Open Veterinary Journal, (2024), Vol. 14(9): 2215-2223
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Research Article
DOI: *10.5455/OVJ.2024.v14.i9.9*

Submitted: 28/04/2024 Accepted: 03/08/2024 Published: 30/09/2024

Detection of biofilm formation and antibiotics resistance of *Staphylococcus* spp. isolated from humans' and birds' oral cavities

Noor A. Al-Taii*, Nagham M. Al-Gburi and Nuha K. Khalil

Zoonotic Research Unite, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

ABSTRACT

Background: *Staphylococcus* spp. are widely distributed in nature and can cause nosocomial, skin infections, and foodborne illness, and it may lead to severe financial losses in birds by causing systemic infection in numerous organs.

Aim: This study was conducted to determine the prevalence of *Staphylococcus* spp. in humans and birds in Baghdad city.

Methods: Seventy-six oral cavity swabs were collected, including 41 from birds and 35 from breeders. All samples were examined by bacteriological methods and identified by using the VITEK technique, the samples were then further studied to test the ability of biofilm formation, and multidrug-resistant (MDR) factors and MAR index were tested with the use of seven antibiotics.

Results: Among the 76 oral swabs, 37 samples were positive (48.68%) for *Staphylococcus* spp.: 7 human samples (20%) and 30 bird samples (73.17%). In humans, *Staphylococcus lentus* was the most prevalent (42.85%) followed by *Staphylococcus aureus* (28.57%), *Staphylococcus hominis* and *Staphylococcus sciuri* were at (14.29%) to each. In birds, *Staphylococcus pseudintermedius*, *Staphylococcus gallinarum, S. lentus*, *Staphylococcus haemolyticus*, *Staphylococcus* spp, *S. sciuri,* and *Staphylococcus xylosus* were detected in 36.67%, 16.67%, 10%, 10%, 13.33%, 3.33% and 3.33%, respectively. *Staphylococcus* isolates from the human samples demonstrated that only *S. lentus* was resistant 33.33% to ME, OX, and SXT. Furthermore, one of them was MDR and high MAR index value. The antimicrobial pattern of *Staphylococcus* spp. isolated from birds was as follows *S. pseudintermedius* isolates demonstrated 100% resistance to CN, CIP, SXT, and MDR (100%) and high MAR indices value; *S. xylosus* was resistant 100% against ME, CN, SXT, and Do and it was MDR with high MAR index; *S. lentus* was resistant 25% against ME, OX, C, and SXT, whereas, *S. gallinarum* was resistant 33.33% against ME and OX. The results demonstrated that biofilm formation of the *Staphylococcus* spp. isolated from human samples were weak biofilm formers: *S. lentus, S. hominis*, and *S. aureus*, while other *S. aureus* (50%) was moderate. In birds, the majority of the isolates had non-biofilm-producing capabilities, while 80% of *S. lentus* and 100% of *S. xylosus* showed moderate biofilm formation.

Conclusion: Healthcare problem was observed in this study due to high MDR and MAR index among *Staphylococcus* spp. isolated from pet birds to their owners and vice versa.

Keywords: *Staphylococcus* spp., Oral cavity, Antibiotic resistance, Biofilm.

Introduction

Staphylococci are Gram-positive cocci, facultative anaerobic bacteria that demonstrate non-motile arrangement similar to a grape-like cluster. These bacteria can produce coagulase enzymes and can coagulate blood plasma; therefore, it is divided into two groups: coagulase-positive and coagulase-negative *staphylococci* (CoNS) (Todar, 2008). In humans and animals including birds, numerous *Staphylococcal* species are obligate cutaneous and mucosal flora, including those of the upper respiratory tract, digestive system, and genitourinary tract (Heilmann *et al*., 2019). Besides being widely distributed, *Staphylococci* are also easily transmitted among humans, animal species, and between humans and animals. Mainly carriers, clinically sick humans and animals, tainted food, water, and equipment, as well as an environment where animals are packed together, are sources of infection. Numerous transmission pathways have been identified, including direct contact with the body, via the hands, contact with excretions or non-living items (fomites), consumption of tainted food and drink, aerosols, and vectors (Ferreira *et al*., 2011; Hayyawi, 2012). The oral cavity contains a variety of microbial environments that foster the development of microbial communities on the mucosal surfaces (such as those in the lips, cheek, palate, tongue, and teeth). As these birds are employed for entertainment, companionship, or psychological support, the habit of keeping them

***Corresponding Author:** Noor A. Al-Taii. Zoonotic Research Unite, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. Email: *noor.ham@covm.uobaghdad.edu.iq*

as pets has grown around the world, and the variety of pet bird species has expanded to include psittacine birds (Wieler *et al*., 2011). Due to their frequent human contact, pet birds may become infected with a number of highly important zoonotic pathogens (Wagner *et al*., 2014). Pet animals are also thought to be a possible source for the spread of multidrug-resistant (MDR) zoonotic microorganisms (Hu *et al*., 2011).

