial. The study found isolates' sensitivity to gentamycin, ciprofloxacin, tetracycline, and amikacin was favorable, while resistance to azithromycin, hloramphenicol, and ampicillin was high, suggesting limited treatment options. High rates of β-lactamase resistance pose a significant challenge to antibiotic treatment, necessitating research and innovation to counteract this. Advocating responsible antibiotic use and strong infection control measures is crucial. The study confirmed the identification of *S. aureus, P.aeruginosa*, and *E. coli* isolates from Iraq using molecular techniques, enhancing knowledge of bacterial diversity and potential infection control strategies.

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Author's Contribution:

AK, SQ and SJ made contributions to the conception and design of the study, were involved in data collection and drafted the manuscript. AK, SQ and HI carried out the molecular diagnostic tests and prepared the alignment of nucleotide sequences and conducted the molecular genetic analysis. AK carried out the serological tests and data analysis and performed the statistical analysis. AK, SQ and SJ revised the manuscript critically and together prepared the final draft of the manuscript, etc. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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