

Otodectic, Bacterial Etiology, and Antimicrobial Resistance Patterns of Otitis Externa in Cats from Northern Palestine: A Cross-Sectional Study

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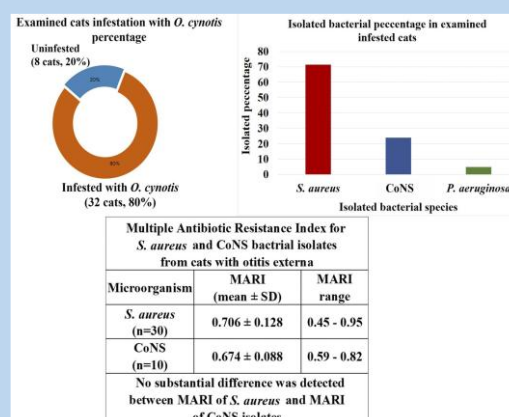
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Abstract: Otitis externa in cats is a common external ear canal inflammation. Usually caused by parasites and subsequent bacterial infections. In this study *Otodectes cynotis* (*O. cynotis*) infestation was examined. In addition, bacterial pathogens and antimicrobial resistance (AMR) patterns and multiple antibiotic resistance indices (MARI) of *Staphylococcus* isolates from cats with otitis externa from Northern Palestine were also examined. AMR in companion animal bacterial infections raises therapeutic and public health problems. Ear samples were taken from 40 cats with otitis externa, from which *O. cynotis* was detected. Bacterial isolates were identified using standard microbiological methods. Antimicrobial susceptibility testing against 22 antimicrobial agents using disk diffusion test was performed. MARI was estimated for *Staphylococcus* isolates.

In total, 80% (32/40) of the examined cats were infested with *O. cynotis*. Most of the 42 bacterial isolates were *Staphylococcus aureus* (*S. aureus*) (71.4%, 30/42), followed by coagulase-negative Staphylococci (CoNS) (23.8%, 10/42) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (4.8%, 2/42). Mixed *S. aureus* and *P. aeruginosa* infections was 5% (2/40). All *Staphylococcus* isolates were multidrug-resistant. *S. aureus* isolates had a mean MARI of 0.706 ± 0.128 (range: 0.45-0.95), while CoNS isolates had 0.674 ± 0.088 (range: 0.59-0.82). All isolates were penicillin- and ampicillin-resistant. *S. aureus* and CoNS MARI values were not significantly different ($U = 178.0$, $p = 0.292$). Neither host breed nor sex had a significant effect on bacterial isolation rates.

Due to *O. cynotis*' high incidence and multidrug resistance, parasitological diagnosis and antibiotic susceptibility testing are necessary in cats with otitis externa. The limited sample size and the fact that cats were sourced from a single veterinary clinic should be viewed with caution. Cat otitis externa incidence and antibiotic resistance in Northern Palestine need extensive multicenter investigation. Our findings highlight the necessity of antimicrobial stewardship, antibiotic resistance assessments, and parasitological analyzes in Northern Palestine for One Health.



Keywords: Antibiotic resistance, Antimicrobial stewardship, Cat otitis externa, Multidrug resistance, One Health, *Otodectes cynotis*, Palestine, *Staphylococcus*.

Introduction

One of the most common clinical diseases in feline medicine globally is otitis externa, which is defined as inflammation of the external auditory canal [1, 2]. This inflammation presents across a broad clinical spectrum, ranging from mild irritation and excessive cerumen accumulation to severe pain, tissue ulceration, and potential systemic complications [3, 4]. The etiology is complex and multifactorial, driven by primary causes such as foreign bodies,

hypersensitivities, and parasitic infestations. Anatomical conformation and environmental conditions act as predisposing risk factors, while secondary bacterial and fungal pathogens serve as perpetuating factors that exacerbate and sustain the inflammatory state [2, 5]. One of the major causative factors of otitis externa in cats worldwide is *Otodectes cynotis* (*O. cynotis*), which is the common ear mite of cats and dogs and a parasitic arthropod that inhabits the external ear canal. This infestation is

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particularly prevalent in young animals and those living outdoors [3, 6]. Previous studies have found that the incidence of *O. cynotis* infection among cat populations varies, ranging from 0.9% to 83.7%, based on study groups, management approaches, and regional factors [4]. The presence of *O. cynotis* creates an inflammatory milieu that predisposes the ear canal to secondary bacterial and fungal infections, complicating both treatment strategies and clinical management [2, 7].

Numerous studies have examined the bacterial pathogens linked to otitis externa in cats, as well as their prevalence and/or resistance to various classes of antimicrobial agents, such as coagulase-positive *Staphylococcus* (CoPS), coagulase-negative *Staphylococcus* (CoNS), *Proteus* spp., *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), and others [8-16].

Staphylococcus species, particularly *Staphylococcus aureus* (*S. aureus*), are the most commonly isolated pathogens from affected cats' ears, and they play a major role in the persistence and exacerbation of otitis externa [15, 16]. Other frequently reported bacterial agents include *Streptococcus* spp., *P. aeruginosa*, and a range of other Gram-negative bacterial species [9, 12]. According to the One Health paradigm, antimicrobial resistance (AMR) in bacterial strains from companion animals has become a major problem in veterinary medicine and public health [11, 17]. Because otitis externa in cats involves the interaction of animal health, antibiotic use, and potential public health concerns, the One Health concept is particularly relevant to this study. Antimicrobial resistance (AMR) profiles and bacterial infections in cats are significant for improving veterinary diagnosis and treatment, as well as recognizing the possible role of companion animals in the development and spread of AMR pathogens.

The rise of methicillin-resistant strains of multidrug-resistant (MDR) *Staphylococcus* species constitutes a serious treatment challenge and a potential issue for zoonotic transmission [11, 18-22]. Healthy cats' ears may be a reservoir for antibiotic-resistant CoPS and CoNS which are key sources of AMR and possible zoonotic diseases which can transmit to humans [23, 24]. The global spread of AMR is generally accepted as a serious threat to human and animal health, necessitating a One Health strategy that integrates perspectives on human, animal, and environmental health [25]. Developing evidence-based treatment plans and carrying out successful antimicrobial surveillance programs require an understanding of the local epidemiology of the bacteria causing cat otitis externa and their profiles of antibiotic resistance [16]. Despite the widespread significance of cat otitis externa, little is known about its etiology and patterns of antibiotic resistance in a variety of nations, particularly Palestine.

