Journal of Zoonotic Diseases
https://jzd.tabrizu.ac.ir/
Published by the University of Tabriz
Online ISSN: 2717-2910

Bacterial isolation from domestic cats: Detection of biofilm formation and the *ica* gene

Amna Yarub Hameed[1*], Abeer Mahmood Faraj[2], Alaa Saeed Sheet[3], Shahad Ismael Saeed[4]

[1]Department of Medical Laboratory Technologies, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq
[2]Department of Pharmacy Techniques, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq
[3]Department of Radiology Techniques, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq
[4]Department of Optometry Techniques, Mosul Medical Technical Institute, Northern Technical University, Mosul, Iraq

Article type:
Original Article

Keywords:
Domestic cats
Biofilm
Antibiotic resistance
Zoonosis
VITEK 2

Article history:
Received:
June 2, 2026
Revised:
July 18, 2026
Accepted:
August 23, 2026
Available online:
August 24, 2026

Abstract

This study was intended to isolate and to identify bacterial species in domestic cats, to determine their capability to form biofilms, and identify the presence of *ica* genes (*icaA* and *icaB*). There were a total of 22 samples collected at three anatomical locations namely; the oral cavity, skin and forepaws. The isolation of bacteria was carried out in the conventional culture media, which was then subjected to phenotypic and biochemical identification and VITEK 2 automated identification system to identify the species accurately. The findings indicated that the presence of bacteria growth in all samples of the skin and paws (100%) and the presence of bacteria in oral samples (54.54%). Various kinds of bacterial species were obtained. *Staphylococcus sciuri* is the most common (30%), followed by *Staphylococcus xylosus* (15%). Antibiotic susceptibility test showed that a majority of isolates are susceptible to a wide spectrum of antibiotics though some patterns of multidrug resistance were also observed. Congo Red Agar (CRA) test proved that 70 percent of the isolates were able to form biofilm. At the molecular level, *icaA* gene was not found in any of the isolates and *icaB* gene was found in 77.7% of the samples tested. These results show that domestic cats can be a source of bacteria that are biofilm forming and having antibiotic resistance and this can pose a threat of the spread of the disease to humans as a zoonotic disease carrier.

Introduction

Cats are some of the most popular companion animals in the world. They are kept as domestic animals and as stray animals roaming freely in the urban and rural setting. In addition to the value of pets, cats have long been appreciated in

***Corresponding author:** *amina.yarub@ntu.edu.iq*
https://doi.org/10.22034/jzd.2026.22106
https://jzd.tabrizu.ac.ir/article_22106.html
Cite this article: Hameed A.Y., Alsabaawi A.M.F., Sheet A.S., Saeed S.I., Bacterial isolation from domestic cats: Detection of biofilm formation and the *ica* gene. Journal of Zoonotic Diseases, 2026, 10 (3): 1338- 1350.

agricultural societies due to their capacity to regulate rodent infestations, which was enabled by their sensitive sense of smell and chemical communication (1). Over the past decades, the number of pets has grown significantly because of changes in demographics and the changing modern lifestyle. Among the most significant factors, one can single out the appearance of one-person households, urbanization, and the ageing population that seeks companionship. These social tendencies are also accompanied by the increased focus on the emotional well-being, which has also contributed to the growth of the demand in the companion animals as the means of psychological support (2, 3). Although the psychological and physical health benefits of owning pets are well-documented, the close and protracted nature of human being and companion animals presents the possibilities of health risks especially those that are related to zoonotic diseases. Research continuously shows that cats and dogs (the most popular pets kept) can be a host to a wide range of potentially pathogenic microorganisms, including multiple antibiotic-resistant bacteria. Animals can infect people during direct physical contact, use an ectoparasite, or aerosolize contaminated particles, which increases the risk of immunocompromised people, infants, and older people (4). It has been widely documented that domestic cats have the ability to be a reservoir of a number of major zoonotic pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), other *Staphylococcus species* (*S. pseudintermedius*, *S. intermedius*, and *S. sciuri*) and other species of fungi, parasites, and viruses (5).

Most bacteria have several virulence factors that enable them to escape the host immune system and are resistant to antimicrobial agents. The most clinically relevant of them is the capacity to develop biofilms - structured communities of microorganisms that are enclosed within a self-produced exosystem of polymeric macromolecules that adheres to the biotic and abiotic surfaces. This type of biofilm growth gives the bacteria a high level of protection against host immunity and a high resistance to antibiotic treatment (6).

The formation of biofilms is often linked to the expression of polysaccharide intercellular adhesin (PIA/PNAG), a polysaccharide made of 1, 6 -N-acetylglucosamine. This polysaccharide is synthesized under the control of ica operon, which codes the major enzymatic apparatus that governs the intercellular adhesion and biofilm structure. Several genera of bacteria-like organisms, such as Staphylococcus, Streptococcus, Salmonella, Pseudomonas and Escherichia coli - have been reported to be able to form biofilms in a wide range of host environments. Significantly, research has demonstrated that cats can be the major source of multidrug-resistant bacterial strains harboring virulence-associated genes, such as icaA and icaB, that are directly connected with biofilm formation (7).