Antimicrobial medications, most frequently-lactam antibiotics, are used to treat Staphylococcal infections in both humans and animals. These antibiotics were initially quite successful against *Staphylococci*, but in the mid-1940s-lactamase-producing *Staphylococcus* isolates appeared, and within a few years their prevalence rose sharply (Liu *et al*., 2012; Geenen *et al*., 2013). As a result of their ability to manufacture beta-lactamases and develop resistance to mobile genetic elements, plasmids, and transposons, *Staphylococci* species may play a significant role in the evolution of MDR bacteria (McCallum *et al*., 2010). Because of the barrier created by the biofilm's matrix, the formation of biofilms by *Staphylococcus* species is a critical mechanism that increases antimicrobial resistance. This barrier decreased antibiotic penetration and/or diffusion and increased the high rate of multi-resistant phenotypes. Additionally, the ability of MDR or extensively drug-resistant strains to produce biofilms enhances overall resistance and potentially leads to incurable conditions (França *et al*., 2021; Tsopmene *et al*., 2023).

Thus, the aim of this study was to isolate *Staphylococcus* spp. and to evaluate their susceptibility to antimicrobial agents and biofilm production in Baghdad city.

Materials and Methods

Samples

Seventy-six oral swabs, including 41 oral swabs were collected from presumably healthy pet birds bred at home (ornamental and poultry) as follows: Pigeon (10), Cokatial (3), Chicks (pullet) (3), Myna (1), Budgie (love birds) (7), Canary (2), Parrot (9), Buluble (3), and Duck (3), in addition to 35 oral swabs from the breeders of these birds. The swab samples were collected using swabs containing a transport medium (nutrient agar). Each sample was marked with the date of collection and transferred to the University of Baghdad/College of Veterinary Medicine/Zoonosis Research Unit's bacteriology laboratory.

Isolation and identification

The swabs were incubated at 37ºC for 24 hours, then sub-cultured on mannitol salt agar (HiMedia/India) and incubated at 37ºC for 24 hours, then suspected *Staphylococcus* colonies (ferment and non-ferment of Mannitol) were streaking on blood agar (HiMedia/India) and incubated at 37°C for 24–48 hours, the plates were examined for the presence (creamy, greyish white, or yellow colonies) and hemolytic activity on blood agar. Pure colonies were then cultivated on nutrient agar plates for catalase test, and Gram stain (Quinn *et al*.,2002). Further identification was done by VITEK®2 system, briefly, pure colonies were transferred into a polystyrene tube containing 3 ml of saline to obtain a density equivalent to 0.5–0.63 utilizing VITEK2 DensiChek spectrophotometer. Then, the tube containing the bacterial suspension was put into VITEK 2 cassette and the identification Gram-positive bacilli colorimetric reagent card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube and transferring the cassette inside the VITEK 2 instrument, the results were read after 18–24 hours.

Antimicrobial susceptibility test, MDR, and multiple antibiotics resistance (MAR) index

A bacterial suspension containing 1×10^8 CFU was prepared, then a sterile swab was inserted into the bacterial suspension, and the swab was pressed and rotated firmly against the inside of the tube to remove the excess fluid, the swab was streaked entirely on Muller Hinton agar in three directions and allowed to stand for 3–5 minutes, then the discs were placed onto agar using sterile forceps with gentle pressed to firm the discs on agar, and the plates were incubated at 37ºC for 18–24 hours. After that, inhibitory zones surrounding these antibiotic discs in millimeters (mm) were measured (CLSI, 2022). Seven antibiotic disks (Merseyside, U.K.) were used including: Methicillin (ME10 µg), Oxacillin (Ox 5 µg), Gentamycin (CN 10 µg), Trimethoprim/Sulphamethoxazole (SXT 25 µg), Chloramphenicol (C10 µg), Ciprofloxacin (CIP 10 µg), and Doxycycline (DO 10 µg). MDR was detected according to the work of (Magiorakos *et al*.,2012), the isolates that were resistant to three or more different antibiotic classes were considered MDR. The MAR was measured by dividing (a): Number of Antibiotics that have been resistant by a specific isolate by (b): Number of antibiotics used against that isolate, the isolates that showed measures equal to or more than (0.2) were considered high risk (Magiorakos *et al*., 2012).

Detection of biofilm formation

Biofilm formation was detected using crystal violet assay according to (Christensen *et al*., 1985; Stepanović *et al*., 2007; Sandhu *et al*., 2016) some modifications; bacterial suspension (1×10^6CFU) in trypticase soya broth containing glucose and sucrose 1%. 200 µl of the suspension was transferred into three wells, just trypticase soya broth was used in the negative control well, then incubated at 37ºC for 48 hours and non-adherent cells were removed, through washing the microtiter plate twice with distilled water, let dry in the air, heat fixing in the oven at 45ºC. The adhering cells were stained with crystal violet 1% for half an hour, the staining was erased by washing it off with water, letting it air dry, and then resolving it in 200 µl of 95% ethanol. Afterward, the re-solubilized crystal violet's optical density (OD) was determined using a

plate reader at 570 nm (OD_{570}) (ELIZA). The findings of biofilm production using the following calculation were classified by the OD cut-off (ODC) calculation, which showed whether or not the isolates were capable of generating biofilms.

