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Bacterial Etiology of Otitis Externa in Cats

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Abstract | Otitis externa is a common inflammatory condition affecting the external ear canal of domestic cats, often complicated by secondary bacterial infections. Understanding the bacterial etiology is crucial for effective therapeutic management. To identify and characterize bacterial pathogens associated with otitis externa in cats and evaluate their distribution patterns across demographic and geographic variables. A cross-sectional study was conducted on 100 cats presenting with clinical signs of otitis externa from Baghdad and Anbar provinces. Ear swabs were collected for bacterial isolation and identification using conventional microbiological methods, followed by PCR confirmation using species-specific primers. Five bacterial species were identified including *Staphylococcus aureus* (35.6%), *Pseudomonas aeruginosa* (22.2%), *Escherichia coli* (20%), coagulase-negative *Staphylococcus* (17.8%), and *Klebsiella pneumoniae* (4.4%). No significant differences were observed between geographic regions (p = 0.856). Age analysis revealed a decreasing trend in *S. aureus* prevalence with increasing age (p = 0.114). PCR confirmation achieved detection rates of 93.8% to 100% across all species. *S. aureus* and *P. aeruginosa* were the predominant bacterial pathogens in feline otitis externa. The high prevalence of these potentially zoonotic organisms emphasizes the importance of proper diagnosis and treatment protocols in veterinary practice.

Keywords | Otitis externa, Cats, Bacterial infection, *Staphylococcus aureus*, *P. aeruginosa*, Iraq

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INTRODUCTION

Otitis externa, or external ear infection, is the most common dermatologic pathology in small animal practice, with domestic cats being more affected worldwide (Nardoni *et al*., 2014). Otitis externa is an inflammatory condition of the external ear canal, defined as the area from the external acoustic meatus to the tympanic membrane, with clinical signs of erythema, edema, pain, and purulent exudate (Rosser, 2004). Estimates of the prevalence of otitis externa in general practice cat populations range from 2% to 6.6%, while estimates of up to 13% have been reported in specialty referral patients (O'Neill *et al*., 2014; Pressanti *et al*., 2014). This suggests that otitis externa is often a substantial clinical burden in small animal veterinary medicine (Gotthelf, 2014; Ali *et al*., 2024; Al-Sailawi *et al*., 2024).

Feline otitis externa is a multifactorial condition with diverse pathogenesis, integrating the influence of predisposing factors, the primary cause, and perpetuating factors that may contribute to the establishment or maintenance of the inflammatory process (Kennis, 2013). Predisposing factors include anatomical abnormalities (i.e. stenotic ear canals, excessive hair within the ear canal), breed differences (i.e. heredity seen in breeds such as Persian, Himalayan), or environmental factors (*i.e.* high humidity, excessive moisture, or poor ventilation in the ear canal conducive to microbial growth) (Bond *et al*., 1995).

Primary causes of otitis externa in cats include a range of conditions, but parasitic infestations are a particular cause of feline cases. *Otodectes cynotis*, commonly referred to as ear mites, are implicated in ~50% of all otitis externa cases in cats, especially in young animals or when cats have access to the outdoors (Powell *et al*., 1980). Allergic reactions, specifically food allergies and atopic dermatitis, are another prevalent primary cause; studies have found that as many as 43% of cats with food allergies will concurrently develop otitis externa (Zur *et al*., 2002). Foreign bodies, although less common in cats than in dogs, can occasionally induce otitis externa, especially grass awns and other plant materials (Little *et al*., 1991).

The usual progression from primary inflammation to complicated otitis externa involves secondary bacterial colonization and infection, which is an essential factor in the progression of the disease (Griffin, 2006). The typical feline ear canal has a delicate microbial environment that mainly composed of gram-positive cocci, with Staphylococcus species being the most commonly cultured organisms from healthy ears (Hariharan *et al*., 2006), however, when there is disruption to the ear canal environment due to inflammation, elevated temperatures, altered pH, and moisture, the regular microbial community shifts to opportunistic bacterial overgrowth (Bensignor *et al*., 2006).