Considering these facts, the current research was aimed at researching the incidence of multidrug-resistant biofilm-producing isolates of domestic cats, and determining the occurrence of the ica gene as a major virulence factor related to the potential of transmitting zoonotic diseases. While cosmetic and human-derived biofilm and *ica*-gene surveys have been previously reported, data on companion-animal isolates from the Middle East, and specifically from Iraq, remain scarce. To our knowledge, this is the first study to combine phenotypic biofilm assays (Congo Red Agar) with molecular screening of *icaA* and *icaB* genes across multiple anatomical sites (oral cavity, skin and forepaws) of domestic cats in the Mosul region, and the first local report of a high prevalence of *icaB* in the near-complete absence of *icaA*, a pattern that has not, to our knowledge, been previously described in feline isolates and that points to an *ica*-independent biofilm pathway warranting further investigation.

Materials and Methods

Sample collection

There were 22 samples taken from domestic cats. The sampling process involved samples of three different anatomical locations to give a complete microbiological description of the host animals:

Oral swabs

Sterile cotton-tipped swabs were inserted into the oral cavity of every cat and swabbed against the mucosal surfaces to get representative oral microbiota samples. The method will allow both resident commensal flora and opportunistic pathogens that exist in the oral cavity to be recovered. Oral swabs are a well-known non-invasive technique of measuring microbial colonization of companion animals.

Skin swabs

Sterile cotton swabs were used to collect surface swabs of the skin of each cat. The swabs were used with equal force on specific parts of the skin to harvest as much as possible. Skin sampling is also of special relevance in companion animals because the skin is in direct contact with environmental contaminants and is a primary colonization site for staphylococci.

Paw imprint sampling

The paw prints were prepared by pressing the front and rear paws of all the cats on the Blood Agar plates. This is the method of imprinting that offers a direct measurement of bacterial contamination of the exterior extremities. Paws are also a highly pertinent sampling area in the detection of possibly zoonotic bacteria since they are regularly in contact with the environment. This is also beneficial as it does not use swabs, and a more accurate capture of the in-situ microbial load is made possible (8).

Primary culture

All samples were plated directly onto Blood Agar (BA) - a rich and non-selective general-purpose media that is capable of supporting the growth of the broadest number of bacterial species including fastidious species. Aerobic incubation of inoculated plates was done at a temperature of 37°C for 24 to 48 hours. After incubation, plates were assessed on the basis of the bacterial proliferation, morphology of the colony, and hemolytic patterns.

Subculture on selective and differential media

Following primary incubation, colonies with discrete morphological features were subcultured in a series of selective and differential culture media to enable species identification and presumptive classification:

Salmonella–Shigella agar

SSA is a very selective media, which is specific to inhibit the development of most of the non-enteric Gram-negative microorganisms and the selective development of *Salmonella* and *Shigella* species. The medium is composed of bile salts and sodium citrate as an inhibitor, lactose and neutral red indicator to distinguish the lactose fermenting and non-fermenting colonies. Salmonella-suspected colonies are black-centered in color because of the production of hydrogen sulfide.

MacConkey agar

MCA is a selective and differential media that is applied in the isolation and differentiation of Gram-negative enteric bacteria in terms of their lactose fermentation ability. MCA contains the bile salts and crystal violet, which inhibits Gram-positive bacteria and the neutral red pH indicator, which allows lactose fermenters (pink/red colonies) and non-fermenters (colorless colonies) to be identified. This is a medium that cannot be done without in the identification of members of the family *Enterobacteriaceae* (2, 9).

Mannitol Salt agar

MSA is a selective media that takes advantage of Sstaphylococci tolerance to high amounts of salt (7.5% NaCl) to block the growth of other most organisms. The mannitol substrate and phenol red indicator allows distinction between mannitol-fermenting organisms including: *Staphylococcus aureus* (that forms yellow colonies with the formation of acid). Non-fermenters such as coagulase-negative staphylococci, which maintain pink to red coloration around their growth. The selective media were incubated at 37 ° C aerobically (24-48 hours) (9).

Bacterial identification

Phenotypic identification

Macroscopic observations of the colonies growing in all the media were tabulated. This involved the evaluation of colony morphology (circular, irregular), size, color, texture (smooth, rough), elevation (flat, convex, umbonate) and margin morphology (entire, undulate, serrate). Blood Agar hemolytic activity was categorized as alpha (α) hemolysis (partial), beta (β) hemolysis (complete, clear zone), or gamma (γ) hemolysis (no change). The first level of bacterial identification is the phenotypic characterization (10)

Microscopic identification

Colonies growing on the different culture media were used to prepare bacterial smears, where the sterile loop was used to touch the individual colonies and deposit the material on clean glass slides. The smears were dried using air and fixed using heat of a Bunsen burner flame. The gram staining was then done as per the standard protocol of Tripathi and Sapra (11) with crystal violet application, iodine mordant of Gram, acetone-alcohol decolorization, and safranin counterstain application. The stained smears were observed under oil immersion (x1000) under the light microscope. The step was used to identify the Gram reaction (positive or negative), cellular morphology (cocci, rods, spirilla), and cellular arrangement (clusters, chains, pairs) which combined with each other give important diagnostic data to classify the species.

