



Fecal Carriage of *S. aureus* and the *mecA* Gene in Resident Wild Birds and Its Zoonotic Potential

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ABSTRACT

Resistant *Staphylococcus (S.) aureus* in general and MRSA, in particular, have received great attention in both veterinary and human health sectors. The importance of fecal carriage of staphylococci is rarely encountered. This study aimed to investigate the role of wild birds in Giza governorate, Egypt in spreading resistant *S. aureus* from winter 2019 to summer 2021. Cloacal swabs and fecal droppings were collected from different species of wild birds (rock pigeons, laughing doves, cattle egrets, and hooded crows). Isolation and identification of *Staphylococcus* spp. were performed using Columbia agar base with 5% defibrinated sheep blood and mannitol salt agar. Moreover, molecular detection of the *coa*, *nuc*, and *mecA* genes has been investigated via the polymerase chain reaction (PCR) assay. Out of 166 fecal samples examined, staphylococci had been confirmed in 100 samples (60.2%), with *S. aureus* representing 70% of the obtained staphylococci; however, non-aureus staphylococci represented the remaining 30% of the isolates. The *mecA* gene carriage was (57.1%) in *S. aureus*. This study highlighted the zoonotic potential of staphylococci isolated from resident wild birds in Giza, Egypt. Presences of such pathogenic microorganisms with their resistance traits around and in the human habitat add to the microbial community present around human dwellings in the study area. They may play a role in the spreading of various illnesses.

Keywords: *Coa* gene, *mecA* gene, MRSA, PCR, Wild birds.

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INTRODUCTION

Staphylococcus (S.) aureus is one of the clinically significant pathogens of humans and animals. This bacteria has been linked to various illnesses of various severity and has been found to be life-threatening, with fatality rates higher than those of AIDS, viral hepatitis, and tuberculosis combined (Van Hal *et al.*, 2012). The ability of Livestock-associated *S. aureus* to infect human populations and the community-associated *S. aureus* adds a considerable burden on the healthcare system (Dweba *et al.*, 2019).

Although antimicrobial resistance is usually more frequent in poultry, it has also been detected in bacteria isolated from wild birds (Benskin *et al.*, 2009). Free-living birds that are often encountered in the human environment naturally harbor harmful antibiotic-resistant microorganisms (Literak *et al.*, 2010; Wang *et al.*, 2017). This is especially noticed in crows due to their growing numbers and continuous

mobility that aids in the spreading of harmful pathogens (Literak *et al.*, 2010).

Nowadays, antimicrobial resistance has been identified by the world health organization (WHO) as one of the most serious threats to public health and food security (WHO, 2020). Moreover, methicillin-resistant *S. aureus* (MRSA) strains are a significant global health issue (Gajdacs and Zsoldiné Urbán, 2019). The *mecA* gene that encodes PBP2a (penicillin-binding protein 2a) is responsible for the majority of MRSA infections, and its detection helps in the identification of methicillin resistance in *S. aureus* (Shrestha *et al.*, 2002). According to CLSI 2020 guidelines, *S. aureus* strains that test positive for the *mecA* should be reported as MRSA (CLSI, 2020).

Furthermore, methicillin-resistant coagulase-negative staphylococci (MRCNS) have long been identified to cause human and animal diseases (Chen *et al.*, 2016). Meanwhile, special attention has been paid

to other coagulase-positive staphylococci and non-aureus staphylococci representing a significant threat. Although they are usually found as commensals in humans and animals, however exchange of these bacteria between animals and humans can sometimes cause severe or even lethal infections (**González-Martín et al., 2020**).

Despite being recognized as major reservoirs or carriers for transmission during the last decade, there has been a rising interest in MRSA incidence in wildlife. Still, little data are available (**Silveira et al., 2021**). Understanding MRSA's general epidemiology at the national level is essential for healthcare professionals and policymakers to support successful preventive and control initiatives. Pigeons, doves, and hooded crows are among wild birds extensively dispersed in Egypt. Because of their colonial nesting behavior around human dwellings, the vast majority of their excrement concentrate in these areas, contributing to the microbial load that may contain potentially harmful pathogens to human health.

Despite extensive research on MRSA in humans and animals, there is still a scarcity of data about the level of infection, carriage, and the zoonotic importance of this bacterium from wildlife. Here we report the carriage of the *mecA* gene in *S. aureus* isolated from droppings and cloacal samples of resident wild birds in Giza, Egypt, and highlight its zoonotic importance to public health.

MATERIALS AND METHODS

Ethical statement

Protocols for sample collection and laboratory examination for this study were reviewed and approved by Faculty of Veterinary Medicine, Cairo University's Institutional Animal Care and Use Committee (No. VetCU10102019087).

Sampling

A total of 166 fecal samples were collected from resident wild birds (rock pigeons, laughing doves, cattle egrets, and hooded crows) from different regions in Giza governorate, Egypt, during the period from winter 2019 to summer 2021. Traps were used to capture the birds and either cloacal swabs or the top surface of fresh droppings were obtained before the release of the birds from the net. Occasionally, a professional hunter was hired to shoot some crows and doves when the birds identified the traps at the collection sites and avoided them. Samples were transported in an ice box as soon as possible and a microbiological examination was performed within 24

h in Zoonoses Department Research Laboratory, Faculty of Veterinary Medicine, Cairo University.