S. aureus is one of the most clinically significant bacterial pathogens in feline otitis externa; the prevalence of *S. aureus* varies between regions, with estimates ranging from 15% to 40% (Al-Mohana *et al*., 2019; Habeeb *et al*., 2020). *S. aureus* is a gram-positive coccus that possesses numerous virulence factors, including adhesins that facilitate attachment to disrupted epithelium, toxins that contribute to tissue damage, and enzymes that enable invasion and persistence (Foster, 2005). In addition, *S. aureus* also presents with zoonotic potential, as infected cats may serve as a source of disease for humans, particularly in homes with immunocompromised individuals (Al-Saffar *et al*., 2021).

P. aeruginosa is another significant bacterial pathogen involved in feline otitis externa, particularly in the chronic and recurrent type (Mohammed and Al-Ani, 2020). This opportunistic pathogen flourishes within the humid, warm environment of the inflamed ear canal and possesses innate antimicrobial resistance mechanisms as well as biofilm-forming capabilities (Al-Jundiy and Mahmood, 2022). It has been demonstrated in studies that infections by *P. aeruginosa* associated with otitis externa elicit more severe clinical signs, a longer treatment course, and a higher propensity for recurrence than infections caused by other bacterial pathogens (Nuttall and Cole, 2007).

E. coli has a similarly recognized association in feline otitis externa and has been reported at recent isolation rates of 10% - 25% from infected ears (Hussein *et al*., 2021). *E. coli* species are historically classified as commensal organisms in the gastrointestinal tract; in rare cases, these pathogenic strains of *E. coli* can establish an infection in the external ear canal, typically when pre-existing immune compromise or concurrent systemic illness is present (Al-Mayahi and Ali, 2019). *E. coli* detected in ear infections may also indicate fecal contamination of potential interest to feline caregivers, especially in multi-cat settings, to emphasize the importance of proper hygiene (Johnson and Stell, 2000).

Coagulase-negative *Staphylococcus* species are often regarded as commensal; following the same pattern as previously identified bacteria, they can assume a pathogenic role concerning otitis externa in vulnerable cats, such as those with an underlying allergic condition or in states of immunocompromise (Khalaf *et al*., 2022). Coagulase-negative *Staphylococcus* species are increasingly recognized for their ability to form biofilms and exhibit antimicrobial resistance, which contributes to treatment failures and the development of chronic infections (Rasheed *et al*., 2020). *K. pneumoniae* is noted less frequently than *E. coli* or a coagulase-negative Staphylococcus and is classified in veterinary dermatology as an emerging concern due to its association with severe infections and multidrug-resistant patterns (Lee *et al*., 2016). The distribution of bacterial pathogens in otitis externa geographically varies considerably due to factors such as climate, population density, patterns of antimicrobial use, and hygiene practices (Rosychuk, 1994). In the Middle East, particularly in Iraq, specific data on the bacterial etiology of feline otitis externa are scarce, despite the presence of numerous domestic cats and the veterinary burden of skin and ear diseases (Al-Gburi al., 2021; Jasim *et al*., 2022).

The accurate identification of bacterial pathogens is crucial for optimal therapeutic management of otitis externa. Culture-based approaches have been used in bacteriological examinations and are reliable; however, they require time, and in some cases, may not detect fastidious or pathogenic organisms that are present in low numbers (Buckley *et al*., 2000). Molecular diagnostic techniques, such as polymerase chain reaction (PCR) using species-specific primers, have been used since the 1990s and have transformed bacterial identification by being rapid, sensitive, and specific for target organisms (Ahmed and Hassan, 2020). The current gold standard for bacterial identification utilizes the 16S ribosomal RNA gene, enabling researchers to identify species-specific variable regions (Clarridge, 2004).

Age-associated patterns of susceptibility to feline otitis externa have been demonstrated in several studies, where

younger cats are at a higher risk of developing the disease, possibly due to lower immunological maturity or greater complications resulting from a parasitic load, as well as increased behavioral risks from external or contaminated environments (Al-Dabbagh *et al*., 2021, Mahmood and Al-Jumaily, 2019). Older cats may develop otitis externa in association with neoplasia, immune-mediated diseases, or metabolic diseases such as hyperadrenocorticism (Zur *et al*., 2011).