Biochemical identification

The identification tests carried out were biochemical identification tests based on the standard procedures outlined in Riedel et al. (12). These experiments are based on the fact that, in some metabolic processes, there is a difference in the ability of bacteria to react:

Blood hemolysis assessment

To assess hemolytic potential of the isolates, Blood Agar was utilized in the assessment of the lysis of erythrocytes. The observed pattern of hemolysis (alpha (partial, greenish discoloration), beta (complete, clear zone), or gamma (no hemolysis)) also gives information regarding the toxin-forming abilities of the bacterium and helps conclude on presumptive species identification (13).

VITEK 2 automated identification system

The VITEK 2 System (BioMerieux) is a diagnostic platform that is fully automation based and a high-throughput system, making use of miniaturized biochemical reaction panels in sealed cards to identify bacterial species and their susceptibility profiles to antibiotics simultaneously. In the case of each isolate, a bacterial suspension of known turbidity (equivalent to 0.5 -0.63 McFarland standard) was made and placed onto the proper VITEK 2 cards (GP card to Gram-positive organisms: GN card to Gram-negative organisms). The system is based on the optical reading technology of fluorescence to follow the dynamics of a biochemical reaction and to compare the results to an extensive internal database of reference profiles of thousands of bacterial species. The results of the identification are given as a confidence percentage of the final species name. All the tests were done at Pharma Specialized Laboratory, Mosul, in strict compliance with the recommended procedures and standardized diagnostic tests of the manufacturer.

Biofilm formation detection

The modified Congo Red Agar (MCRA) method was used to determine the Gram-negative bacteria. The ability to form biofilms was determined with the help of the Modified Congo Red Agar (MCRA) technique, described by Mariana et al (14). This method is a phenotypic technique that identifies slime formation, which is one of the major elements of biofilm structure, through the color of colonies grown on a specially designed agar media. The test principle is rooted on the affinity of Congo Red stain with the extracellular polysaccharide matrix that is produced by biofilm forming strains leading to typical dark brown or black colonies with crystalline texture. The strains that do not produce it are normally pink or red.

DNA extraction

The bacterial isolates were subjected to the standard Boiling Method (heat lysis method) to extract the genomic DNA. To lyse the cells, a bacterial colony from an overnight culture was suspended in 200 µL of sterile distilled water and then incubated at 100 ° C in a boiling water bath. Centrifugation at 12,000 rpm for 5 minutes was then performed to remove cellular debris. The supernatant obtained having released DNA was stored at -20 o C until it was used as a template in PCR amplification. The boiling technique is an easy, affordable, and fast method instead of commercially offered extraction kits, and it has been considerably tested to be used in PCR-based molecular diagnostics.

PCR Detection of Biofilm-Associated ica Genes

The detection of biofilm-associated *ica* genes was performed by PCR. Conventional PCR was used to detect the presence of biofilm-associated genes *icaA* and *icaB*. The *ica* operon codes the most important enzymatic machinery of polysaccharide intercellular adhesin (PIA), an indispensable structural element of staphylococcal biofilms. The presence of *icaA* and *icaB* in particular makes it possible to evaluate the ability to develop biofilm under PIA dependence.

Primer sequences

The specific primer pairs (5′ to 3′) described by Mirzaee et al. (15) was used for amplification of the target genes; icaA gene, Forward (F): 5′-ACACTTGCTGGCGCAGTCAA-3′ and Reverse (R): 5′-TCTGGAACCAACATCCAACA-3′; and icaB gene, Forward (F): 5′-AGAATCGTGAAGTATAGAAAATT-3′ and Reverse (R): 5′-TCTAATCTTTTTCATGGAATCCGT-3′. Both target genes were optimized for specific and efficient amplification with PCR, using a thermal cycler. The PCR cycling program included 30 cycles of denaturation (95°C, 30 s) and annealing (50°C, 30 s) followed by an initial denaturation step (95°C, 5 min).

The first step of denaturation is used to ensure that the template of the double-stranded DNA is fully separated. The 30-cycle amplification procedure that consists of repeated denaturation, annealing and extension cycles leads to exponential amplification of the target sequence. The last extension process provides full synthesis of the PCR products.

Agarose gel electrophoresis

The PCR products were subjected to horizontal agarose gel electrophoresis on 1.5% agarose gels stained in 1x TAE buffer. The concentration of 1.5% was selected to give the best resolution to the expected amplicon sizes (about 188 bp of *icaA* and 900 bp of *icaB*). Ethidium bromide (EtBr) was used to stain gels, and is a fluorescent intercalating agent binding to double-stranded DNA. After the electrophoresis, a UV transilluminator was used to view the gels at 80-100 V over a period of 45-60 minutes. Gels were run with a DNA molecular weight ladder to enable proper sizing of amplified bands. The presence of the bands at the desired molecular weights proved that positive results were obtained.

Statistical analysis

Categorical data (bacterial growth positivity by anatomical site, biofilm status, and antibiotic susceptibility category) were expressed as frequencies and percentages. Differences in bacterial growth-positivity rates between the three sampling sites (oral cavity, skin and forepaws) were compared using the chi-square test for the overall comparison and Fisher's exact test for pairwise 2×2 comparisons, given that some cell counts were small. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism (version 9) / IBM SPSS Statistics (version 26).

Results

Characteristics of the sampled cats

The findings indicated that Persian and Himalayan breeds were the most common of the sampled cats with each occupying the largest proportions of the overall sample (38.36% and 36.36% respectively).