This study aimed to examine the presence of *O. cynotis* infestation as a main causative agent for otitis externa in cats, detect and identify bacterial pathogens linked to cat otitis externa, and assess the AMR profiles of isolated bacteria, in cats from Northern Palestine. This study's findings will enhance the understanding of the disease and provide clinicians in the region with essential information in order to guide suitable diagnostic and therapeutic approaches. Additionally, this study assessed the degree of multidrug resistance by using the multiple antibiotic resistance index (MARI) to offer evidence-based recommendations for the diagnosis and management of this prevalent clinical condition in the region.

Materials and Methods

Ethical Approval and Consent to Participate

This study was reviewed and approved by the Institutional Review Board (IRB) for Animal Research at An-Najah National University (Approval Ref. No. [2026-08-04 IACUC]). All procedures involving animals were conducted in accordance with institutional guidelines and international standards for ethical research. Consent to participate was not applicable, as the study did not involve human subjects.

Study Design and Population

The study population consisted of 40 cats (30 Persian cats and ten domestic shorthairs (local/street cats) with different ages, and sexes, that were submitted to a small-animal clinic in the city of Jenin, Palestine. The animals showed signs of an outer ear infection.

Clinical Evaluation

Standard guidelines for cats' clinical assessment and veterinary physical assessment were strictly followed during cats' clinical evaluation and veterinary examination. This was conducted by a licensed veterinarian at a veterinary clinic Vetecare Center in Jenin, Northern Palestine.

Each cat got a thorough medical examination, with special attention paid to the ears and associated tissues. Head shaking, ear discharge (defined as ceruminous or purulent) with a foul smell, edema, erythema, bleeding, and ulceration were among the clinical signs that were methodically documented. Acute moist dermatitis at the base of the ear and aural hematomas were noted in severe cases. Because clinical data were retrieved from routine veterinary clinic records completed by different attending veterinarians, minor clinical signs were inconsistently recorded across cases. Therefore, although we couldn't list every symptom in detail, we made sure to track the main clinical signs.

Parasitological Analysis

Bilateral ear swab specimens were obtained from the external auditory canals utilizing sterile cotton-tipped applicators. Specimens obtained from each ear were later pooled for examination. Microscopic analysis was conducted using the procedure established by Al-Khafaji et al [6]. Specimens were positioned on a clean glass slide with 1-2 drops of xylene. The prepared slides were examined under a light microscope at 4× and 10× magnifications to identify mites, eggs, or fecal material. Representative microscopic images were captured for documentation and to support morphological identification.

The detection of mites, eggs, or fecal matter was determined to be indicative of *O. cynotis* infestation.

Bacterial Sample Collection, Isolation and Identification

Collection, isolation and identification of bacterial pathogens were carried out in a small animal clinic, Jenin, Palestine. For the purpose of bacterial culture and identification, sterile cotton-tipped swabs designed exclusively for bacterial sample collection were used to collect ear swabs. In order to gather enough material from the infected region, the swabs were gently rotated after being softly inserted into the external ear canals. Following collection, the swab samples were enriched by incubating in sterile 5 mL of tryptone soy broth for 6-10 hours at 37°C. Enriched cultures were streaked onto a variety of culture media, such as nutrient agar, blood agar, eosin methylene blue (EMB) agar and mannitol salt agar (MSA), in order to separate

and examine the cultural characteristics of the pathogenic microorganisms. The bacterial isolates were examined for Gram staining characteristics, motility detection and colonial morphology on different cultural media such as pigmentation, hemolytic patterns and fermentation. The bacterial isolates were analyzed biochemically using catalase, citrate utilization, urease, coagulase, oxidase, indole, and Methyl Red-Voges-Proskauer (MR-VP) tests.

S. aureus identification involved characterizing isolates through positive catalase and coagulase tests, mannitol fermentation, and the presence of golden-yellow colonies on blood agar. CoNS were identified by positive catalase and negative coagulase assays.

The isolates of *P. aeruginosa* were characterized by their Gram-negative rod morphology, motility, positive results for oxidase, catalase, and citrate utilization, and negative outcomes for lactose fermentation, urease, indole, Methyl Red and Voges-Proskauer assays. Presumptive identification of *P. aeruginosa* was based on the characteristic production of blue-green pigment on the culture medium, accompanied by a distinctive grape-like odor.

Antimicrobial Susceptibility Testing

In accordance with the Clinical and Laboratory Standards Institute (CLSI) instructions for veterinary pathogens, the Kirby-Bauer disk diffusion technique was used on Mueller-Hinton agar plates to evaluate antibiotic susceptibility [26]. Each isolate was inoculated onto the surface of the agar medium using a standardized inoculum that was equivalent to a 0.5 McFarland turbidity standard. The following antimicrobial disks were tested: ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), enrofloxacin (ENR, 5 µg), levofloxacin (LEV, 5 µg), gentamicin (CN, 10 µg), neomycin (N, 30 µg), kanamycin (K, 30 µg), cephalixin (CL, 30 µg), ceftriaxone (CRO, 30 µg), ceftiofur (FUR, 30 µg), ceftioxin (CX, 30 µg), amoxicillin (AX, 25 µg), penicillin (P, 10 U), amoxicillin/clavulanic acid (AMC, 30 µg), ampicillin (AMP, 10 µg), doxycycline (DO, 30 µg), sulfamethoxazole/trimethoprim (SXT, 25 µg), chloramphenicol (C, 30 µg), florfenicol (FFC, 30 µg), fosfomycin (FO, 50 µg), spectinomycin (SH, 100 µg), and erythromycin (E, 15 µg). Following incubation at 37°C for 18-24 hours, the diameter of the inhibitory zone was measured to the nearest millimeter for each antimicrobial disk and classified as resistant, intermediate or susceptible based on CLSI instructions for veterinary pathogens. Multidrug resistance (MDR) was assigned to isolates that were resistant to a minimum of three distinct antimicrobial drug classes.

Determination of Multiple Antibiotic Resistance Index (MARI)

The MARI of the pathogenic microbial isolates from cats with otitis externa was used to assess their AMR profiles. For each bacterial isolate, the MARI was calculated using the following formula: $MARI = D/E$, where D is the number of antibacterials to which the isolate was resistant, and E is the total number of antimicrobials investigated for the isolate. To distinguish between sources of exposure that are high-risk and those that are low-risk, a MARI of 0.20 was employed. The formula uses for calculating the index of a sample with multiple isolates is $R/(E \times F)$, where R is the total AMR score of all the isolates in the sample, E is the number of antimicrobials tested for each isolate, and F is the total number of isolates in the sample [27, 28].