The gender predisposition for feline otitis externa is controversial, with some studies reporting a higher incidence in male cats, while others find no difference between the sexes (Cole *et al*., 1998; Petrov *et al*., 2013). More consistency is observed in the predisposition for breeds of, predominantly long-haired cats, with breeds such as Persian, Himalayan, and Maine Coon cats reported to display a higher incidence, potentially due to their anatomical conformation, including a narrow canal and more hair in the external ear (August, 1988).

The economic consequences of otitis externa are greater than the initial treatment costs for the condition (Hill *et al*., 2006). It incorporates additional fees for other diagnostic procedures, extra visits, and/or management of complications (otitis media or facial nerve paralysis). Moreover, the frequency of recurrent treatment typically associated with bacterial otitis externa reinforces the risk of developing antimicrobial resistance and ongoing treatment costs, even though otitis externa is, to a significant extent, a preventable disease (Paterson, 2016).

Preventative methods for feline otitis externa focus on ear hygiene, prompt management of incidents for predisposing conditions, and environmental modifications to minimize risk factors (Saridomichelakis *et al*., 2007). However, it is contingent on who understands the bacterial pathogens in that area, as well as the risk factors associated with bacterial otitis externa (Al-Rubaie and Mohammad, 2020).

The purpose of this study is to help address the knowledge gap regarding bacterial etiology of otitis externa in cats, particularly in an Iraqi context, and authoritatively provide necessary data for evidence-based therapeutic decision-making regarding the cat and contribute to a global understanding of this essential condition in cats, particularly in terms of bacterial identification from the traditional microbiological perspective in comparison to molecular confirmation. This study was designed to examine streams of epidemiological data and incorporate demographic and geographic patterns of bacterial otitis externa into the understanding of the condition.

MATERIALS AND METHODS

Study design and setting

A cross-sectional observational study was conducted between October 2024 and April 2025 across multiple veterinary clinics and animal hospitals in Baghdad and Anbar provinces, Iraq. The study protocol was approved by the Institutional Review Board of the College of Veterinary Medicine at the University of Fallujah, and informed consent was obtained from all pet owners before sample collection.

Study population and sample size

The study population consisted of domestic cats presenting with clinical signs suggestive of otitis externa. A sample size calculation was performed using the formula for cross-sectional studies with finite populations, considering a 95% confidence interval, a 5% margin of error, and an expected prevalence of 30% based on previous regional studies. The calculated minimum sample size was 81 cats, which was increased to 100 to account for potential sample losses during processing.

Inclusion and exclusion criteria

Inclusion criteria

- Domestic cats of any breed, age, and sex
- Clinical signs of otitis externa, including ear discharge, odor, erythema, or pruritus
- Owner consent for sample collection and participation
- No antimicrobial therapy within the preceding 14 days

Exclusion criteria

- Cats with ruptured tympanic membranes confirmed by otoscopic examination
- Recent ear cleaning within 48 hours of sample collection
- Concurrent systemic antimicrobial therapy
- Cats with neurological signs suggesting inner ear involvement

Clinical examination and data collection

All cats underwent a comprehensive clinical examination by qualified veterinarians. Otoscopic examination was performed using standard veterinary otoscopes to assess ear canal morphology, degree of inflammation, type and quantity of discharge, and tympanic membrane integrity. Clinical data recorded included signalment (age, sex, breed), geographic location, duration of clinical signs, previous treatments, and concurrent medical conditions.

Age categories were defined as: Kittens (<6 months), juveniles (6-12 months), and adults (>12 months). Breed classification included Sherazi (Persian type), Himalayan, and local mixed breeds. The geographic distribution

encompassed urban and suburban areas within Baghdad and Anbar provinces.