The lower and equal proportions (9.09% each) were recorded for Scottish, British, and Chinchilla breeds, Table 1.

Table 1. Characteristics of the Sampled Domestic Cats

Variable	Category	Number (n=22)	Percentage (%)
Breed	Persian	8	36.36%
Vaccination Status	Vaccinated	18	81.81%
Type of Food	Dry food only	14	63.63%

Vaccination status

The proportion of sampled cats that were vaccinated was large (81.81%, n=18), with 18.18 (n=4) not vaccinated. The percentage of vaccination coverage is a respectable indicator of the owner health awareness, but the portion of unvaccinated individuals remains an issue that could be considered the potential public health threat.

Dietary habits

Food records indicated that 63.63% of the cats were fed on commercial dry food only and the remaining 36.36% on a mixed diet of commercial dry food topped with homemade meals. Commercial diets have been linked to better oral health and standard nutritional balance, whereas home-cooked diets bring diversity to nutrient balance.

Age distribution

The large proportion of cats (90.9%) was in the 1-5-year range, which is the young adulthood of cats; a stage of physiological stability and optimal immune function. Only 9.09% of cats were younger than one year.

Bacterial isolation by sampling site

Growth positivity by anatomical site

Microbiological analysis showed that there were significant differences in the rates of bacterial isolation in the three sampled sites. There was also 100% positivity of bacterial growth on skin and forepaw samples, which confirmed that the body surface and extremities of domestic cats sustain a large continuous bacterial load that is made up of commensal and potentially pathogenic species. On the contrary, oral samples had a significantly lower positivity rate of 54.54%, and four out of five of the oral samples were found to have no bacterial growth under the conditions used in the culture.

This difference was statistically significant: overall comparison across the three sites by chi-square test ($\chi^2 = 23.57$, df = 2, $p < 0.0001$), and pairwise Fisher's exact tests confirmed that both skin and forepaw positivity rates were significantly higher than the oral cavity rate ($p = 0.0005$ for each comparison), while skin and forepaw rates did not differ from one another ($p = 1.000$).

Bacterial species identified

Forty bacterial isolates were obtained from all the sampling sites. A combination of phenotypic, biochemical, and VITEK 2 automated techniques was used to identify thirteen different bacterial species. The predominant species were *Staphylococcus sciuri* (30%), followed by *S. xylosus* (15%). The remaining eleven species were each isolated at a comparable, lower frequency (5% each), together accounting for the rest of the 40 isolates (Table 2).

Table 2: Bacterial Species Isolated from Domestic Cats and Their Relative Frequencies

Bacterial Species	Number of Isolates	Percentage (%)
Staphylococcus sciuri	12	30%
Staphylococcus xylosus	6	15%
Staphylococcus warneri	2	5%
Staphylococcus lentus	2	5%
Staphylococcus hominis	2	5%
Staphylococcus vitulinus	2	5%
Staphylococcus felis	2	5%
Enterococcus cecorum	2	5%
Enterococcus gallinarum	2	5%
Kocuria rhizophila	2	5%
Kocuria rosea	2	5%
Rothia kristinae	2	5%
Leuconostoc mesenteroides subsp. cremoris	2	5%
TOTAL	40	100%

Antibiotic susceptibility profiles

Antibiotic susceptibility testing was performed using the VITEK 2 automated system, which provided minimum inhibitory concentration (MIC) values and interpretive categories (Sensitive [S], Intermediate [I], or Resistant [R] for a broad panel of clinically relevant antibiotics. The results for key species are summarized in Table 3.

The general results indicated that most of the isolates were vulnerable to a large number of antibiotics examined. Nonetheless, certain patterns of resistance were discovered, especially with coagulase-negative staphylococci (CoNS). It was particularly noted that Benzylpenicillin and Fusidic Acid resistance were also noted in several species of *Staphylococcus*, and *E. gallinarum* was intrinsically resistant to Vancomycin at low levels. *S. hominis* was multidrug resistant and the profile showed resistance to Oxacillin, Benzylpenicillin and Fusidic Acid, and positive result of a Cefoxitin screen result indicative of methicillin resistance.

Table 3. Antibiotic Susceptibility Profile of Bacterial Isolates (S=Sensitive, I=Intermediate, R=Resistant). Data are shown for the three most frequently isolated species (n = 12, 6 and 2 isolates for S. sciuri, S. xylosus and S. warneri, respectively, out of 40 total isolates); a dash (—) indicates that the corresponding antibiotic was not tested for that isolate.

Species	Benzylpen.	Oxacillin	Fusidic Acid	Linezolid	Vancomycin	Teicoplanin	Rifampin	Ery.	Levoflox.
S. sciuri	R	S	R	S	S	S	S	S	S
S. xylosus	R	S	R	S	S	S	S	S	S
S. warneri	R	S	S	S	S	S	S	I	S

Biofilm detection by Congo Red agar

To assess the biofilm forming ability of all 40 bacterial isolates, the modified congo red agar (MCRA) test was used. The results were categorized as positive (black, dry, crystalline

colonies), weak (dark or brownish colonies) or negative (pink/red colonies).