1-ODC = average OD of negative control + (3 × standard deviation of negative control)

2- OD isolates = average OD of isolate—ODC

Biofilm formation was categorized as follows:

OD ≤ ODc: no biofilm production

ODc< OD ≤ 2 × ODc: weak biofilm production

2 × ODc< OD ≤ 4 × ODc: moderate biofilm production

4 × ODc< OD: strong biofilm production.

Statistical analysis

The SAS, version 9.6thed (SAS, 2018) was used to identify significant variations in proportions, in addition to the chi-square test.

Ethical approval

Not needed for this study.

Results

Of the 76 oral swabs examined from humans and birds, 37 (48.68%) were positive for *Staphylococcus* spp, 7/35 (20%) were from humans, and 30/41 (73.17%) were from birds with significant differences (Table 1). In humans, out of the 7 isolates; *Staphylococcus lentus* was the most dominant 3 (42.85%) followed by *Staphylococcus aureus* 2 (28.57%), *Staphylococcus hominis*, and *Staphylococcus sciuri* 1 (14.29%) to each, while, 30 isolates from birds, *S. pseudintermedius* was the most isolate spp. at 11 (36.67%), *Staphylococcus gallinarum*, *S. lentus*, *Staphylococcus haemolyticus*, *Staphylococcus* spp *S.sciuri* and *Staphylococcus xylosus* were detected at rate 5 (16.67%), 5 (16.67%), 3 (10%), 4 (13.33%), 1 (3.33%), and 1 (3.33%), respectively. In addition, *S. lentus* and *Staphylococcus sciuri* were detected in both human and bird samples (Table 2).

According to bird species, Pigeon, Cokatial, Chicks (pullet), and Myna were 100% positive for *Staphylococcus* followed by love birds at 71.42%, parrots at 66.66%, and Canary at 50%, while not detected in ducks. In Pigeon, *Staphylococcus pseudintermedius* were detected at 100%; from Cokatial was *S. haemolyticus* at 66.66% and *S. xylosus* at 33.33%; from Chicks (pullet) was *S. gallinarum* at 66.66% and *S. lentus* 33.33%; Parrot 66.66% *Staphylococcus* spp. and *S. lentus* was 33.33%; from Myna and Canary was *S. gallinarum* 100%, and from bulbule was *S. pseudintermedius* at 100% (Table 3).

Antibiotic resistance

Staphylococcus isolates from humans showed that all isolates were sensitive 100% to all seven antibiotics, except *S. lentus* which was resistant at 66.66% against ME, OX, and SXT, and one of them was MDR, and the MAR index was 0.43 (Table 4). For *Staphylococcus* isolates from birds, the antimicrobial pattern was as follows: *S. sciuri* and *S. haemolyticus* were absolute (100%) sensitive to all antibiotics, *S. pseudintermedius* were resistant 100% to CN, CIP, SXT, and Do and all of them were MDR 100%, and MAR index for all were 0.57, *S. xylosus* was resistant 100% against ME, CN, SXT and Do, and it was MDR and the MAR index was 0.43, *S. lentus* was resistance 20 % against ME, OX, and Do, 40% against C, and MAR index range 0.14–0.28 (only one isolate) was high MAR index 0.28, while *Staphylococcus* spp. were resistant 33.33% to ME, OX, and C, one isolate was MDR and MAR index was one isolate reported MAR index 0.57, *Staphylococcus gallinarum* was resistant 33.33% against two antibiotics which are ME and OX with low MAR index (Table 5).

Biofilm production

In humans 2/3 of *S. lentus* isolates (66.67%) showed weak biofilm production and 1 (33.33%) was non-biofilm producer, while 1 (100%) isolate of *S. hominis* was weak, the 2 isolates of *S. aureus* was weak 1 (50%) and the other was moderate 1 (50%) (Table 6). In birds most isolates show non-biofilm formation; *S. pseudintermedius* 11 (100%), *S. lentus* 1(20%) and (100%) from *Staphylococcus haemolyticus* (3), *S. sciuri* (1), *S. gallinarum* (2) while 4 (80%) of *S. lentus* and 1 (100%) of *S. xylosus* show moderate biofilm formation (Table 7).

Table 1. Positive carriage of *Staphylococcus* in humans and birds.

Source	No	Positive (%)	X^2 value (*p*-value)
Human	35	7 (20%)	7.8,452 (005,096)
Birds	41	30 (73.17%)	
Total	76	37 (48.68%)	

Significant differences at $p < 0.05$.

Table 2. Percentages of *Staphylococcus* spp. isolated from humans and birds.

***Staphylococcus* spp.**	**Positive No. (%)**	
	Human (7)	**Birds (30)**
S. pseudintermedius	0	11 (36.67)
S. lentus	3 (42.85)	5 (16.67)
S. gallinarum	0	5 (16.67)
S. sciuri	1 (14.29)	1 (3.33)
S. haemolyticus	0	(10)
s. hominis	1 (14.29)	0
S. xylosus	0	1 (3.33)
S. aureus	2 (28.57)	0
Staph. spp	0	4 (13.33)

Table 3. Distribution of *Staphylococcus* spp in birds according to species.