Sample collection

Ear swabs were collected using sterile cotton-tipped applicators designed explicitly for bacterial sampling. The sampling technique involved gently inserting the swab into the external ear canal without forcing it past any obstructions, followed by gentle rotation to collect adequate material from the infected site. Care was taken to avoid contamination from the external ear pinna or surrounding skin.

Two swabs were collected from each affected ear: one for immediate bacterial culture and identification, and a second for molecular analysis. Samples were labeled with unique identifiers and transported to the laboratory within 2 hours of collection in Stuart's transport medium to maintain bacterial viability.

Bacterial isolation and identification

Primary culture

Ear swabs were inoculated onto multiple types of media, including blood agar, MacConkey agar, and mannitol salt agar plates. Plates were incubated at 37°C under both aerobic and microaerophilic conditions for 24 to 48 hours. Bacterial growth was assessed daily, and isolates were sub-cultured for purification and identification.

Bacterial identification

Preliminary identification was performed using standard microbiological techniques, including Gram staining, catalase testing, oxidase testing, and coagulase testing for staphylococci. Biochemical identification was completed using commercially available identification systems and manual biochemical panels, following the manufacturer's instructions.

S. aureus identification was confirmed by positive catalase and coagulase tests, as well as the fermentation of mannitol. Coagulase-negative Staphylococcus species were identified by positive catalase and negative coagulase tests, with further speciation using additional biochemical tests.

P. aeruginosa identification was based on gram-negative rod morphology, positive oxidase test, characteristic blue-green pigmentation, and distinctive grape-like odor. *E. coli* was identified by its gram-negative rod morphology, positive indole test, methyl red test, Voges-Proskauer test, and citrate test (IMViC pattern).

K. pneumoniae identification was confirmed by gram-negative rod morphology, positive capsule formation, lactose fermentation, and a negative indole test.

Molecular confirmation by PCR

DNA extraction

Bacterial DNA was extracted from pure cultures using the boiling method. Briefly, bacterial colonies were suspended in sterile distilled water, heated at 100°C for 10 minutes, and centrifuged at 12,000 rpm for 5 minutes. The supernatant containing DNA was used directly for PCR amplification.

PCR amplification

Species-specific PCR was performed for each bacterial isolate using validated primer sets as shown in Table 1.

PCR reactions were performed in 25 µL volumes containing 12.5 µL master mix, 1 µL each primer (10 pmol/µL), 2 µL template DNA, and nuclease-free water. Thermal cycling conditions included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (55-60°C depending on primers, 30 seconds), and extension (72°C, 1 minute), with final extension at 72°C for 7 minutes.

Table 1: Primer used in present study.

Primer name	Seq.	Annealing Temp. (°C)	Product Size (bp)
Klpn_16s-F	GACGATCCCTAGCTGGTCTG	60	95
Klpn_16s-R	GTGCAATATTCCCCACTGCT		
E.coli_16s-F	GTTAATACCTTTGCTCATTGA		340
E.coli_16s-R	ACCAGGGTATCTAATCCTGTT		
acr-F	TTGATTCACCAGCGCGTATTGTC	55	570
acr-R	AGGTATCTGCTTCAATCAGCG		
fbp-F	CCTACCTGTTGGTCTTCGACCCG	58	284
fbp-R	GCTGATGTTGTCGTGGGTGAGG		
S. aureus 16s-F	-F 5'-GGCCGTGTTGAACGTGGTCAAATCA-3'		
S. aureus 16s-R	R5' -TIACCATTTCAGTACCTTCTGGTAA-3'	55	270

Gel electrophoresis

PCR products were analyzed by agarose gel electrophoresis using 1.5% agarose gels stained with ethidium bromide. Electrophoresis was performed at 100 V for 60 minutes, and the results were visualized under UV transillumination. A 100 bp DNA ladder was used as a molecular weight marker.

Quality control

Quality control measures included the use of positive and negative controls for all PCR reactions, duplicate testing of 10% of samples, and blind re-identification of selected isolates. Laboratory personnel were blinded to clinical information during bacterial identification procedures.