The outcome was that 28 (70%) isolates were strongly positive to slime production that is to say a strong biofilm-forming ability. Four isolates (10% percent) were weakly reactive indicating low or moderate biofilm potential. Eight (20%) isolates were negative. The four weak positive isolates were found to be

S. xylosus. Some of the isolates that were fully negative included two strains of *Kocuria rhizophila*, *Rothia kristinae*, *E. cecorum*, and *S. sciuri*. These data prove that most of the isolates in domestic cats have an active biofilm-forming ability, which is one of the main virulence aspects of enhanced resistance to antibiotics and environmental stress factors.

Molecular detection of biofilm-associated ica genes

The molecular analysis of the *icaA* and *icaB* genes was performed on nine bacterial isolates. The samples were selected according to the representation of the species, the abundance of the colony, and the purity of the culture. PCR amplification of the target genes was followed by agarose gel electrophoresis.

icaA gene

All nine isolates that were tested failed to yield the icaA gene. The overall lack of *icaA*-specific amplification bands in all of the samples implies that this gene is not present in the genome of these strains, or it is present at a lower concentration than the limit of the assay.

icaB gene

Unlike *icaA*, *icaB* was found in 7 out of 9 tested isolates (77.7%), and 2 of the tested isolates (22.2%), did not give any results. Agarose gel was used to determine whether the amplicon was of the expected size of about 900 base pairs through the comparison with the DNA molecular weight ladder, Figure 1.

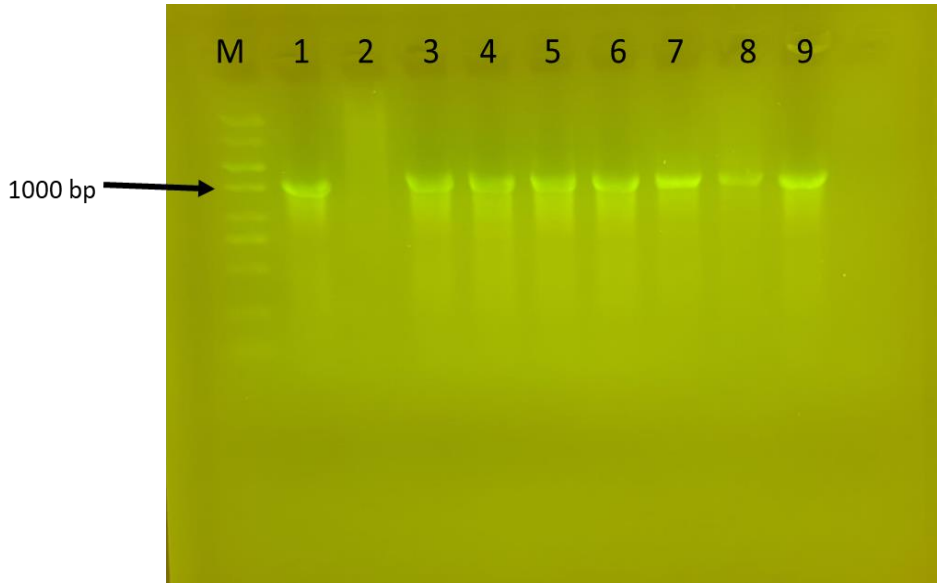

Fig. 1. Agarose gel electrophoresis results showing the presence of *icaB* gene amplicon (~900 bp) in positive isolates**.** Lane M: 100-bp DNA molecular weight ladder; lanes 1-9 correspond to the nine bacterial isolates selected for molecular screening (see Section 4.5); a positive band of approximately 900 bp indicates amplification of the *icaB* amplicon; a no-template negative control was included in a separate lane and showed no amplification.

Discussion

The sampled population (81.8% vaccinated, 63.6% fed commercial dry food, 90.9% aged 1-5 years, and 72% of Persian/Himalayan breed) is broadly comparable to cat populations described in European and Israeli surveys, and this similarity supports the relevance of the microbiological findings beyond the local setting. The unvaccinated minority (18.2%) and the predominance of adult, physiologically mature animals are noteworthy mainly because both factors could independently influence bacterial colonization patterns and should be considered as potential confounders, rather than as findings in their own right, when interpreting the bacteriological results that follow (8, 16, 17).

The 100% isolation rate of skin and paw surfaces confirms that the skin and paw areas are always colonized by a heterogeneous bacterial community in domestic cats. This aligns with the results of Phumthanakorn et al., who reported that external body surfaces of companion animals are a focal point of unceasing environmental contact, which makes them extremely vulnerable to microbial colonization by commensal and transient organisms (18).

The relatively low rate of oral isolation (54.54%) could be explained by a number of factors such as the antimicrobial effects of feline saliva (lysozyme and peroxidase systems) that prevent bacterial growth, competition with the resident oral flora, and the alkalinity of the feline oral environment. These observations are similar to those reported by Stepanovic et al, which indicated skin isolation rates of more than 95% and oral positivity of about 60% (18).

S. sciuri occurred most often (30%), which is expected due to its established status as an endemic component of the cutaneous microbiota of domestic and wild animals. The prevalence of 30% in the present study concurs with the information given by Shida. Significantly, *S. sciuri* carries a chromosomal counterpart of the *mecA* gene, which codes penicillin-binding protein 2a (PBP2a) and is linked to methicillin resistance. This renders S. *sciuri* as a possible source of genetic reservoir of resistance genes transmission to more virulent species including *S. aureus* (19).