Birds spp (no)	*Staphylococcus* spp %						
	pseudintermedius	*lentus*	*haemolyticus*	*xylosus*	*sciuri*	*gallinarum*	spp
Pigeon (10)	100	0	0	0	0	0	0
Cokatial (3)	0	0	66.66	33.33	0	0	0
Chicks (pullet) (3)	0	33.33	0	0	0	66.66	0
Myna (1)	0	0	0	0	0	100	0
Budgie (love birds) (7)	0	40	20	0	20	20	0
Canary (2)	0	0	0	0	0	100	0
Parrot (9)	0	33.33	0	0	0	0	66.66
Buluble (3)	100	0	0	0	0	0	0
Duck (3)	0	0	0	0	0	0	0

Table 4. Antimicrobial resistance of *Staphylococcus* spp. isolated from human.

A.M	*S. lentus* (3)		*S. hominis* (1)		*S. sciuri* (1)		*S. aureus* (2)	
	R%	S %	R %	S %	R%	S %	R%	S%
Me	33.33	66.66	0	100	0	100	0	100
CN	0	100	0	100	0	100	0	100
CIP	0	100	0	100	0	100	0	100
O X	33.33	66.66	0	100	0	100	0	100
C	0	100	0	100	0	100	0	100
SXT	33.33	66.66	0	100	0	100	0	100
Do	0	100	0	100	0	100	0	100

A.M=antimicrobial, R=resistance, S=sensitive, Methicillin (ME), Oxacillin (Ox), Gentamycin (CN), Trimethoprim/ Sulphamethoxazole (SXT), Chloramphenicol (C), Ciprofloxacin (CIP), Doxycycline (DO).

Table 5. Antimicrobial resistance of *Staphylococcus* spp isolated from birds.

A.M.	*Staphylococcus* spp													
	Pseudintermedius 11		*Xylosus* 1		*Lentus* 5		*Haemolyticus* 3		*Sciuri* 1		*Gallinarum* 4		Spp. 4	
	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%
Me	0	100	100	0	20	80	0	100	0	100	33.33	66.66	33.33	66.66
CN	100	0	0	100	0	100	0	100	0	100	0	100	0	100
CIP	100	0	100	0	0	100	0	100	0	100	0	100	0	100
O X	0	100	0	100	25	75	0	100	0	100	33.33	66.66	33.33	66.66
C	0	100	100	0	25	75	0	100	0	100	0	100	33.33	66.66
SXT	100	0	0	100	25	75	0	100	0	100	0	100	0	100
Do	100	0	0	100	0	100	0	100	0	100	0	100	0	100

A.M=antimicrobial, R=resistance, S=sensitive,Methicillin (ME), Oxacillin (Ox), Gentamycin (CN), Trimethoprim/ Sulphamethoxazole (SXT), Chloramphenicol (C),Ciprofloxacin (CIP), Doxycycline (DO).

Discussion

In this study, birds were more positive for *Staphylococcus* spp than humans with significant differences, and most isolates belonged to CoNS. In humans, the most prevalent isolate was *S. lentus* followed by *S. aureus*, while in birds, *S. pseudintermedius*, *S. gallinarum,* and *S. lentus* were the most prevalent isolates. There are a few studies about the prevalence of *Staphylococcus* in the oral cavity in pet birds and humans to compare.

Table 6. Percentages of biofilm formation of human isolates.

Biofilm phenotype (OD570)	*Staphylococcus* spp.			
	***lentus* (3)**	***hominis* (1)**	***sciuri* (1)**	***aureus* (2)**
Weak	66.67	100	0	50
Moderate	0	0	100	50
Non	33.33	0	0	0

Table 7. Percentages of biofilm formation of *Staphylococcus* spp of birds isolates.

***Staphylococcus* spp. (no)**	Biofilm phenotype (OD570)		
	Weak	**Moderate**	**Non**
S. pseudintermedius (11)	0	0	100
S. xylosus (1)	0	100	0
S. lentus (5)	0	80	20
S. haemolyticus (3)	0	0	100
S. sciuri (1)	0	0	100
S. gallinarum (4)	0	0	100

Staphylococcus aureus was isolated from human samples only in the current study; in other studies, *S. aureus* was detected at 84% among farm workers (Nanoukon *et al*.,2017) and was the most identified spp. at 33.8% from dental cavities (Alzahrani *et al*.,2022); in addition, it was detected at 100% and 5% from tonsillitis and rhino sinusitis, respectively (Kasim and Al-Zubaidy 2022). *Staphylococcus aureus* was not isolated from birds which contrasts with previous results indicating its presence at 15.89% within the internal organs of poultry properly (Marek *et al*.,2016). Abunna *et al*., (2022) *S. aureus* was 62.5% carried out on ostensibly healthy hens, farm workers, and hen litter at chicken farms.