Statistical analysis

Data were analyzed using SPSS version 26.0. Descriptive statistics were calculated for all variables, including frequencies, percentages, means, and standard deviations. Chi-square tests or Fisher's exact tests were used to compare categorical variables, while continuous variables were analyzed using appropriate parametric or non-parametric tests depending on data distribution. Statistical significance was set at $p<0.05$.

Associations between bacterial species and demographic variables (age, sex, breed) and geographic location were assessed using cross-tabulation and chi-square analysis. Age-related trends were evaluated using chi-square tests for trend. All statistical tests were two-tailed, and 95% confidence intervals were calculated where appropriate.

RESULTS

Study population characteristics

Table 2 show the study population consisted of 100 feline subjects, with a nearly equal gender distribution (54% male, 46% female). Breed composition was predominantly Sherazi (90%), with smaller representations of Himalayan (8%) and local breeds (2%). The age distribution showed 42% of kittens under 6 months, 38% of juveniles (6-12 months), and 20% of adults (>12 months), establishing a representative sample across the various developmental stages.

Table 2: Demographic characteristics of study population.

Category	Subgroup	Count	Percentage
Sex	Male	54	54%
	Female	46	46%
Breed	Sherazi	90	90%
	Himalayan	8	8%
	Local breed	2	2%
Age Groups	<6 months	42	42%
	6-12 months	38	38%
	>12 months	20	20%

Bacterial isolation results

A total of 45 bacterial isolates were recovered from the 100 ear samples, representing five distinct bacterial species. *S. aureus* was the most frequently isolated organism (n=16, 35.6% of isolates), followed by *P. aeruginosa* (n=10, 22.2%), *E. coli* (n=9, 20%), coagulase-negative *Staphylococcus* (n=8, 17.8%), and *K. pneumoniae* (n=2, 4.4%).

Geographic distribution

Geographic analysis shown in Table 3, revealed no significant regional differences in bacterial isolation rates. The total number of bacterial isolates mirrored the sample distribution (Baghdad: 68.9% vs Anbar: 31.1%, $p = 0.856$), indicating that geographic location did not significantly affect bacterial prevalence patterns.

Age-related distribution

Age-related trends in bacterial isolation (Table 4) showed *S. aureus* prevalence decreased with age: 62.5% (<6mo) vs 25.0% (6-12mo) vs 12.5% (>12mo), though not statistically significant ($p=0.114$). *Klebsiella* was exclusively isolated in the 6-to 12-month age group (100% of cases, $p = 0.099$).

Table 3: Bacterial isolation by geographic region.

Region	*S. aureus* (n=16)	Coag. Staph (n=8)	*Pseudomonas* (n=10)	*E. coli* (n=9)	*Klebsiella* (n=2)	Total Isolates
Baghdad	11 (68.8%)	6 (75.0%)	7 (70.0%)	6 (66.7%)	1 (50.0%)	31 (68.9%)
Anbar	5 (31.2%)	2 (25.0%)	3 (30.0%)	3 (33.3%)	1 (50.0%)	14 (31.1%)
p-value	0.920	0.724†	1.000†	0.892†	1.000†	0.856

†Fisher's exact test p-values

Table 4: Age group differences in bacterial isolation.

Age group	*S. aureus*	*Coag. Staph*	*Pseudomonas*	*E. coli*	*Klebsiella*
<6 months	10 (62.5%)	3 (37.5%)	4 (40.0%)	6 (66.7%)	0 (0%)
6-12 months	4 (25.0%)	3 (37.5%)	4 (40.0%)	2 (22.2%)	2 (100%)
>12 months	2 (12.5%)	2 (25.0%)	2 (20.0%)	1 (11.1%)	0 (0%)
p-value	0.114	0.604†	0.873†	0.188†	0.099†

†Fisher's exact test p-values

PCR CONFIRMATION RESULTS

PCR analysis demonstrated high detection rates for all targeted bacterial species: *S. aureus* (15/16, 93.8%), coagulase-negative *Staphylococcus* (8/8, 100%), *P. aeruginosa* (10/10, 100%), *E. coli* (9/9, 100%), and *K. pneumoniae* (2/2, 100%) (Table 5; Figure 1).