The second most prevalent isolate was *S. xylosus* (15%), which was very similar to the prevalence obtained by Shida (16.4%), but lower than the prevalence reported by Razali et al. (34.28%). The difference between studies could be due to the age of the hosts, the diets, geographical location, and the culture methodologies which were used in each case (20).

Minor staphylococcal species such as *S. warneri, S. lentus, S. vitulinus, S. felis,* and *S. hominis* were individually recovered at 5% and are typical occasional commensal residents of the feline skin. The significantly lower incidence of *S. felis* (12.7%) than the 26.1% reported by Moon et al. could be due to site-specific differences in sampling, geographic differences, or the health condition of the population sampled (11).

E. gallinarum (5%) was found to be a part of the feline microbiota in accordance with the established relationship with gastrointestinal flora and fecal contamination. The presence in the external surfaces could be attributed to environmental pollution by the habitat of the cats. Isolation of *E. cecorum* is characteristic because it is mostly related with poultry and is not a typical occurrence among domestic cat. It can be present as a result of common exposure to the environment with birds or polluted environmental surfaces (21).

Recovery of *Kocuria rhizophila*, *Kocuria rosea*, *Rothia kristinae*, and *Leuconostoc mesenteroides* subsp. cremoris is also a significant observation because these are not considered to be part of the established feline microbiome. Such organisms are mostly related to a source in the environment, such as soil, water, and human skin, or a particular animal host. They could be in the feline specimens

due to contamination of the environment, human-to-animal microbial interchange by direct handling, contact with contaminated surfaces, sensitive VITEK 2 detection technology or sharing of living space with other animal species. It is most likely that these organisms are temporary environmental polluters and not permanent members of the feline microbiota. However, their resolution reveals the significance of investigating human-animal-environment microbial exchange processes, and it deserves further investigation (12, 22, 23).

Most of the bacterial isolates obtained in clinically healthy cats were susceptible to most of the antibiotics tested, which in general is in agreement with the data of Elnageh et al, who found 100 percent susceptibility of *Staphylococcus* isolates to Nitrofurantoin, Tetracycline, Teicoplanin, Rifampin, and Linezolid. Importantly, though, in the current study, there were prevalent cases of resistance to Benzylpenicillin and Fusidic Acid in several Staphylococcus species, a tendency that aligns with the earlier reports that *Staphylococcus* have *fusC* gene-mediated resistance to Fusidic Acid. The only isolate not resistant to Fusidic Acid was *S. warneri* (14, 23).

All isolates of *S. sciuri* were susceptible to Oxacillin and this is in line with literature. Staphylococcus hominis was multidrug-resistant (MDR), resistant to Oxacillin, Benzylpenicillin, and Fusidic Acid, and positive Cefoxitin screen - results suggest the presence of methicillin resistance mediated by the *mecA* gene (24).

E. gallinarum had intrinsic low-level Vancomycin resistance, a characteristic that is well-characterized in this species and is due to the *vanC* gene that is encoded in its chromosome. *VanC* gene expresses a D-Ala-D-Ser ligase that changes the Vancomycin target D-Ala-D-Ala to D-Ala-D-Ser which lowers the antibiotic binding affinity significantly. This resistance mechanism is not similar to the acquired *vanA* and *vanB* resistance of high-level Vancomycin-resistant enterococci and has been identified as a species-defining characteristic of *E. gallinarum* (25).

K. rosea was found to be of a multidrug-resistant phenotype and showed resistance to Amoxicillin, trimethoprim, as well as Clindamycin. Although Vancomycin tends to be effective against *Kocuria* species, some strains have been reported to develop low-level Vancomycin resistance. The findings support the thesis that animals with a clinical condition could be MDR carriers in a silent reservoir, and they could spread to human contacts and the environment (4).

Results of MCRA indicated that the biofilm-forming capacity of the isolates was highly heterogeneous, and it is indicative of genetic and regulatory variation between bacterial species and strains. The existence of variability in the production of the slime layer and the quorum sensing-associated behaviors which are essential intermediates of the biofilm formation were also evident in the data. These qualitative variations confirm a model where biofilm formation is not an inherently determined species characteristic but is actively controlled by environmental signals and strain-specific genetic variations (26).

The obtained findings agree with the data of Asante et al., who reported a high level of biofilm-forming capacity variation between *S. xylosus* and *S. hominis* isolates. In a similar manner, Vasileiou et al. demonstrated that the phenotype of biofilm formation can be heterogeneous in the populations of *S. sciuri*, which confirms the opinion that this characteristic is strain- and condition-specific (27).

The observation that the *K. rosea* and *K. kristinae* showed biofilm-forming ability but the *K. rhizophila* was always negative is in line with the results provided by Al-Khamesi et al. (26). Such inter-species variations are probably the manifestation of cell wall composition variation and the difference in the expression of cellular regulatory pathways that regulate the production of extracellular polysaccharide (EPS). This overall negativity of *K. rhizophila* can either imply that the

bacteria do not have any biofilm-promoting genes or that post-transcriptional silencing occurs in the conditions it has been examined.