Statistical Analysis

Even though the small sample size may constrain the study's statistical power, statistical analysis was carried out with cautious interpretation of numerical trends between breeds/sexes. SPSS software version 26.0 (IBM Corp., Armonk, NY, USA) was employed to analyze the data. Pearson's chi-square test was employed to analyze categorical variables such as breed, sex, and pathogen isolation, shifting to Fisher's exact test when predicted cell counts decreased below 5. Binary logistic regression was implemented in order to assess the independent effects of breed and sex, as well as their interaction on *S. aureus* colonization. Odds ratios (ORs) with 95% confidence intervals were used to interpret the results. Statistical significance was defined as a P-value of less than 0.05. The descriptive statistics provided for each group of bacteria include percentages and frequencies. The MARI data's normal distribution was evaluated using the Shapiro-Wilk test. The MARI values of *S. aureus* and CoNS isolates were assessed using the non-parametric Mann-Whitney U test because it was assumed that the distributions would not follow a normal distribution.

Results

Clinical Examination

During the comprehensive clinical evaluation, the 40 cats with otitis externa showed a range of symptoms. Head shaking and varying degrees of ear drainage were the most often observed clinical signs. Ceruminous discharge, which is waxy and ranges in color from brown to black, and purulent discharge, which shifts from yellow to green, were identified as the two primary forms of discharge. In addition to purulent discharge and bacterial illness, malodor coming from the afflicted ears was a typical symptom. Upon palpation of the ear base, many affected cats showed signs of discomfort, and swelling and pain were noted in the external ear canal in many cases. Prominent characteristics that indicated active inflammatory processes were redness, or erythema of the external ear canal. A narrowing of the ear canal, known as stenosis, and ulceration of the epithelial lining were observed in more advanced cases. Some occurrences of ear canal hemorrhage were reported, which was usually linked to acute inflammation or self-inflicted trauma. Scratching ears or the area around them was a notable clinical finding in a number of instances, suggesting self-inflicted harm due to severe pruritus. As a result, the area surrounding the ear's base would frequently develop excoriations and acute moist dermatitis (Figure 1). In more serious instances, problems such as aural hematomas were noted. Some cats exhibited a head tilt, which is a sign of severe pain or discomfort in the affected ear. From moderate cases with little discharge and head shaking to severe cases with widespread inflammation, discomfort, and secondary problems, the combination and severity of clinical symptoms differed among the affected cats.



Figure 1: Otitis externa in a cat showing scrapes and waxy discharge.

Detection of *O. cynotis*

Microscopic examination of ear swab samples demonstrated an elevated incidence of *O. cynotis* infestation. In total, 80% (32 out of 40) of the cats exhibited a positive result for *O. cynotis*, suggesting that *O. cynotis* might act as an important contributing factor to otitis externa in the investigated cats. Indicators of an active infestation included the presence of eggs, distinctive feces, or mites (Figure 2).

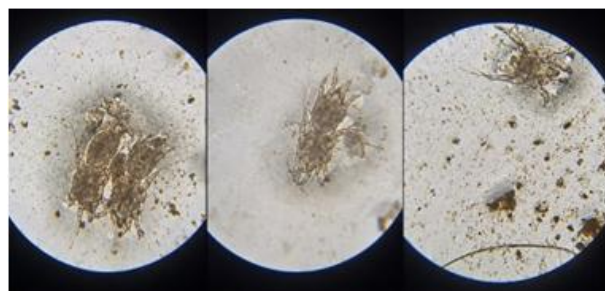


Figure 2: Swabs from otitis externa cases under the microscope, showing the presence of mites and their debris.

Bacterial isolation rates by breed and sex

The sex distribution of the sampled cats was balanced within each breed. There were 15 males and 15 females among the Persian breed, and five males and five females in the domestic shorthair breed. The disparity in sample numbers among groups suggests a possible source of bias that may influence the reliable nature of our research results.

As shown in table 1, out of all the bacteria that were isolated, the most common recovered organism was *S. aureus* representing 30 isolates among the 42 isolates. *S. aureus* was isolated more frequently from Persian cats, and within each breed, *S. aureus* isolated from male cats more than female cats. The second most prevalent category of staphylococci was CoNS which represented ten isolates. Of these, 8 were found in Persian cats, whereas no male domestic cat showed positive results. *P. aeruginosa* was isolated from only two male Persian cats.

In comparison to domestic shorthairs, Persian cats showed a higher rate of bacterial isolation across all species. Moreover,

in most bacterial groups, more isolates from male cats were recovered than female cats.

Table (1): Frequency of bacterial isolates among cat breeds, sex, and bacterial species.

Bacteria	breed	Sex	Isolates Number	sum
<i>S. aureus</i>	Persian cat	Male	12	30
		Female	10	
	Domestic cat	Male	5	
		Female	3	
CoNS	Persian cat	Male	3	10
		Female	5	
	Domestic cat	Female	2	
<i>P. aeruginosa</i>	Persian cat	Male	2	2
Total isolates			42	

CoNS: coagulase-negative *Staphylococcus*

Across all three bacterial categories, there was no statistically significant relationship between sex or breed and bacterial isolation in all tested animals. Although the isolation rate of *S. aureus* in male cats showed a greater isolation rate (85%) than females (65%), this disparity did not approach a statistically significant level ($\chi^2 = 2.133$, $df = 1$, $p = 0.144$, OR = 3.05, 95% CI: 0.66-14.14). There was no significant difference in the isolation rates of Persian cats (73.3%) and domestic shorthairs (80.0%) ($\chi^2 = 0.178$, $df = 1$, $p = 0.673$) (Table 2).

According to logistic regression, the effect of sex on *S. aureus* carriage was the same across breeds, and there was no interaction between breed and sex ($p = 0.782$). Isolation rates for CoNS were low in every category. In terms of numbers, Persian cats had a little greater isolation rate (8/30, 26.7% vs. 2/10, 20.0%), and it seemed that females had a higher rate (7/20, 35.0% vs. 3/20, 15.0%). Statistical testing proved unreliable (expected frequencies < 5 in numerous cells) because of the extremely small number of isolates. Although a Fisher's Exact test would yield more accurate results, the small sample sizes make it impossible to draw any significant conclusions.

Table (2): Correlation between breed, sex and bacterial isolation in cats with otitis externa.

Variable factor	Isolated Bacteria	Statistical Test	Statistic	p-value	OR value (95% CI)
Breed	<i>S. aureus</i>	χ^2	0.178	0.673	0.69 (0.12-3.95)
	CoNS	χ^2	0.178	0.673	1.45 (0.25-8.35)
	<i>P. aeruginosa</i>	Fisher's exact	-	0.402	-
Sex	<i>S. aureus</i>	χ^2	2.133	0.144	3.05 (0.66-14.14)
	CoNS	χ^2	2.133	0.144	0.33 (0.07-1.52)
	<i>P. aeruginosa</i>	Fisher's exact	-	0.147	-

OR: odds ratio; CI: confidence interval.

