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Molecular study on methicillin-resistant *Staphylococcus aureus* strains isolated from dogs and associated personnel in Jordan

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ABSTRACT

Objective: To determine the prevalence, genetic relatedness, and pattern of antimicrobial susceptibility in methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) isolated from household dogs, farm dogs, and stray dogs, compared to isolates from their associated personnel.

Methods: MRSA was isolated from 250 nasal swabs (150 swabs from dogs and 100 swabs from humans). PCR assays were used to detect the presence of both the *nuc* and *mecA* genes, which confirmed the identity of *S. aureus* isolates and the presence of methicillin resistance, respectively. Disk diffusion was used to determine the antibiotic susceptibility against 15 antimicrobial agents along with an *E*-test that determined the minimum inhibitory concentration for oxacillin. Pulsed field gel electrophoresis was conducted to determine the genetic relatedness of MRSA isolates from dogs to those from associated and unassociated personnel.

Results: The prevalence of *S. aureus* in dogs and humans was 12.7% and 10.0% respectively, while the prevalence of MRSA isolates in dogs and humans was 5.3% and 5.0%, respectively. The prevalence of MRSA isolates in household dogs, farm dogs, and stray dogs was 7.8%, 4.7%, and 0.0%, respectively. MRSA isolates demonstrated a significantly higher rate of multi-resistance against three or more antimicrobial agents than methicillin-susceptible *S. aureus* (MSSA). Trimethoprim-sulphamethoxazole and chloramphenicol were the most effective antibiotics against all MRSA isolates. Pulsed field gel electrophoresis revealed a strong association between dog MRSA isolates and MRSA isolates from strongly associated personnel.

Conclusions: MRSA is prevalent in house dogs, as well as in dog rearing centers and among their strongly associated personnel. A strong association was found between the MRSA isolates from dogs and those from humans who are in close contact. In addition, MRSA isolates showed a high rate of multi-resistance compared to MSSA isolates.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) is a major health care-associated pathogen worldwide and has increased in incidence dramatically over the last decade [1,2]. Companion animals have been implicated more frequently as potential reservoirs of MRSA than other livestock [3,4]. In several studies, a 0%–4% prevalence rate of MRSA in dogs has been reported [5–7]. Other reports demonstrated MRSA at a higher prevalence (~9%) in pets and veterinary staff [8,9], and the nasal carriage of MRSA plays a key role in the epidemiology and pathogenesis of community-associated infections [10,11].

In Jordan, MRSA is widely prevalent in Jordanian hospitals and represents a serious public health problem. The nasal carriage rate of *S. aureus* among the Jordanian healthy young population was 40%, and 19% of the nasal *S. aureus*, and 57% of clinical isolates were resistant to oxacillin [12]. A retrospective study conducted at King Abdullah University Hospital in North Jordan showed that 152 *S. aureus* isolates collected from different infections revealed that the overall rate of MRSA

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was 34%, with a rate of 57%–70% in the adult intensive care unit [13]. To our knowledge, no studies on MRSA in companion animals in Jordan were found in the literature that demonstrated its prevalence and health hazard. Therefore, this study was conducted to document the prevalence of MRSA in dogs and their associated personnel, as well as to determine their genetic relatedness and antimicrobial resistant profile.

2. Materials and methods

2.1. Sample collection, transportation, and preparation

In total, 250 nasal swabs were collected from 150 dogs and 100 humans during a period between March and the end of October 2009. The 150 dog samples were collected from household dogs, stray dogs, and farm dogs from the middle and northern parts of Jordan. The total numbers of household dogs, stray dogs, and farm dogs were 77, 30, and 43, respectively, as illustrated in Table 1.

The 100 human nasal swab samples were collected from personnel strongly associated with dogs (5 from dog owners, 50 from employees who feed, take care of, treat, and train dogs daily at the Spana Welfare Center, Humane Center for Animal Welfare, and K-9 Center, 25 from intermediately associated personnel, including veterinarians working in clinics and veterinary students, and 20 from unassociated personnel who have never been in contact with dogs). A sterile cotton swab moistened with normal saline was inserted into the nares and gently rotated to make contact with the nasal septum. For dogs, smaller swabs were inserted to a distance of about 0.5–1.0 cm. All swabs were placed in a transport medium and stored at 4 °C until cultured within 6-h collection at the Microbiology Research Laboratory, Faculty of Veterinary Medicine, Jordan University of Science and Technology.