Table 5: PCR confirmation results.

Bacterial species	Number tested	PCR positive	Detection rate (%)
S. aureus	16	15	93.8
Coagulase-negative Staph	8	8	100
P. aeruginosa	10	10	100
E. coli	9	9	100
K. pneumoniae	2	2	100

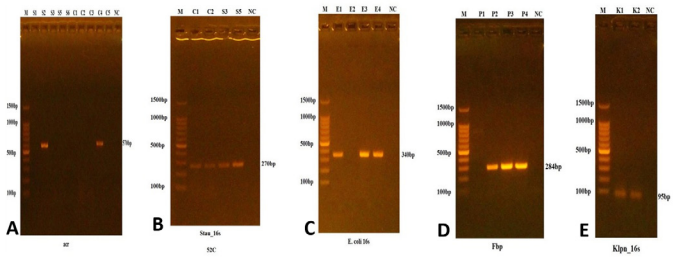

Figure 1: Results of the amplification of the *acr gene* of *Staphylococcus* spp. (A), 16S gene of *S. aureus* (B), *E. coli 16S* gene of *E. coli* (C), the *fbp* gene of *P. aeruginosa* (D) and *Klpn_16s* gene of *K. pneumoniae* (D). The sample species were fractionated on a 1.5% agarose gel by electrophoresis and stained with Ethidium Bromide. M: 100bp ladder marker. Lanes S1-C5 resemble 570 bp PCR products, NC: negative control.

DISCUSSION

The current study provides valuable insights into the bacterial nature of otitis externa in cats in the Iraqi context. This research can shed light on the bacterial phenomena associated within the context of a complex microbial environment, which includes potentially pathogenic organisms with significant clinical and zoonotic concerns. The occurrence of *S. aureus* as the most crucial bacterial pathogen (35.6%) aligns well with the global perspective, as this organism frequently isolated and reported in the majority of feline otitis externa cases (Fernández *et al.*, 2014; Pressanti *et al.*, 2014).

The high frequency of *S. aureus* in our cat population is noteworthy, especially given that it is known to be both a commensal and pathogenic organism in cats. This Gram-positive coccus possesses numerous virulence factors that facilitate adhesion to damaged epithelium, tissue invasion, and survival in the ear canal environment (Lowy, 1998). The decrease in frequency and severity of *S. aureus* in older cats, as observed in our study, although not statistically significant (p = 0.114), suggests that there may be an age-dependent frequency of *S. aureus* in different populations, which warrants further exploration. Other studies in various geographical areas have suggested that some younger cats may be at a greater risk due to their immature immune systems and behaviors (Bourély *et al.*, 2019; Al-Gburi *et al.*, 2021).

Furthermore, the zoonotic potential of *S. aureus* is another clinical concern because infected cats serve as a context for human transmission, especially in households with close contact (Boost *et al.*, 2011). Transmission of methicillin-resistant *S. aureus* (MRSA) to owners through pets has been discussed in the literature (Morris *et al.*, 2006). Documentation of MRSA strain transmission from cats to their owners underscores the importance of good infection control practices in treatment plans. The relatively high isolation rate found in our study suggests that feline otitis externa may be an important, yet under-recognized, source of staphylococcal exposure for households in Iraq. *P. aeruginosa* was the second most prevalent pathogen (22.2% of isolates) identified in our study and is well-known for its association with chronic and recurrent cases of otitis externa. The heritable aspects of this gram-negative organism are concerning, including its inherent resistance to antimicrobials and ability to form biofilms in the ear canal (Driscoll *et al.*, 2007; Bjarnsholt *et al.*, 2009). Aerobic bacteria, such as *P. aeruginosa*, are typically associated with more severe cases of otitis externa, requiring longer-term treatments and higher recurrence rates compared to other bacterial pathogens (Khosravi *et al.*, 2005). In our study, it was apparent that *P. aeruginosa* is not age-dependent, as evidenced by our cases, which showed a normal distribution across age groups (p = 0.873), unlike the age-dependent pattern observed with *S. aureus*.