-The odds ratio cannot be computed since there were no *P. aeruginosa* isolates in the Domestic Shorthair category as well as in females.

Bacterial Identification and Bacteriological Analysis

A total of 42 distinct bacterial isolates were identified from the 40 cats, suggesting that some cases had mixed bacterial pathogens. Conventional microbiological methods were used to successfully categorize the bacterial isolates into *S. aureus*, CoNS and *P. aeruginosa*.

S. aureus was the most frequent form of isolated bacteria, representing 71.4% of the total isolates. These showed Gram-positive cocci, had a growth on MSA medium with positive mannitol fermentation, golden-yellow colonies on blood agar and these isolates were positive catalase and coagulase tests. Additionally, 23.8% of the bacterial isolates were proven to be CoNS because they were Gram-positive cocci that grew on MSA without mannitol fermentation and were coagulase-negative and catalase-positive.

P. aeruginosa was detected in a small fraction of the sample (4.8%). The identification of this organism was accomplished using a group of tests. These 2 isolates were Gram-negative rod shape, positive oxidase and catalase, negative indole, methyl red and Voges-Proskauer negative, positive citrate utilization, and negative urease. Due to their characteristic blue-green coloring and grape-like aroma, these isolates were positively identified as *P. aeruginosa*. Table 3 shows the distribution of microbial isolates from cats with otitis externa in Northern Palestine.

Table (3): Distribution of microbial isolates collected from cats with otitis externa infection in North of Palestine.

Microorganism	No. of infected cats	No. of microorganism isolates (%)
<i>S. aureus</i>	30	30 (71.4)
CoNS	10	10 (23.8)
<i>P. aeruginosa</i>	2	2 (4.8)
total	40	42 (100)

CoNS: coagulase-negative *Staphylococcus*

Two cases of cats' otitis externa (5%), showed the co-infection pattern of *P. aeruginosa* and *S. aureus* (Table 4).

Table (4): Bacterial co-infection patterns in cats with otitis externa infections.

Bacterial Pattern	No. of isolates	Percentage of Infection
<i>S. aureus</i>	28	70
CoNS	10	25
<i>S. aureus</i> and <i>P. aeruginosa</i>	2	5
Total	42	100

CoNS: coagulase-negative *Staphylococcus*

Antimicrobial Resistance Patterns in *Staphylococcus* Isolates

There was a concerning degree of resistance to several kinds of antibiotics in both *S. aureus* and CoNS isolates. It is clear that β -lactam resistance mechanisms are prevalent because all of the obtained isolates showed full resistance to ampicillin and penicillin.

The majority of the *S. aureus* isolates (n = 30) showed high resistance to the following antibiotics: erythromycin (86.7%), cefoxitin (90%), enrofloxacin (93.3%), sulfamethoxazole/trimethoprim (86.7%), and chloramphenicol (86.7%). The resistance rates to other antimicrobial agents included amoxicillin had the highest resistance rate of 80%, followed by florfenicol (76.7%), gentamicin (66.7%), kanamycin (66.7%), doxycycline (63.3%), and neomycin (60%). Fosfomycin, cephalexin, ceftiofur, and amoxicillin/clavulanic acid had the lowest resistance rates of 43.3%, 23.3%, 23.3% and 13.3%, respectively, suggesting that these antibiotics may still be effective against a large number of isolates (Table 5).

Overall, 100% resistance to penicillin, ampicillin, spectinomycin, ciprofloxacin, norfloxacin, and enrofloxacin was observed in all CoNS tested isolates. In addition, chloramphenicol, florfenicol, and erythromycin showed a resistance rate of 90% each, while amoxicillin, sulfamethoxazole/trimethoprim showed 80% resistance for each. On the other hand, neomycin and cefoxitin showed rates of resistance of 70% for each.

Although high resistance rates were clear against many antimicrobial agents, some drugs, such as fosfomycin (40%), cephalexin (30%), doxycycline (30%), ceftriaxone (10%) and amoxicillin/clavulanic acid (10%) have lower resistance rates as shown in Table 5.

Table (5): The antibiotic sensitivity profile of the *S. aureus* and coagulase-negative *Staphylococcus* isolates recovered from cats with otitis externa infection in Northern Palestine.

Antibiotic class	Antibiotic	<i>S. aureus</i> (n = 30)			CoNS (n = 10)		
		R (%)	S (%)	I (%)	R (%)	S (%)	I (%)
Fluoroquinolone	Ciprofloxacin CIP	25 (83.3)	5 (16.7)	0 (0)	10 (100)	0 (0)	0 (0)
	Norfloxacin NOR	25 (83.3)	4 (13.3)	1 (3.3)	10 (100)	0 (0)	0 (0)
	Enrofloxacin ENR	28 (93.3)	2 (6.7)	0 (0)	10 (100)	0 (0)	0 (0)
	Levofloxacin LEV	25 (83.3)	5 (16.7)	0 (0)	7 (70)	3 (30)	0 (0)
Aminoglycoside	Gentamicin CN	20 (66.7)	8 (26.7)	2 (6.7)	4 (40)	3 (30)	3 (30)
	Neomycin N	18 (60)	11 (36.7)	1 (3.3)	7 (70)	2 (20)	1 (10)

	Kanamycin K	20 (66.7)	6 (20)	4 (13.3)	4 (40)	5 (50)	1 (10)
Cephalosporins	Cephalexin CL	7 (23.3)	21 (70)	2 (6.7)	3 (30)	7 (70)	0 (0)
	Ceftriaxone CRO	15 (50)	13 (43.3)	2 (6.7)	1 (10)	6 (60)	3 (30)
	Ceftiofur FUR	7 (23.3)	23 (76.7)	0 (0)	3 (30)	5 (50)	2 (20)
	Cefoxitin CX	27 (90)	3 (10)	0 (0)	7 (70)	3 (30)	0 (0)
Aminopenicillin	Amoxicillin AX	24 (80)	6(20)	0 (0)	8 (80)	2 (20)	0 (0)
	Penicillin P	30(100)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)
	Amoxicillin/clavulanic acid AMC	4 (13.3)	25 (83.3)	1 (3.3)	1 (10)	9 (90)	0 (0)
	Ampicillin AMP	30 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)
Tetracycline	Doxycycline DO	19 (63.3)	9 (30)	2 (6.7)	3 (30)	7 (70)	0 (0)
Sulfonamides	Sulfamethoxazole/trimethoprim SXT	26 (86.7)	4 (13.3)	0 (0)	8 (80)	2 (20)	0 (0)
Amphenicoles	Chloramphenicol C	26 (86.7)	3 (10)	1 (3.3)	9 (90)	1 (10)	0 (0)
	Florfenicol FFC	23 (76.7)	6 (20)	1 (3.3)	9 (90)	1 (10)	0 (0)
Phosphonic Antibiotic	Fosfomycin FO	13 (43.3)	15 (50)	2 (6.7)	4 (40)	6 (60)	0 (0)
Aminocyclitol	Spectinomycin SH	28 (93.3)	2 (6.7)	0 (0)	10 (100)	0 (0)	0 (0)
Macrolides	Erythromycin E	26 (86.7)	2 (6.7)	2 (6.7)	9 (90)	1 (10)	0 (0)

R: resistant; S: susceptible; I: intermediate.