Staphylococcus lentus and *S. sciuri* were identified in both humans and birds in this work, the two spp. *Staphylococcus lentus* suggested colonization in the respiratory tract, and investigated among farm animals and occupational people when exposed to livestock, *S. lentus* identified at 33.33% from inflamed teeth and gingiva of human's oral cavity, and rhinosinusitis at 15% (De Martino *et al*., 2010; Alash and Mohammed 2019; Kasim and Al-Zubaidy, 2022). Also, it was detected at 14.5% in the tracheal of wild birds, and 13.90% in internal organs of poultry (Marek *et al*., 2016; Ruiz-Ripa *et al*., 2020). In contrast, *S. sciuri* has been formerly found to cause infections in animals, it was isolated from internal organs of poultry at 3.9%, tracheal carriage in wild birds at 60%, and from nocturnal birds of prey (Marek *et al*.,2016; Alash and Mohammed, 2019; Ruiz-Ripa *et al*., 2020; Silva *et al*., 2022). While infection and colonization of humans with *S. sciuri* have been described as rare phenomena (Shittu *et al*.,2004; Severin *et al*., 2010).

Staphylococcus xylosus is a commensal bacterium of the skin of small mammals and farm animals. In this finding, *S. xylosus* was identified in bird samples, a previously detected *S. xylosus* from wild birds and poultry (Rueanghiran *et al*., 2017; Alash and Mohammed, 2019; Kasim and Al-Zubaidy, 2022), also it was reported in humans with urinary tract infections and from chronic infection of the lacrimal tract humans (Al Mathkhury *et al*., 2018; Sidibe *et al*.,2022). A study revealed that *S. xylosus*, *S. sciuri*, and *S. lentus* were found to induce mild subclinical disease with histopathological lesions in the internal organs of infected poultry (Shokery *et al*., 2018; Ruiz-Ripa *et al*., 2020).

Staphylococcus hominis was identified in humans and *S. haemolyticus* in birds in the current study, *S. haemolyticus* is associated with hospital-acquired infections, it was reported in nocturnal birds of prey and humans with rhinosinusitis (Panda and Singh, 2018; Kasim and Al-Zubaidy, 2022; Silva *et al*., 2022). In contrast, *S. hominis* is the third most frequently isolated spp. from patient blood infections, meningitis, rhinosinusitis, food poisoning, and mastitis in cattle, and it was discovered that *S. hominis* caused illness in hens (Kasim and Al-Zubaidy, 2022; Azimi *et al*., 2020; Sorour *et al.,* 2023).

Staphylococcus pseudintermedius was isolated from birds in this work, it is known that veterinary *S. pseudintermedius* strains commonly colonize the skin and mucous membranes of animals, most often dogs and cats, *S. pseudintermedius* methicillin-resistant strains have emerged as a major challenge for veterinary dermatologists in particular owing to their extensive multidrug resistance and their behavior as

nosocomial pathogens. It has been recognized as an opportunistic and zoonotic pathogen that is able to colonize humans and cause severe diseases, especially in immunocompromised hosts, it has also been proven that this spp. is transmitted to humans in contact with animals (Chrobak-Chmiel *et al*., 2018; Kasim and Al-Zubaidy, 2022; Silva *et al*., 2022; Moses *et al*., 2023). *Staphylococcus gallinarum* was isolated from birds in the current work, *S. gallinarum* is one of the etiological agents that causes bumble foot disease in birds and it was detected from broiler feces and is found to induce disease in experimentally infected chickens (El Sawi and Mohamed, 2015; Rueanghiran *et al*., 2017; Sorour *et al.,* 2023). In addition, it was isolated from human patients (Yu *et al*., 2008; Ohara-Nemoto *et al*., 2008).

In this finding, *Staphylococcus* isolates from humans and birds showed differences in their susceptibility to antimicrobial agents, most of the isolates were MDR and had high MAR index values. These results partially agree or disagree with other results. Marek *et al*. (2016) found that *Staphylococcus sciuri* isolated from poultry was resistant to SXT at 25%, Do at 42%; *S. lentus* was resistant to SXT at 55.5%, and DO at 44.4%. CoNS recovered from wild birds including *S. sciuri*, *S. lentus*, *S. xylosus*, and other spp. were resistant to CN 3%, SXT 1%, C 1%, CIP 4%, and the isolates were MDR 34% (Ruiz-Ripa *et al*., 2020). The results of Silva *et al*. (2022) showed *S. sciuri* isolates carried the mecA gene, which is known to be responsible for methicillin resistance, and *S. haemolyticus* was resistant to SXT (Ugwu *et al*., 2015). *Staphylococcus* spp. isolates including *S. gallinarum* and *S. lentus* from broiler feces were resistant to OX 87%, CN 0%, C 3%, and SXT 13% (Rueanghiran *et al*., 2017). *Staphylococcus gallinarum*, *S. hominis,* and other spp. from chicken were reported to resist 100% to ME and DO, and 84% against SXT with MDR (Sorour *et al*., 2023). Bacteria having a MAR index≥ 0.2 originate from a high-risk source of contamination where several antibiotics are used (Sandhu *et al*., 2016). These findings reflect that *S. lentus, S. pseudintermedius*, and *S. xylosus* isolates are likely to be from high-risk sources and originate from an environment where several antibiotics are used and the results investigate that pet birds play a source in the emergence and, spread of antibiotic resistance (Phumthanakorn *et al*., 2017).