The presence of *P. aeruginosa* in the environment, coupled with its innate ability to survive in various situations, makes this organism particularly troublesome in the veterinary realm (Kerr and Snelling, 2009). This organism is capable of producing numerous virulence factors that contribute to tissue damage and chronic inflammation typically associated with pseudomonal otitis externa (Strateva and Yordanov, 2009). Management of *P. aeruginosa* otitis externa generally requires targeted antimicrobial therapy based on susceptibility testing due to the organism's inherent resistance profile, making empirical treatment often egregious (Pye *et al.*, 2013).

Notably, *E. coli* was isolated from 20% of the samples, which signifies a noteworthy finding, as this organism is increasingly recognized as a pertinent pathogen associated with otitis externa, particularly in cases of underlying immune compromise or concurrent gastrointestinal

disease (Kaper *et al*., 2004). While historically regarded as a commensal of the intestinal tract, virulent strains of *E. coli* can cause infection at extra-intestinal sites, including the external ear canal (Johnson and Russo, 2005). In ear specimens, *E. coli* may also suggest the potential for fecal contamination, particularly in the presence of multiple cats or poor hygiene practices) Al-Mayahi and Hussain (2021). Also, pathogenic *E. coli* was isolated from other animals by same authors (Hasan *et al*., 2019; Kareem *et al*., 2020). This organism was resembling to another bacteria called Salmonella which were isolated from pet animals (Hasan *et al*., 2018; Abdulgafor *et al*., 2018).

The age distribution of *E. coli*, with a higher prevalence in younger cats as evidenced by 66.7% in <6 months versus 11.1% in >12 months, although not statistically significant (p= 0.188), indicates a potential role of behavioral or immunological factors that may predispose younger animals to infection. This may reflect differential environmental exposures in kittens or developmental differences in local immune responses (Piatti *et al*., 2008).

A 17.8% incidence of coagulase-negative *Staphylococcus* species was observed, underscoring the growing clinical relevance of these organisms in feline otitis externa. Although coagulase-negative Staphylococcus species have traditionally been viewed as innocuous commensals, there is growing evidence regarding their role in chronic infections, especially in cats with immunocompromised conditions and underlying allergic conditions (Huebner and Goldmann, 1999; von Eiff *et al*., 2002). Coagulase-negative *Staphylococcus* species can form biofilms and subsequently become resistant to antimicrobial agents, likely contributing to treatment failures and chronic infections, making their recognition clinically significant (Fey and Olson, 2010). The low rate of *K. pneumoniae* (4.4% of isolates) differs from international reports, which found much higher rates; however, differences in environmental exposures and patterns of antimicrobial use may account for this discrepancy (Podschun and Ullmann, 1998). Nonetheless, the exclusive isolation of this organism in the 6–12-month age group, although not statistically significant (p = 0.099), is certainly unusual and warrants further consideration. *K. pneumoniae* is increasingly associated with severe infections and multidrug resistance; therefore, its isolation in a low-prevalence area is clinically relevant (Patel Bonomon, 2013).

The lack of significant geographic differences in bacterial isolation prevalence between Baghdad and Anbar provinces (p = 0.856) is surprising, considering the differences in geographic features (environmental conditions, population density, and access to veterinary care) in these regions. This suggests that the patterns of bacterial colonization in cats with otitis externa may be influenced more by host factors and pathogen features, rather than geographic characteristics, within the context of Iraq (Al-Rubaie and Mohammad, 2020). This is in contrast to studies in other regions, which have reported significant geographic variations in bacterial prevalence, possibly due to local environmental conditions or variations in antimicrobial use patterns in the areas studied (Beco *et al*., 2013; Hillier *et al*., 2006).

The lack of significant variations in isolation rates between males and females suggests that sex may not play a crucial role in bacterial colonization in cats with otitis externa, a finding supported by other studies (Hassan *et al*., 2019; Bajwa, 2019). Similarly, the lack of significant differences based on breed is limited by the predominance of Sherazi cats (90%) in the study population, which may suggest that breed does not significantly influence bacterial susceptibility in this population.