Multidrug Resistance Patterns

According to this study, all of the *Staphylococcus* isolates recovered from cats with otitis externa were highly resistant to multiple drugs. A total of 30 *S. aureus* and 10 CoNS isolates fulfilled the criteria for MDR since they were resistant to three or more classes of antibiotics.

Overall, 46.7% of *S. aureus* isolates (14/30) showed resistance to nine antibiotic classes, making this pattern the most common in *S. aureus*. Seven isolates (23.3%) exhibited resistance to eight antibiotic classes; five isolates (16.7%) showed resistance to seven classes, three isolates (10%) were resistant to ten different classes of antibiotics, while only one isolate, accounting for 3.3% of the total, was resistant to six classes (Table 6).

For CoNS isolates, five isolates (50%) showed resistance to seven classes of antibiotics, four isolates (40%) demonstrated resistance to nine classes, and one isolate (10%) exhibited resistance to eight classes (Table 6).

Table (6): Occurrence of multidrug-resistant *Staphylococcus* isolates collected from cats with otitis externa infection in Northern Palestine, based on the number of resistant antibiotic groups.

Microorganism	No. of resistant antibiotic groups	No. of isolates (%)	Total isolates	MDR (%)
<i>S. aureus</i>	6	1 (3.3)	30	100
	7	5 (16.7)		
	8	7 (23.3)		
	9	14 (46.7)		
	10	3 (10)		
CoNS isolates	7	5 (50)	10	100
	8	1 (10)		
	9	4 (40)		

CoNS: coagulase-negative *Staphylococcus*

Multiple Antibiotic Resistance Index (MARI)

All 30 *S. aureus* isolates demonstrated a high degree of multidrug resistance, with a mean MARI of 0.706 ± 0.128 ranging from 0.45 to 0.95. Resistance to 17 antimicrobials was the predominant resistance pattern, detected in 6 (20.0%) isolates, which was followed by resistance to 12 and 16 antimicrobial agents, each recorded in 5 (16.7%) isolates. Additionally, 4 isolates (13.3%) exhibited resistance to 14 antimicrobial agents, while another 4 isolates (13.3%) exhibited resistance to 19 antimicrobials. One isolate exhibited the highest MARI score (0.95), showing resistance to 21 of the 22 antibiotics tested. The aggregate antibiotic resistance score for *S. aureus* isolates was 466, indicating significant AMR within this bacterial population (Table 7).

The average MARI value of the 10 CoNS isolates was 0.674 ± 0.088 , ranging between 0.59 and 0.82. The most prevalent MARI pattern was resistance to 13 and 14 antimicrobials, identified in 3 (30.0%) isolates each, whereas resistance to 18 antimicrobial agents was noted in 2 (20.0%) isolates. The aggregate antibiotic resistance score for these isolates was 148 (Table 8).

Table (7): Antibiotic resistance patterns and the MARI for 30 *S. aureus* isolates collected from cats with otitis externa infection in Northern Palestine using 22 antimicrobial agents.

<i>S. aureus</i> isolates			
Number of resistant antibiotics	*MARI of isolate	No. of isolates (%)	Aggregate antibiotic resistance score
10	0.45	2 (6.7)	20
12	0.55	5 (16.7)	60
14	0.64	4 (13.3)	56
15	0.68	1 (3.3)	15

16	0.73	5 (16.7)	80
17	0.77	6 (20.0)	102
18	0.82	2 (6.7)	36
19	0.86	4 (13.3)	76
21	0.95	1 (3.3)	21
Total		30 (100)	466

*MARI: Multiple Antibiotic Resistance Index.

Table (8): Antibiotic resistance patterns and the MARI for 10 CoNS isolates collected from cats with otitis externa infection in Northern Palestine using 22 antimicrobial agents.

CoNS isolates			
Number of resistant antibiotics	*MARI of isolate	No. of isolates (%)	Aggregate antibiotic resistance score
13	0.59	3 (30.0)	39
14	0.64	3 (30.0)	42
15	0.68	1 (10.0)	15
16	0.73	1 (10.0)	16
18	0.82	2 (20.0)	36
Total		10 (100)	148

*MARI: Multiple Antibiotic Resistance Index.

The Shapiro-Wilk test for normality revealed a normal distribution for *S. aureus* isolates ($p = 0.168$) but not for CoNS isolates ($p = 0.039$). Thus, the comparison of MARI values between the two groups was conducted utilizing the Mann-Whitney U test. No substantial difference was detected between MARI of *S. aureus* and MARI of CoNS isolates ($U = 178.0$, $p = 0.292$).

Discussion

Otitis externa in cats is a common and clinically significant condition in small animal medicine [2, 4, 15]. The signs found in this study, such as head shaking, ceruminous or purulent ear discharge, foul odor, swelling, and pain in the external ear canal, are similar to those in previously published studies [3, 4, 29, 30]. In more serious cases, complications like aural hematomas and acute moist dermatitis were reported by [2], who also noted that inadequately treated otitis externa can progress to tympanic rupture, abscess formation, and even systemic infections. All cats examined in the present study exhibited clinical manifestations indicative of otitis externa. This finding is inconsistent with previous studies, which reported varying prevalence rates ranging from 2% to 79% among the cats examined [1, 7, 31, 32].

Ear mite infestation is a leading cause of otitis externa in cats [2, 33]. The high occurrence (80%) of *O. cynotis* infection found in this study, raises the possibility that this parasite may play a major role in the cats' otitis externa, which makes cats more vulnerable to bacterial and fungal infections later on. Prevalence rates between 6% and 63.3% have been documented in other investigations [2-4, 6, 10, 13] which showed lower occurrence rates than the present study. In general, variation in pathogen isolate distribution depends on several factors such as the populations studied (stray or owned), methodological approaches, animal management practices, and geographical regions, climate and sanitary conditions [2-4, 6, 10, 13, 16, 30,

33, 34]. Based on the results of this investigation, *O. cynotis* is likely a highly contagious ectoparasite that spreads mostly through close animal contact [2], causing pruritus, head and ears shaking, and other common clinical signs seen in an infested cat [33]. These abnormalities can alter the ear canal microenvironment, which can lead to the growth of opportunistic bacteria. Ear swab microscopy is still the gold standard for fast, inexpensive, and accurate diagnosis of mites, eggs, and distinctive feces. Eliminating infestation, reducing discomfort, preventing secondary infections, and minimizing the recurrence of otitis externa require early diagnosis and proper acaricidal treatment [2, 33].