Biofilm formation is considered to be one of the most important virulence factors in *Staphylococci*, especially for *S. aureus* and *S. epidermidis*, as it allows them to adhere to tissues and indwelling medical devices (Otto, 2013; Wuytack *et al*., 2020). Our results show that isolated *Staphylococcus* spp. were weak biofilm producers, these results are in contrast with Silva *et al*. (2022) in which several isolates of *Staphylococcus* spp. from food-producing fowl were potent biofilm makers, raising potential public health issues given that biofilm formation poses a significant risk to the food business and causes significant financial losses for the livestock sector. One of the most common causes of illnesses linked to biofilms is *S. aureus* which is thought to be the cause of persistent human infections (Cascioferro *et al*., 2021). Gourari-Bouzouina, *et al*.,(2024) demonstrated the ability of *S. aureus* to highest production of biofilm and metabolic activity by using Scanning electron microscopy. The biofilm formation test reveals that 100% of *Staphylococcus* isolated from human urine isolates 12.69% were strong biofilm producers, 77.77% were moderate biofilm producers, and 9.52% were low biofilm producers (Tsopmene *et al*., 2023).

Conclusion

The current study showed that different *Staphylococcus* spp. are widely distributed in birds and their owners according to samples isolated from different regions in Baghdad, isolated *S. pseudintermedius* which is an important zoonotic spp from the birds may be considered a major problem in health care. High resistance against antimicrobial agents, MDR, and high risks due to high MAR index values among the isolates lead to the transfer of this resistance from birds to owners and vice versa, and from birds to other animals, and also may transfer to other microorganisms. Therefore, care must be taken when dealing with birds, and not use medications randomly.

Acknowledgments

Appreciation goes to Zoonotic Researches Unit, Veterinary Medicine College, University of Baghdad, Iraq.

Authors' contributions

Al-Gburi and Altaii: Designed the study.Al-Gburi, Altaii, and Khalil: Planned the study and analyzed the results, supervised the study, corrected and reviewed and approved the final manuscript.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Data availability

All data are provided in the manuscript.

References

Abunna, F., Adugna, B., Tufa, T.B., Ayana, D., Gutema, F.D., Waktole, H., Regassa, F.and Abdi, R.D. 2022. Detection and antimicrobial resistance of *Staphylococcus* Species from chicken, chicken litter, and humans in addis Ababa, Vet. Med. Int. 2022, 9084334.

Al Mathkhury, H.J.F., Flaih, M.T. and Alghrairy, Z.K.A. 2018. Pathological study on *Staphylococcus Xylosus* isolated from patients with urinary tract infections. ANJS. 11(2), 123–130.

Alash, S.A. and Mohammed, M.Q. 2019. Antibacterial activity of some mouth wash solutions against *Staphylococcus* lentus isolated from mouth infections. Iraqi J. Sci. 60(12), 2583–2589.

Alzahrani, M.R., Guan, B.J., Zagore, L.L., Wu, J., Chen, C.W., Licatalosi, D.D., Baker, K.E. and Hatzoglou, M. 2022. Newly synthesized mRNA escapes translational repression during the acute phase of the mammalian unfolded protein response. PLoS One 17(8), e0271695.

Azimi, T., Mirzadeh, M., Sabour, S., Nasser, A., Fallah, F. and Pourmand, M.R. 2020. Coagulase-negative staphylococci (CoNS) men- ingitis: a narrative review of the literature from 2,000 to 2,020. New Microbes New Infect. 37, 100755.

Cascioferro, S., Carbone, D., Parrino, B., Pecoraro, C., Giovannetti ,E., Cirrincione, G. and Diana, P 2021. Therapeutic strategies to counteract antibiotic resistance in MRSA biofilm-associated infections. Med Chem. 16(1), 65–80.

Christensen, G.D., Simpson, W.A., Younger, J.J., Baddour, L.M., Barrett, F.F. and Melton, D.M. 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. 22(6), 996–1006.

Chrobak-Chmiel, D., Golke, A., Dembele, K., Ćwiek, K., Kizerwetter-Świda, M., Rzewuska, M. and Binek, M. 2018. *Staphylococcus pseudintermedius*, both commensal and pathogen. Med. Weter. 74(6), 362–370.

CLSI. 2022. Performance Standards for Antimicrobial Susceptibility Testing; document M100. 32nd ed. PA: Clinical and Laboratory Standards Institute, USA.

De Martino, L.D., Lucido, M., Mallardo, K., Facello, B., Mallardo, M., Iovane, G., Pagnini, U., Tufano, M.A. and Catalanotti, P. 2010. Methicillin-resistant Staphylococci isolated from healthy horses and horse personnel in Italy. J. Vet. Diagn. Investig. 22, 77–82.