The molecular investigation showed that the *icaA* and *icaB* genes dissociated differently: *icaA* was not found in the nine isolates, but *icaB* was observed in 77.7% of isolates tested. This result is particularly different compared to the earlier reports. Indicatively, Deniz et al. (28) identified *icaA* in 99.17% of the coagulase-negative staphylococci, whereas only 28.92 percent of them contained *icaB*. On the same note, Armoon et al. (17) have found *icaA* to be prevalent in 72% of *S. aureus* isolates. The lack of *icaA* in the entire study can also be due to the difference in the origin of host (animal vs. human), geographic variation, or evolutionary divergence in the structure of *ica* operon across cat-associated strains.

It is noteworthy how high the prevalence of *icaB* was identified to be in the current study (77.7%). IcaB protein is a deacetylase that is known to alter PIA by eliminating acetyl groups that make bacterial adherence and biofilm stability more stable. The study of Bi et al. (29) showed that both the *IcaA* and *IcaB* collaborate to catalyze the production of PIA and proved that *icaB* on its own might have enough functional capacity to promote the formation of biofilms even without the presence of *icaA*.

The *icaB* deficiency in *S. felis* and *Leuconostoc* spp. isolates is consistent with the existing literature that supports that these species might have alternative biofilm formation pathways, which are *ica*-independent. Such mechanisms can include surface-associated proteins (e.g., biofilm-associated protein [Bap]) or extracellular DNA (eDNA), or teichoic acids, which can also act as structural scaffolds of biofilm matrix assembly and do not involve the production of PIA. This observation is consistent with other researchers who have shown that some MRSA isolates had a full biofilm-forming ability but had no *ica* genes, which further supports the notion of *ica*-independent biofilm formation (22).

The combination of the molecular data of the current study leads to the following conclusions: (1) the existence of *icaB* correlates with the increased possibility of biofilm formation; (2) the lack of *icaA* does not exclude biofilm formation; and (3) the formation of biofilms in the bacterium of domestic cats is a complex phenomenon including *ica*-dependent and *ica*-independent genetic processes. Further research ought to examine the entire *ica* operon (*icaC* and i*caD*) and other biofilm promoting genes in order to develop a more in-depth picture of the molecular epidemiology of biofilm formation in animal associated bacteria.

Conclusion

The results indicate that the skin, paws and oral cavity of domestic cats are frequently colonized by the *Staphylococcus* species, particularly *S. sciuri* and *S. xylosus*, and that the Vitek 2 system is useful in identifying the species level. A significant proportion of these isolates were able to form biofilms and resistant to multiple antibiotic classes, suggesting that cats may be a source for biofilm-forming and antibiotic-resistant bacteria. Interestingly, only the presence of the icaB gene was enough to cause the biofilm formation, without the presence of the other genes of the ica operon. These results indicate possible zoonotic transmission but are hypothesis-generating because of the small number of sites in this study, and would require multi-site studies with paired human and environmental isolates to confirm.

Acknowledgments

The authors are grateful to the Northern technical university, Mosul Medical technical institute, Mosul, Iraq, in sponsoring and recording this work. Pharma Specialised Laboratory, Mosul is also given special thanks to gain entry to VITEK 2 analytical services.

Conflict of Interest

There were no conflicts of interest.

Ethical approval

The Ethical Committee for Research Involving Human Participants in the Medical Technical Institute of Mosul approved this study with approval No. MMTRC-2025-013 (24/06/2025). All the procedures followed institutional and international ethical standards, such as the Declaration of Helsinki.

Artificial Intelligence Statement

AI tools were only used for language editing and formatting support in the process of manuscript preparation. The study plan, data gathering, statistical analysis and interpretation of data did not involve an AI system. The scientific content, methodology and conclusions are the responsibility of the author only.