Breed isolation rates were not significantly different ($p = 0.674$). Isolates were recovered more frequently from males than from females, but the difference was not statistically significant ($p = 0.144$), and logistic regression showed no breed-sex interaction ($p = 0.782$). The indicated numerical discrepancies should be interpreted with caution, as they may represent sample variance instead of biological variations. An elevated number of bacteria in Persian cats could be biologically plausible, given their long, thick fur surrounding the outer ear canal, which decreases ventilation, maintains humidity, and encourages the accumulation of wax and debris. When coupled with inflammatory or parasitic conditions such as *O. cynotis* infection, these variables may alter the usual ear milieu and facilitate bacterial colonization. Following multivariable analysis, Elmoslemany et al. [11] found sex does not predict methicillin- or multidrug-resistant staphylococcal carriage in cats. Similarly, Miszczak et al. [23] reported no correlation between staphylococcal isolation and sex, breed, or age in healthy or sick cats, nor did sex affect disease prevalence in companion cats [31]. Males could exhibit higher bacterial prevalence owing to variations in behavior. Several recent studies have clarified the direct role of testosterone in *S. aureus* infections [35, 36]. Additionally, male cats, especially those permitted outdoors, have broader territorial boundaries, wander more, and fight more, increasing environmental exposure and bacterial transmission.

CoNS were the second most common bacterial category in this study. CoNS are typical components of skin microbiota and may indicate colonization instead of infection. The dermal and mucosal layers of cats harboring CoNS, which may turn into opportunistic infections following injury to the ear canal. Although females demonstrate a higher rate of numerical isolation compared to males, the relatively low number of isolates obstructed meaningful statistical analyses. The recognition of CoNS is clinically important since these opportunistic infections serve as reservoirs for antibiotic resistance genes in companion animals [15, 23]. Furthermore, recent findings indicate that methicillin resistance is often more prevalent in CoNS compared to CoPS, underscoring the epidemiological significance of these bacteria in veterinary practice. Elmoslemany et al. [11] reported that CoNS colonize cats and harbor AMR genes. The complete lack of CoNS in male domestic shorthairs necessitates further investigation, possibly suggesting breed-specific differences in skin barrier efficacy or competing microbial dynamics that warrant future metagenomic studies [37]. The distinctive isolation of *P. aeruginosa* from two male Persian cats, notwithstanding its infrequent occurrence (4.8%), is a noteworthy finding. This opportunistic bacterium flourishes in the warm, moist environment of the inflamed ear canal and possesses intrinsic antimicrobial resistance mechanisms alongside its

capacity to create biofilms. Research studies have demonstrated that *P. aeruginosa* infections associated with otitis externa result in more severe clinical symptoms, need prolonged therapeutic regimens, and show a higher propensity for recurrence than infections caused by alternative bacterial pathogens [15, 38]. Neither breed nor sex substantially influences bacterial isolation in cat otitis externa, indicating that these host characteristics are not sufficient reasons for bacterial prevalence. Conversely, a multifaceted interaction of local ear conditions, host immune response, parasite presence, interaction with domestic dogs, antibiotic administration, environmental sanitation, and management strategies may influence bacterial colonization [14, 15, 23, 37]. Small sample size, especially for domestic shorthair cats and rare bacterial species, limited statistical ability to detect tiny differences across groups. Therefore, bigger multicenter studies with more demographic and clinical factors are needed to determine how breed and sex affect bacterial colonization and infection in cats' otitis externa.

The most common bacterial isolate in this investigation was *S. aureus* (71.4%, 30/42) followed by CoNS (23.8%, 10/42) and *P. aeruginosa* (4.8%, 2/42). The incidence of mixed *S. aureus* and *P. aeruginosa* infections was 5% (2/40). The predominance of staphylococci in general, and *S. aureus* in particular in cases of otitis externa in this study was in agreement with many studies in different regions. Karakaya et al. [16] found that *Staphylococcus* was the most prevalent genus (33.3%) among 75 isolates from cats with otitis externa in Türkiye, with *S. felis* (8%) and *S. epidermidis* (8%) being the most common species. Ahmed and Hasan [15] found that 56.3% of Egyptian cats with otitis externa had staphylococci, with *S. aureus* being the most common. Our research (71.4%) had a higher *S. aureus* prevalence than several regional studies. Hiblu et al. [10] found that 22.7% *S. aureus* isolates from cats with otitis externa in Libya and 31.8% CoNS. Incidence rate of *S. aureus* was found in 44.4% of Iraqi samples and CoNS in 33.3% [13]. Geographic changes in bacterial populations, sampling methodologies, or host characteristics may explain this difference. The 23.8% CoNS isolation rate in this study matches regional reports. Dégi et al [8] found 25.9% of Romanian cat otitis externa isolates were CoNS, and Hiblu et al. [10] found a percentage of 31.8% in Libya. According to Núñez et al. [24], the most prevalent CoNS isolates from healthy cats in Chile were *S. felis* (38.8%), *S. epidermidis* (22.2%), and *S. simulans* (16.6%), with 22.2% of strains resistant to several antibiotics. Our findings and others indicate that CoNS are important opportunistic infections in cats' otitis externa [8, 24]. *P. aeruginosa* was found in 4.8% of isolates in this study, while it was found in 13.6% in Libya [10], 18.5% in Romania [8], and 6.7%-15.6% in Iraq [15, 16]. Our study found a reduced prevalence, possibly due to environmental exposure, cleanliness, or infection chronicity. Mixed infections with *S. aureus* and *P. aeruginosa* were found in 5% of our cases, similar to the reported 7.9% [16]. Many otitis externa cases are polymicrobial, as Dégi et al. [8] found mixed infections in 40% of *S. aureus*-positive samples.