Isolation and identification of *S. aureus*

Isolation and identification of *S. aureus* were carried out as described earlier (**Quinn et al., 2011**). Fecal specimens were incubated aerobically into 9 ml of brain heart infusion broth (Oxoid, Hampshire, UK) at 37°C for 12-24 h. For each sample, two loops from the incubated broth were streaked on Columbia agar base supplemented with 5% defibrinated sheep blood (Oxoid, Hampshire, UK) and mannitol salt agar (Oxoid, Hampshire, UK). Both plates were incubated at 37°C ± 1°C and the characteristic growth on each medium was recorded after 24-48 h.

Isolates were presumed to be staphylococci based on colony morphology, catalase response, Gram staining, and oxidative-fermentative tests. Following the identification of the genus *Staphylococcus*, the enzyme coagulase was identified in all isolates using slide and tube methods (**Quinn et al., 2011**). Coagulase-negative isolates that showed resistance to methicillin (based on detection of the *mecA* gene, see below) were submitted to species identification using the API-Staph Kit (BioMerieux, France) as described before (**Petzer et al., 2013**). A single pure colony from each identified strain was kept on brain heart infusion broth for additional testing and PCR analysis.

Molecular confirmation of *S. aureus* isolates and detection of the *coa* and *mecA* genes

All staphylococci isolates were refreshed on mannitol salt agar plates at 37°C overnight before DNA extraction. A single bacterial colony was picked from each plate and placed in 200 µl deionized distilled water. The QIAamp Mini DNA Extraction Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA according to the manufacturer's instructions. Primer sequences of the *coa* gene specific for coagulase production, the *nuc* gene specific for *S. aureus*, and the *mecA* gene specific for methicillin resistance in staphylococci were used in conventional PCR protocols previously described (Table 1).

The reactions were carried out in 25 µl reaction mixtures containing 5 µl of DNA as a template, 1 µl (20 pmol) of each primer, 12.5 µl of 1× PCR master mix (Dream Taq Green PCR Master Mix, Fermentas Life Science) and 5.5 µl molecular grade water. Expected amplification bands were photographed using Gel documentation system (Alpha Innotech) after PCR amplification products had been electrophoresed through 1.5% agarose gel (Sigma, USA) with ethidium bromide (0.5 µg ml⁻¹) (Sigma, USA) in 1x TBE buffer.

Table 1: Primer sequences and expected product sizes of *S. aureus* target genes.

Target gene	Sequence	Cycling conditions	Amplified product size	Reference
<i>nuc</i>	5'-GCGATTGATGGTGATACGGTT-3' 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	Initial denaturation: 94°C/5 min. 35 cycles: 94°C/30 s, 55°C/30 s, 72°C/60 s Final extension: 72°C/10 min.	270 bp	(Al-Amery et al., 2019)
<i>coa</i>	5'ATAGAGATGCTGGTACAGG-3' 5'GCTTCCGATTGTTTCGATGC-3'	Initial denaturation: 94°C/45 s 30 cycles: 94°C/20 s, 57°C/15 s, 70°C/15 s Final extension: 72°C/2 min.	750 bp	(Hookey et al., 1998)
<i>mecA</i>	5'-GTGAAGATATACCAAGTGATT-3' 5'-ATGCGCTATAGATTGAAAGGAT-3'	Initial denaturation: 94°C/4 min. 35 cycles: 94°C/60 s, 55°C/60 s, 72°C/60 s Final extension: 72°C/10 min.	147 bp	(Zhang et al., 2005)

RESULTS

Out of 166 fecal samples from wild birds, based on phenotypic (culture characteristics, slide and tube coagulase testing), API kit testing and genotypic identification of the *coa* gene, staphylococci were detected in 100 fecal samples (60.2%). *S. aureus* predominated among the recovered *Staphylococcus* spp. (70 isolates, 70%), while other non-aureus staphylococci were detected at a lower level (30 isolates, 30%) (Table 2). *S. cohnii* was the most frequent (16%) coagulase-negative *Staphylococcus* spp. (table 2). The occurrence of the *mecA* gene in *S. aureus* isolates carried by wild birds was (57.1%, 40/70).

Table 2. *Staphylococcus* spp. isolates detected based on coagulase testing, API kits, and *coa* and *nuc* gene detection:

<i>Staphylococcus</i> spp.	Staphylococci isolates	Total n=100 (%)
<i>S. aureus</i> (n=70)	<i>S. aureus</i>	70 (70)
CN staphylococci (n=30)	<i>S. chromogenes</i>	4 (4)
	<i>S. simulans</i>	3 (3)
	<i>S. haemolyticus</i>	3 (3)
	<i>S. epidermidis</i>	4 (4)
	<i>S. cohnii</i>	16 (16)

DISCUSSION

This study aimed to investigate the fecal carriage of resistant *S. aureus* by wild birds in Giza, Egypt. Some studies that have been conducted in Egypt have surveyed the presence of several zoonotic pathogens in wild birds (Ahmed et al., 2019; El Taweel et al., 2020; Nabil et al., 2020); however, very few studies have reported the presence of staphylococci from wild birds and its probable zoonotic importance.