2.2. Isolation and identification of S. aureus

All nasal swabs were cultured on mannitol salt agar (Oxoid, UK) and incubated aerobically at 37 °C for 24–48 h. The cultures were then examined for the presence of *S. aureus* (yellow colonies) and for a microscopic appearance after Gram staining. The presumptive *S. aureus* isolates were further examined for pigments and coagulase production, by using the tube method [14].

Table 1
Distribution of the dogs' nasal swab samples according to the dogs' locations and type of rearing system in Jordan.

Middle zone	Household dogs	Stray dogs	Farm dogs*
Amman governorates			
Swelieh city	5	4	6
Jaweh town	0	5	5
Sahaab town	0	6	7
Spana Welfare Center	13	0	0
Dogs Police K-9 Center	27	0	0
Humane Center for Animal Welfare	14	0	0
Al-Zarga governorates	0	4	8
Northern zone			
Ramtha dogs Police K-9 Center	18	0	0
Jarash governorates	0	4	6
Ajlune governorates	0	3	4
Irbid governorates	0	4	7
Total	77	30	43

*: Dogs kept with sheep and goat flocks.

2.3. Molecular identification of S. aureus isolates

2.3.1. DNA extraction and identification of the nuc gene

The extraction protocol was done according to the Wizard genomic DNA purification kit (Promega cooperation, Technical manual genomic DNA purification part TM0580, Madson, USA). Then, presumptive *S. aureus* isolates were tested by PCR amplification of the *nuc* gene [15]. PCR amplification was conducted at a final volume of 25 μL [12.5 μL of Go Taq master mix (Promega, USA), 5 μL (2.5 pmol) of each primer F (GCGATTGATGGTGA TACGGTT) as well as R (AGCCAAGCCTTGACGAACT AAAGC), 2.5 μL of a bacterial DNA sample and 5 μL nuclease free water]. The PCR amplification was conducted as follows: 5 min at 94 °C, 35 cycles for 30 s at 94 °C, 45 s at a corresponding annealing temperature of 55 °C, and 45 s at 72 °C, and a final extension of 10 min at 72 °C. The PCR products were observed on 1.5% agarose gels.

2.4. Identification of MRSA by E-test and antimicrobial susceptibility testing

MRSA isolates were identified by *E*-test (Oxoid, UK), which is a gradient antibiotic stabilized on a plastic strip with 30 graduations to provide an accurate minimum inhibitory concentration (MIC) over a range of 256–0.015 μg/mL. This test was conducted for oxacillin only, according to the manufacturers' instructions (Oxoid, UK) and guidelines. Mueller-Hinton agar (Difco, Detroit, MI, USA) supplemented with 2% NaCl was used for this purpose [16]. Samples for the *E*-test were prepared according to Clinical and Laboratory Standards Institute [17]. Isolates showed MICs equaled to or greater than 4 μg/mL, which were considered MRSAs [18].

The agar disk diffusion susceptibility test of 15 antimicrobials [cefoxitin (10 μg), penicillin (10 IU), cephalexin (30 μg), kanamycin (30 μg), gentamicin (10 μg), tobramycin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), azithromycin (15 μg), erythromycin (15 μg), tetracycline (15 μg), amoxicillin-clavulanic acid (20/10 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg), nalidixic (30 μg), and chloramphenicol (30 μg)] was carried out by using the Clinical and Laboratory Standard Institute guidelines [19]. The *S. aureus* ATCC 25923 strain was used as a control.

2.5. Molecular identification of MRSA

For further confirmation, MRSA isolates were tested by PCR amplification of the *mecA* gene. PCR amplification was conducted at a final volume of 25 μL [12.5 μL of Go Taq master mix (Promega, USA), 5 μL (2.5 pmol) of each primer F (5′-GCA ATC GCT AAA GAA CTA AG) as well as R (5′-GGG ACC AAC ATA ACC TAA TA) [20], 2.5 μL of a bacterial DNA template and 5 μL nuclease free water]. PCR amplification was conducted as follows: denaturation at one cycle of 94 °C for 3 min, 30 cycles at 94 °C for 45 s, annealing at 53 °C for 2 min, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR products were observed by electrophoresis on 1.5% agarose gels (Nusieve Bioproducts, Maine, USA).

2.6. Pulsed field gel electrophoresis (PFGE)

PFGE was used to study the genetic relatedness between the *S. aureus* isolates from dogs and human *S. aureus* isolates. PFGE was performed according to a Canadian standard protocol