The wide spread resistant to penicillin and ampicillin in this study suggests a widespread β -lactamase production or *mecA* genes that give methicillin resistance. This finding was in agreement with other studies in many countries [14, 39, 40], demonstrating the global nature of β -lactam resistance in cats' staphylococci. Since cefoxitin resistance is a proxy marker for methicillin resistance, the high rates (90% in *S. aureus* and 70% in CoNS) are notable. Miszczak et al. [23] found methicillin-

resistant *Staphylococcus* species in healthy and sick pets, while Elmoslemany et al. [11] found it in Saudi Arabian cats. According to Núñez et al. [24], CoNS strains from the external ear canal of healthy cats in Chile were resistant to oxacillin, a marker for methicillin resistance. The elevated occurrence of cefoxitin resistance in this study may be attributed to geographical variations in the use of antibiotics or the clinical characteristics of the isolates (originating from infected ears instead of colonization). Methicillin-resistant staphylococcal strains exhibiting significant cefoxitin resistance raise concerns from both a treatment and zoonotic perspective. The percentages of resistance to erythromycin (86.7% in *S. aureus* and 90% in CoNS) and sulfamethoxazole/trimethoprim (86.7% and 80% in CoNS) were both alarming. Similar bacterial resistance were documented by [8, 10, 14, 16] in other regions. The widespread use of macrolides and sulfonamides in veterinary treatment may have selected for resistant strains through antimicrobial pressure. Enrofloxacin resistance reached 93.3% in *S. aureus* and 100% in CoNS isolates in this study. Both isolate groups were 83.3% to 100% ciprofloxacin and norfloxacin resistant. Fluoroquinolone resistance rates in *Staphylococcus* spp. are substantial, as documented by Araújo et al. [41] in isolates from companion animals in Portugal. Elnageh et al. [39] also found high ciprofloxacin and enrofloxacin resistance in cat and dog staphylococci. Fluoroquinolone resistance in staphylococci from pets of MRSA-infected cases was also noted by Ferradas et al. [19]. The widespread practical use of enrofloxacin to treat cats' diseases may explain its near-complete resistance. It appears that neither penicillin nor ampicillin are effective against *S. aureus* infections in cats because all of the isolates tested were resistant to these antibiotics. CoNS isolated from cats were resistant to penicillin, ampicillin, ciprofloxacin, norfloxacin, enrofloxacin, and spectinomycin. Some antimicrobial drugs have modest resistance rates, suggesting they may remain useful treatment choices. Many antibiotics had significant resistance rates. Amoxicillin/clavulanic acid was the least resistant at 13.3% in *S. aureus* and 10% in CoNS isolates, while ceftiofur was 23.3% and 30%. Martins et al. [12] found that amoxicillin/clavulanic acid and cephalosporins are efficient against causing bacteria of small animal otitis bacteria. Amoxicillin/clavulanic acid also worked well against staphylococci from Turkish cats with otitis externa [16]. Amoxicillin/clavulanic acid treatment may still be effective for CoNS strains from healthy cats [24]. These findings emphasize the significance of susceptibility testing to guide treatment decisions, as these antibiotics may be used empirically. Fosfomycin resistance was moderate as it was 43.3% and 40% in *S. aureus* and CoNS respectively.

Overall, 23.3% of *S. aureus* and 30% of CoNS isolates were cephalixin-resistant. These findings support Martins et al. [12], who reported that cephalixin and fosfomycin may work against some staphylococcal isolates from otitis cases. These antibiotics may provide an alternate therapeutic option after susceptibility is established, due to their low resistance rates. *S. aureus* and CoNS had 66.7% and 40% gentamicin and kanamycin resistance, respectively. A total of 60% of *S. aureus* and 70% of CoNS isolates were neomycin-resistant. These findings align with Rana et al. [14] research on staphylococci resistance to aminoglycosides in cats and Araújo et al. [41] report on prevalent but not universal resistance in companion animal staphylococci. Sips et al. [21] found MDR *S. felis* with aminoglycoside resistance, suggesting zoonotic transmission.

One of the most worrying outcomes of this study was that 100% of *S. aureus* and CoNS isolates were MDR. Clinical isolates from cats with otitis externa are universally MDR, surpassing rates reported in earlier research [11, 14, 41]. The clinical isolates from infected ears rather than colonization and geographical differences in antibiotic treatment may explain the current study's universal MDR. The majority of *S. aureus* isolates (46.7%) were resistant to nine drug classes, followed by 23.3% and 16.7% that were resistant to eight and seven drug classes, respectively. A total of 50% of CoNS isolates were resistant to seven classes and 40% to nine. These multidrug resistance profiles are among the highest reported, compared to other studies [12, 40]. Multidrug resistance may be due to frequent and often empirical antibiotic usage in veterinary medicine, especially for recurrent otitis externa. CoNS isolates are generally disregarded as pathogens but can store antibiotic resistance genes, hence their high MDR rate is alarming. Núñez et al. [24] discovered AMR primarily to oxacillin in 55.5% of CoNS strains isolated from healthy cats, and 22.2% to several antibiotics. *S. felis*, a CoNS species commonly isolated from cats, has been identified as a pathogen in human dermal injuries, prompting apprehensions regarding zoonotic spread. Sips et al. [21] demonstrated the transmission of *S. felis* from cats to humans, underscoring the zoonotic risk posed by resistant staphylococci and the necessity for integrated One Health AMR monitoring and management. Ferradas et al. [19] observed that pets may carry antimicrobial-resistant *Staphylococcus* species, particularly in MRSA-affected households. Rana et al. [14] demonstrates that multidrug-resistant staphylococci in pets pose significant therapeutic challenges and emphasizes the importance of investigating their virulence and resistance profiles. Risk factors such as past antibiotic therapy and hospitalization may be responsible for the increased clinical frequencies of multidrug-resistant staphylococci in cats [11]. Araújo et al. [41] highlighted the significance of observing AMR patterns in companion animals to inform treatment decisions and avoid resistance. Methicillin-resistant *Staphylococcus* spp. were identified in both healthy and diseased pets, indicating that asymptomatic animals might carry resistant strains [23].

All *Staphylococcus* species isolated from Libyan cats and dogs were resistant to at least two antibiotics [39]. Libyan cats' otitis externa bacteria were resistant to conventional antibiotics, stressing the need for antimicrobial management [10]. Cats' otitis externa staphylococci were resistant to penicillin, ampicillin, and other β -lactams, with frequent MDR [8]. Karakaya et al. [16] found that 44% and 100% of the staphylococci and streptococci isolates from cats' otitis externa in Turkey are MDR, with high penicillin, erythromycin, and tetracycline resistance. According to Chandrika et al. [42], *Staphylococcus* causes 68% of cat otitis externa and it is most resistant to amoxicillin. They advised antibiotic sensitivity testing before treatment to ensure proper treatment. Rodrigues et al. [43] found that 28.1% of *Staphylococcus* isolates from companion animals with otitis were methicillin-resistant, with significant MDR rates. Cobo-Angel et al. [44] observed that most antibiotics become less effective in *Staphylococcus* isolates from cats in the northeastern US, with 19% MDR, and 46% MDR for *S. pseudintermedius*. To maintain antibiotic efficacy in veterinary practice, they highlighted selective antibiotic usage and stewardship.