El Sawi, O. and Mohamed, M. 2015. Prevalence and pathogenicity of *Staphylococcus gallinarum* in poultry with special reference to bumblefoot in Khartoum State. [Ph.D. Thesis].: Faculty of Veterinary Science, University of Khartoum, Khartoum, Sudan.

Ferreira, J.P., Anderson, K.L., Correa, M.T., Lyman, R., Ruffin, F., Reller, L.B. and Fowler, V.G. 2011. Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. PLoS One 6(11), e26978.

França, A., Gaio, V., Lopes, N. and Melo, L.D.R. 2021. Virulence factors in coagulase-negative staphylococci. Pathogens (Basel, Switzerland) 10(2), 170.

Geenen, P., Graat, E., Haenen, A., Hengeveld, P., Van Hoek, A., Huijsdens, X. and Van De Giessen, A. 2013. Prevalence of livestock-associated MRSA on Dutch broiler farms and in people living and/or working on these farms. Epidemiol. Infect. 141(5), 1099–1108.

Gourari-Bouzouina, K., Boucherit-Otmani, Z., Seghir, A., Baba Ahmed-Kazi Tani, Z.Z., Bendoukha, I., Benahmed, A., Aissaoui, M. and Boucherit, K. 2024. Evaluation of mixed biofilm production by Candida spp. and *Staphylococcus aureus* strains co-isolated from cystic fibrosis patients in northwest Algeria. Diag. Microbiol. Infect. Dis. 109(3), 116321.

Hayyawi, S.M. 2012. Comparison of microbial isolates isolated from external ear canal of sheep and their susceptibility to antibiotics. Iraqi J. Vet. Med. 36(0E), 41–48.

Heilmann,C., Ziebuhr, W. and Becker, K. 2019. Are coagulase-negative staphylococci virulent. Clin. Microbiol. Infect. 25(9), 1071–1080.

Hu, Q., Tu, J., Han, X., Zhu, Y., Ding, C. and Yu, S. 2011. Development of multiplex PCR assay for rapid detection of Riemerella anatipestifer, *Escherichia coli*, and *Salmonella* enterica simultaneously from ducks. J. Microbiol. Methods. 87(1), 64–69.

Kasim, B.A. and Al-Zubaidy, I.A.H. 2022. Isolation And identification Of *Staphylococcus* species from dog and human. Ann. For. Res. 65(1), 5717–5722.

Liu, Y., Wang, Y. and Wu, C. 2012. First report of the multidrug resistance gene cfr in Enterococcus faecalis of animal origin. Antimicrob. Agents Chemother. 56(3), 1650–1654.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B. and Paterson, D.L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18(3), 268–281.

Marek, A., Stępień-Pyśniak, D., Pyzik, E., Adaszek, Ł., Wilczyński, J. and Winiarczyk, S. 2016. Occurrence and characterization of *Staphylococcus* bacteria isolated from poultry in Western Poland. Berl Münch Tierärztl Wochenschr. 129, 147–152.

McCallum, N., Berger-B¨achi, B. and Senn, M.M. 2010. Regulation of antibiotic resistance in *Staphylococcus aureus*. Int. J. Med. Microbiol. 300(2-3), 118–129.

Moses, I.B., Santos, F.F. and Gales, A.C. 2023. Human colonization and infection by *Staphylococcus pseudintermedius*: an emerging and underestimated zoonotic pathogen. Microorganisms 11(3), 581.

Nanoukon, C., Argemi, X., Sogbo, F., Orekan, J., Keller, D., Affolabi, D., Schramm, F., Riegel, P., Baba-Moussa, L. and Prévost, G. 2017. Pathogenic features of clinically significant coagulase-negative staphylococci in hospital and community infections in Benin. Int. J. Med. Microbiol. 307(1), 75–82.

Ohara-Nemoto, Y., Haraga, H., Kimura, S. and Nemoto, T.K. 2008. Occurrence of staphylococci in the oral

cavities of healthy adults and nasal–oral trafficking of the bacteria. J. Med. Microbiol. 57, 95–99.

Otto, M. 2013. Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Ann. Rev. Med. 64, 175–188.

Panda, S. and Singh, D.V. 2018. Biofilm formation by Ica-negative ocular isolates of *Staphylococcus haemolyticus*. Front. Microbiol. 9, 2687.

Phumthanakorn, N., Chanchaithong, P. and Prapasarakul, N. 2017. Development of a set of multiplex PCRs for detection of genes encoding cell wall-associated proteins in *Staphylococcus pseudintermedius* isolates from dogs, humans and the environment. J. Microbiol. Methods 142, 90–95.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. 2002. Clinical veterinary microbiology. Boston, MA: Harcourt Publishers, pp: 331–344.

Rueanghiran, C., Viriyarampa, S., Thongyuan, S. and Tulayakul, P. 2017. Species diversity and antimicrobial susceptibility properties of *Staphylococcus* isolated from broiler feces in selected farms, Thailand. J. Public Health 47(1), 44–55.