References

1. Yugeta N, Yoshino Y, Sato Y, Hatayama N, Suzuki Y. Fluoroquinolone-resistant *Staphylococcus pseudintermedius* from feline wound infection: biofilm regulation and virulence genes. Sci Rep. 2024;14(1):75165. https://doi.org/10.1038/s41598-024-75165-y.
2. Oliveira CF, de Carvalho JB, Castro LMC, Guedes PEB, dos Santos KC, Andrioli JL, et al. Imprint and spreading techniques for isolation of subungueal fungi in claws of healthy cats. Acta Vet Bras. 2021;15(1):45-52. https://doi.org/10.21708/avb.2021.15.1.10000 .
3. Guardabassi L, Schwarz S, Lloyd DH. Companion animals as reservoirs of antimicrobial-resistant bacteria: new insights. J Antimicrob Chemother. 2024;79(5):987-95. https://doi.org/10.1093/jac/dkae045.
4. Paul NC, Moodley A, Ghibaudo G, Guardabassi L. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in cats. Vet Microbiol. 2023; 275:109612. https://doi.org/10.1016/j.vetmic.2023.109612.
5. Hanselman BA, Kruth SA, Rousseau J, Low DE, Willey BM, McGeer A, et al. MRSA colonization in veterinary personnel. Emerg Infect Dis. 2006;12(12):1933-8. https://doi.org/10.3201/eid1212.060231.
6. Zhang H, Li Y, Wang J, Chen Z, Liu X. Biofilm-associated genes in feline Staphylococcus isolates and zoonotic potential. Front Vet Sci. 2025; 12:1456789. https://doi.org/10.3389/fvets.2025.1456789.
7. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. MRSA in cats and dogs. J Clin Microbiol. 2007;45(5):1679-88. https://doi.org/10.1128/JCM.01708-06.
8. Foreman-Worsley R, Finka LR, Ward SJ, Farnworth MJ. Indoors or outdoors? Owner demographics and decision making for pet cats. Animals. 2021;11(3):324. https://doi.org/10.3390/ani11030324.
9. Elmoslemany A, Elsohaby I, Alorabi M, Alkafafy M, Al-Marri T, Aldoweriej A, et al. Diversity and risk factors of multidrug-resistant staphylococci in cats. Vet Sci. 2021;8(9):189. https://doi.org/10.3390/vetsci8090189.
10. Moon DC, Choi JH, Boby N, Kim SJ, Song HJ, Park HS, et al. Prevalence of bacterial species in cats. Pathog. 2022;11(3):324. https://doi.org/10.3390/pathogens11030324.
11. Tripathi N, Sapra A. Gram staining. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
12. Riedel S, Morse SA, Mietzner T, Miller S. Jawetz, Melnick & Adelberg's Medical Microbiology. 28th ed. New York: Appleton & Lange; 2019.
13. Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, van Duijkeren E, et al. Public health risk of antimicrobial resistance in companion animals. J Antimicrob Chemother. 2017;72(4):957-68. https://doi.org/10.1093/jac/dkw466.
14. Mariana N, Salman S, Neeba V, Zamberi S. Evaluation of modified Congo Red agar for detection of biofilm produced by clinical isolates of methicillin-resistant *Staphylococcus aureus*. Afr J Microbiol Res. 2009;3(6):330-8. https://doi.org/10.5897/AJMR.9000287.
15. Mirzaee M, Najar PS, Ghasemian AM. Detection of icaABCD genes and biofilm formation in clinical isolates of methicillin-resistant *Staphylococcus aureus*. Iran J Microbiol. 2014;6(3):175-81.

16. van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyorala S, et al. Review on MRSA in animals. Vet Microbiol. 2011;153(1-2):3-14. https://doi.org/10.1016/j.vetmic.2011.05.014.
17. Alotaibi GF, Bukhari MA. Factors influencing biofilm formation. Am J Biomed Sci Res. 2021;13(2):123-9. https://doi.org/10.34297/AJBSR.2021.13.002.
18. Youn JH, Park YH, Hang'ombe BM, Sugimoto C, Iseki H, Kim JH, et al. Genetic diversity of Staphylococcus aureus from cats in Asia. J Vet Sci. 2023;24(2): e23. https://doi.org/10.4142/jvs.2023.24.e23.
19. Sato Y, Hatayama N, Suzuki Y, Yugeta N, Yoshino Y. Urinary tract infection due to *Staphylococcus schleifer*i biofilm formation in the subcutaneous ureteral bypass system in a cat. BMC Vet Res. 2025;21(1):566. https://doi.org/10.1186/s12917-025-05020-3
20. Pires dos Santos T, Damborg P, Moodley A, Guardabassi L. Biofilm formation in Staphylococcus pseudintermedius from companion animals. Vet J. 2024; 289:105-12. https://doi.org/10.1016/j.tvjl.2024.105112.
21. Gómez-Sanz E, Torres C, Lozano C, Zarazaga M. MRSA in cats and dogs: prevalence and risk factors. Vet Microbiol. 2023; 272:109528. https://doi.org/10.1016/j.vetmic.2023.109528.
22. Kazemi K, Ghahramani Y, Kalashgrani MY. Nano biofilms: emerging biotechnology applications. Biotechnol Rep. 2022;35: e00789. https://doi.org/10.1016/j.btre.2022.e00789.
23. Weese JS, van Duijkeren E. MRSA and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol. 2010;140(3-4):418-29. https://doi.org/10.1016/j.vetmic.2009.01.039.
24. Moodley A, Guardabassi L. Clonal spread of MRSP in Danish dogs. Vet Microbiol. 2009;133(4):379-83. https://doi.org/10.1016/j.vetmic.2008.07.024.
25. Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, van Duijkeren E, et al. Antimicrobial resistance in companion animals: updated perspectives. J Antimicrob Chemother. 2025;80(2):345-56. https://doi.org/10.1093/jac/dkae012.
26. Yang Y, Hu X, Cai S, Hu N, Yuan Y, Wu Y, et al. Pet cats may shape the antibiotic resistome of their owner's gut. Microbiome. 2023;11(1):235. https://doi.org/10.1186/s40168-023-01535-1.
27. Razali K, Kaidi R, Giarratana F, Ait-Oudhia K. Staphylococci and zoonotic potential: oral carriage and antibiotic susceptibility in stray dogs and cats in Algeria. J Hell Vet Med Soc. 2022;73(1):3621-8. https://doi.org/10.12681/jhvms.27945.
28. Deniz C, Yilmaz E, Aydin F. Distribution of biofilm-related genes among coagulase-negative staphylococci isolates. J Infect Public Health. 2025;18(2):210-8. https://doi.org/10.1016/j.jiph.2024.12.005.
29. Bi, C., et al. (2022). Role of ica operon in biofilm formation in Staphylococcus aureus. Frontiers in Microbiology, 13, 845–856.