The high PCR confirmation rates reported in this study (93.8-100% for all species) reflect the accuracy of standard microbiology identification and demonstrate that molecular confirmation provides additional necessary support for bacterial species identification and use. The integration of phenotypic and genotypic methods enhances confidence in testing and facilitates the accurate identification of a pathogen (Tong *et al*., 2015; Becker *et al*., 2014). The only discordant isolation for *S. aureus* (93.8% confirmed by PCR) could be related to technical issues in the PCR amplification process or lack of awareness of genetic variation in specific nucleotide sequences.

Using species-specific genetic markers confirmed by PCR offers advantages over conventional identification alone, including increased sensitivity, specificity, and a faster turnaround time compared to traditional methods (Lavenir *et al*., 2007). The *16S rRNA* gene targets we used to identify *S. aureus* and *E. coli* have been extensively validated for bacterial taxonomy, based on their universal presence and unique variable regions for each species (Weisburg *et al*., 1991). The fbp gene target for *P. aeruginosa* and the acr gene for Staphylococcus species have also demonstrated high specificities for identifying bacteria (Spilker *et al*., 2004; Zhang *et al*., 2004). The clinical ramifications of our findings encompass not only therapeutic considerations but also infection control, zoonotic disease risks, and antimicrobial stewardship. The opportunistic, potentially pathogenic bacteria we isolated indicated that *S. aureus* and *P. aeruginosa* were of particular concern, suggesting that appropriate antimicrobial therapy should be based on culture and sensitivity testing instead of relying on empirical treatment (Weese *et al*., 2011). Due to the zoonotic potential of many of the identified organisms,

it is essential to observe appropriate hygiene and take protective measures when handling infected cats.

The public health significance of identifying bacterial pathogens in domestic pet cats raises awareness of zoonotic potential risks to the general population, particularly immunocompromised individuals, the elderly, and young children, who are most vulnerable due to their proximity to pets (Stull *et al*., 2015; Chomel and Sun, 2011). The significance of antimicrobial resistance in these organisms also complicates treatment choices and highlights the importance of rational antimicrobial use in veterinary medicine (Guardabassi *et al*., 2004).

CONCLUSION

This study provides the first comprehensive analysis of bacterial etiology in feline otitis externa within the Iraqi context, revealing a complex microbial landscape dominated by clinically significant pathogens. *S. aureus* and *P. aeruginosa* emerged as the most prevalent bacterial species, accounting for over half of all isolates, with additional contributions from *E. coli*, coagulase-negative *Staphylococcus*, and *K. pneumoniae*.

The age-related trends observed, particularly the declining prevalence of *S. aureus* with increasing age, suggest important epidemiological patterns that may inform targeted therapeutic approaches. The absence of significant geographic and gender-based variations suggests that host-pathogen interactions may have a greater influence on bacterial colonization patterns than demographic factors within our study region.

The high PCR confirmation rates validate the accuracy of conventional microbiological methods while demonstrating the value of molecular diagnostics in bacterial identification. These findings provide essential baseline data for evidence-based therapeutic decisions and contribute to the global understanding of feline otitis externa etiology.

The zoonotic potential of the identified bacterial species underscores the importance of implementing proper infection control measures and emphasizes the need for One Health approaches in managing companion animal diseases. Future research should focus on antimicrobial susceptibility patterns, treatment outcomes, and longitudinal studies to better understand the dynamics of bacterial infections in feline otitis externa.

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NOVELTY STATEMENT

This study is the first to comprehensively investigate the bacterial causes of otitis externa in cats in Iraq, identifying *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the most prevalent pathogens. By combining conventional microbiology with PCR confirmation, it provides reliable data on bacterial prevalence, zoonotic risks, and age-related trends, offering crucial insights for veterinary treatment and public health in the region.

AUTHOR'S CONTRIBUTION

Mustafa Salah Hasan: Researcher, statistical analysis. Ahmed Ali Ahmed: Practical work, writing, editing and sampling.

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Data availability

Data are available upon request

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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