Staphylococci have been identified as an appropriate model for "One Health" investigations, as some species and clones have been demonstrated to "jump" through the three ecosystems of interest (human, animals, and the environment) (Abdullahi et al., 2021). In the present study, staphylococci were detected in 60.2% (100/166) of the wild birds' fecal samples, among which *S. aureus* (70%, 70/100) were phenotypically and genotypically identified. Fecal carriage of staphylococci is not frequently encountered among birds generally and wild birds in particular. However, in a recent study, staphylococci were detected (45.9%) in cloacal samples from wild birds from street markets in Rio de Janeiro, Brazil, with *S. aureus* representing 11% of the total staphylococci (Matias et al., 2018).

Also, *S. aureus* has been identified in faeces of corvids, marine and migratory birds in an earlier study (Hubálek, 2004). Moreover, *Staphylococcus* spp. (*S. aureus*, *S. Sciuri* and *S. saprophyticus*) had recently been detected in droppings of two migratory seabird species along the coastal shores of the Gulf of

California, with *S. aureus* detected at a rate of 6.8% in Heermann's Gulls and 5.9% in Elegant Terns (Contreras-Rodríguez *et al.*, 2019). Despite the usual presence of some of these agents as commensals, detecting such pathogenic bacterial species from wild birds around human dwellings highlights their significance in spreading illnesses to inhabitants, especially if these bacteria carry virulence or antimicrobial resistance determinants.

Coagulase-negative staphylococci (CoNS); *S. haemolyticus*, *S. chromogenes*, *S. simulans*, *S. hyicus*, *S. hominis*, *S. saccharolyticus*, *S. carnosus*, and *S. lugdunensis* were isolated from human and domestic mammalian hosts as reported earlier (Becker *et al.*, 2014). Although CoNS may be recovered at a lower level, special attention should be paid to these species due to their opportunistic behaviour. In this regard, *S. haemolyticus* has been linked to septicemia, human endocarditis, and urinary tract infection (Kloos and Bannerman, 1994). Similar to results in the present study, but at a lower level, *S. chromogenes*, *S. haemolyticus*, and *S. simulans* were isolated from cloacal swabs of wild birds in Rio de Janeiro, Brazil (Matias *et al.*, 2018).

The spread of antimicrobial resistance through staphylococci is a significant problem both in veterinary and human medicine, posing a global challenge since some pathogenic species have developed resistance to most antibiotics that limit the therapeutic choices. The appearance of MRSA has become a global public health issue, with these resistant strains identified from wild animal species and birds (Contreras-Rodríguez *et al.*, 2019; Matias *et al.*, 2018; Monecke *et al.*, 2016; Silveira *et al.*, 2021).

Most methicillin resistance in *S. aureus* is controlled by the *mecA* gene that encodes PBP2a (penicillin-binding protein 2a), which is reported to be the primary cause of the penicillin and methicillin resistance (Pournaras *et al.*, 2015). Due to prolonged COVID-19 lockdown, we can't revive staphylococci isolates for antimicrobial sensitivity testing which was an unintentional limitation to this study. However, alternatively, the presence of the *mecA* gene responsible for methicillin resistance in *S. aureus* was assessed.

In the present study, the *mecA* gene was detected in 57.1% (40/70) *S. aureus* isolates. Similarly, the *mecA* gene (22%) had been detected in *S. aureus* and some coagulase-negative staphylococci recovered from wild bird cloacal samples in Rio de Janeiro, Brazil (Matias *et al.*, 2018). On the other hand, along the shores of Gulf of California, although coagulase-positive staphylococci had been recovered from

droppings of migratory seabirds (Heermann's Gulls and Elegant Terns), none of them carried the *mecA* resistance gene (Contreras-Rodríguez *et al.*, 2019). This may be related to the nature of the surrounding environment and the microbial load where birds from these studies are distributed.

Overall, the dissemination of such resistant bacteria is merely through anthropogenic sources such as industrial and household wastewater effluents, runoff from agriculture, and garbage, between wild animals and the human environments. Once transmitted to wild animals, some bacteria can then be responsible for disseminating various resistance genes, mobile genetic elements, and epidemic clones to several places (Rousham *et al.*, 2018; Silveira *et al.*, 2021).

CONCLUSION

This study highlighted the zoonotic importance of staphylococci isolated from resident wild birds in Giza, Egypt. The presence of such pathogenic microorganisms with their resistance traits around and in the human habitat has public health implications. It may play a role in spreading various illnesses and resistant bacteria. There is an urgent need to develop control systems to restrict bacterial spread throughout different ecosystems, minimize the emergence of more antimicrobial resistance, and maintain the efficacy of presently available antibiotics. Implementation of regular monitoring policies in different environments is necessary to have a clear image of the role of other wild species in transmitting certain zoonotic agents to humans.

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Declaration of Conflicting Interests

The authors declare that they have no competing interests.

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