Ruiz-Ripa, L., Gómez, P., Alonso, C.A., Camacho, M.C., Ramiro, Y., de la Puente, J., Fernández-Fernández, R., Quevedo, M.Á., Blanco, J.M., Báguena, G., Zarazaga, M., Höfle, U. and Torres, C. 2020. Frequency and characterization of antimicrobial resistance and virulence genes of coagulase-negative Staphylococci from wild birds in Spain. Detection of tst-Carrying S. sciuri Isolates. Microorganisms 8(9), 1317.

Sandhu, R., Dahiya, S. and Sayal, P. 2016. Evaluation of multiple antibiotic resistance (MAR) index and doxycycline susceptibility of acinetobacter species among inpatients. Indian J. Microbiol. Res. 3(3), 299.

Severin, J. A., Lestari, E.S., Kuntaman, K., Pastink, M., Snijders, S.V., Lemmens-den Toom, N. and Verbrugh, H.A. 2010. Nasal carriage of methicillin-resistant and methicillin-sensitive strains of *Staphylococcus* sciuri in the Indonesian population. Antimicrob. Agents Chemother. 54(12), 5413–5417.

Shittu, A.J., Lin, D., Morrison, D. and Kolawole. 2004. Isolation and molecular characterization of multiresistant *Staphylococcus* sciuri and *Staphylococcus haemolyticus* associated with skin and soft-tissue infections. J. Med. Microbiol. 53, 51–55.

Shokery, H.M., Redwan, I. A.H., Abd El-Ghany,W.A. and Amer M.M. 2018.Molecular detection of antibiotic resistance genes in identified coagulase negative Staphylococci from chickens flocks and hatcheries in Egypt. Egypt J. Vet. Sci. 49(1), 59–70.

Sidibe, M., Napo, A., Dembele, A., Kassogué, O., Diallo, O., Dembele, D.J. and Traore, L. 2022. *Staphylococcus xylosus* isolation of conjunctival secretions in an 8-year-old child at sikasso hospital (Mali): about a case. Open J. Med. Microbiol. 12(2), 49–55.

Silva, V., Correia, E., Pereira, J.E., González-Machado, C., Capita, R., Alonso-Calleja, C. and Poeta, P. 2022. Exploring the biofilm formation capacity in S. *pseudintermedius* and coagulase-negative Staphylococci Species. Pathogens 11(6), 689.

Silva, V., Lopes, A.F., Soeiro, V., Caniça, M., Manageiro, V., Pereira, J.E., Maltez, L., Capelo, J.L., Igrejas, G. and Poeta, P. 2022. Nocturnal birds of prey as carriers of *Staphylococcus aureus* and other Staphylococci: diversity, antimicrobial resistance and clonal lineages. Antibiotics 11(2), 240.

Sorour, H.K., Shalaby, A.G., Abdelmagid, M.A. and Hosny, R.A. 2023. Characterization and pathogenicity of multidrug-resistant coagulase-negative Staphylococci isolates in chickens. Int. Microbiol. 2023, 1–12.

Stepanović, S., Vuković, D., Hola, V., Di Bonaventura, G., Djukić, S., Cirković, I. and Ruzicka, F. 2007. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 115(8), 891–899.

Todar, K. 2008. Todar's Online Textbook of Bacteriology, Madison, WI: University department in Madison. Available via http://www.textbookofbacteriology.net

Tsopmene, U.J., Iwewe, Y.S., Eyong, I.M., Bisso, B.N. and Dzoyem, J.P. 2023. Antibiotic resistance profile, biofilm formation ability, and virulence factors analysis of three *Staphylococcus* spp. isolates from urine. Cureus 15(4), e37877.

Ugwu, C.C., Gomez-Sanz, E., Agbo, I.C., Torres, C. and Chah, K.F. 2015.Characterization of mannitol-fermenting methicillin-resistant staphylococci isolated from pigs in Nigeria. Braz. J. Microbiol. 46, 885–892.

Wagner, S., Gally, D.L. and Argyle, S.A. 2014. Multidrug-resistant *Escherichia coli* from canine urinary tract infections tend to have commensal phylotypes, lower prevalence of virulence determinants and ampC-replicons. Vet. Microbiol. 169(3-4), 171–178.

Wieler, L.H., Ewers, C., Guenther, S., Walther, B. and Lübke-Becker, A. 2011. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these

potential zoonotic pathogens in clinical samples. IJMM 301(8), 635–641.

Wuytack, A., De Visscher, A., Piepers, S., Boyen, F., Haesebrouck, F. and De Vliegher, S. 2020. Distribution of non-*aureus* staphylo- cocci from quarter milk, teat apices, and rectal feces of dairy cows, and their virulence potential. J. Dairy Sci. 103, 10658–10670.

Yu, D., Chen, Y., Pan, Y., Li, H., McCormac, M.A. and Tang, Y.W. 2008. Case report: *Staphylococcus gallinarum* bacteremia in a patient with chronic hepatitis B virus infection. Ann. Clin. Lab. Sci. 38(4), 401–404.