This investigation found extremely high MARI values in staphylococcal isolates from Northern Palestinian cats with otitis externa. *S. aureus* isolates had a mean MARI of 0.706 ± 0.128

(range: 0.45-0.95), while CoNS isolates had 0.674 ± 0.088 (range: 0.59-0.82). Elmoslemany et al. [11] found mean MARI values of 0.22 ranging from 0.09 to 0.64 in Saudi Arabian cat staphylococci isolated from both diseased and healthy cats. Most of the isolates (58.7%) showed a MARI of less than 0.20. However, a MARI over 0.67 in this study, could indicate extraordinarily extensive antimicrobial exposure in this cats' community, that may lead to MDR. The majority of our *S. aureus* isolates (20.0%) were resistant to 17 of 22 antimicrobial drugs. However, Elmoslemany et al. [11] found resistance to 1-7 of antimicrobials. The aggregate resistance scores for *S. aureus* and CoNS were 466 and 148, this showed that our isolates had great resistance. MARI values of *S. aureus* and CoNS isolates were not significantly different ($U = 178.0$, $p = 0.292$), Elmoslemany et al. [11] found that the MAR index of *S. aureus* isolated from diseased and healthy cats was significantly different ($p = 0.003$), but no difference was found for *S. pseudintermedius*, *S. felis*, *S. capitis*, and *S. saprophyticus* recovered from healthy and diseased cats. This indicates that both staphylococcal categories within this ecological niche encounter analogous antimicrobial selection pressures, and that resistance factors are probably disseminated across species barriers through horizontal gene transfer methods such as plasmid-mediated transmission and transposon mobilization. Non-prescription veterinary antimicrobials may facilitate indiscriminate usage and hasten the emergence of multidrug-resistant staphylococci in areas with insufficient regulatory oversight. The absence of veterinary diagnostic services in Northern Palestine may result in empirical antimicrobial treatment without culture and susceptibility assessment, thus increasing the risk factors for antimicrobial resistance. Subtherapeutic doses or not completing the treatment sessions may promote the emergence of resistant bacterial subpopulations influenced by socioeconomic variables, including financial limitations. The dynamics of community-associated MRSA transmission suggest that intimate human-animal interactions may facilitate reciprocal exchange of resistant staphylococci. MARI values of more than 0.5 were recorded for 93.3% of *S. aureus* isolates and 100% of CoNS isolates. This demonstrates that most veterinary antimicrobials may be ineffective for treating staphylococcal infections in cats. A major problem is the potential transmission of multidrug resistant strains of staphylococci to pet owners and veterinarians [45]. The detection of an isolate with MARI 0.95 (resistant to 21 antibiotics) indicates that even the last-line defensive drugs against severe infections are losing their efficacy, and there is an urgent need for surveillance systems for tracking resistance trends and revising antimicrobial strategies.

From a One Health perspective, responsible antimicrobial use in veterinary medicine is important to minimize selection pressure for AMR and to reduce the potential transmission of resistant organisms between animals, humans and the environment. Therefore, the findings of this study contribute to a broader One Health framework by providing baseline evidence on bacterial infections and AMR in companion animals in Northern Palestine, and highlighting the need for integrated surveillance, antimicrobial stewardship and collaboration between the veterinary and human health sectors. Further multi-center studies with larger and more diverse cat populations over longer periods of time are recommended to understand better the epidemiology and the antimicrobial resistance profiles of cat otitis externa in this region.

The results of this research are subject to several constraints which must be considered when interpreting the findings. The sample population was initially somehow limited (40 cats) and all cases were from one veterinary clinic in Jenin. Therefore, the results of the study may not be a true reflection of the larger cat population of Northern Palestine, and ought not to be extrapolated to the whole area. Uneven distribution of breeds and the small number of certain isolates of bacteria, especially *P. aeruginosa* (n = 2), limited the statistical power to detect associations between sex, breed and bacterial isolation. Secondly, light microscopy was used to recognize *O. cynotis* by observing mites, eggs, or fecal matter of this parasite, lacking molecular validation. While this technique based on morphological diagnosis, it is important to recognize the lack of molecular validation as a constraint. Third, bacterial isolates were identified via standard microbiological techniques, lacking molecular validation. Specifically, CoNS isolates were not classified to the species level, which constrains future analysis of the bacterial composition and resistance traits of this group. Fourth, clinical data was sourced from standard veterinary records filled out by various attending veterinarians, and certain minor clinical indicators were documented inconsistently, potentially leading to insufficient reporting of clinical symptoms. Ultimately, the evaluation of antimicrobial susceptibility was conducted using phenotypic disk-diffusion methods, and the examination of antimicrobial resistance genes or particular molecular resistance mechanisms was not performed. Consequently, the resistance phenotypes identified in this research could not be associated with particular genetic factors. Additional multicenter research encompassing larger and more varied cat cohorts, uniform clinical data gathering, molecular validation of parasite and bacterial identification, and molecular profiling of antimicrobial resistance is necessary to yield more representative epidemiological and microbiological insights regarding feline otitis externa in Palestine.

Conclusion

Most of the cases of otitis externa examined (80%) had *O. cynotis*. *S. aureus* and CoNS were predominant in the examined cases. The 100% finding of MDR with near-complete resistance to fluoroquinolones and standard β -lactams is alarming. One of the largest resistance burdens reported in the literature is the universal multidrug resistance detected among all *Staphylococcus* isolates. The mean MARI values indicate high-risk situations. There are severely restricts therapeutic options available when bacteria develop complete resistance to many antimicrobial drugs. These results highlight the critical need to launch local and national antimicrobial stewardship programs, culture-guided antibiotic therapy, and routine parasitological screenings without delay. A coordinated One Health surveillance system involving the veterinary, human, and environmental health sectors is urgently needed to address the growing problem of AMR in companion animals. This is particularly important in light of the high MARI values and zoonotic potential of methicillin-resistant staphylococci.

Disclosure Statement

- **Ethics approval and consent to participate:** The study was reviewed and approved by the Institutional Review Board (IRB) for Animal Research at An-Najah National University (Approval Ref. No. [2026-08-04 IACUC]).
- **Consent for publication:** Not applicable

- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript. Any other information can be supplied after contacting the corresponding authors.

- **Author's contribution:** Study conception and design: SA, GA, MA and GO. Sample collection and preparation: MA and OA. Experimental work and data analysis: SA, GA, MA, GO and OA. Draft manuscript preparation: GA, SA and MA. Preparation of the final manuscript for submission: GA and SA. All authors reviewed the results and approved the final version of the manuscript.

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