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Nasal colonization and antibiotic resistance patterns of *Staphylococcus* species isolated from healthy horses in Tripoli, Libya

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The present study investigated the colonization rates and antimicrobial susceptibility of Staphylococcus species isolated from the nostrils of healthy horses. A nonselective laboratory approach was applied, followed by confirmation using a Phoenix automated microbiological system. Among the 92 horses included in the study, 48.9% (45/92) carried Staphylococcus species of mostly the coagulase-negative staphylococci (CoNS) type yielding 70 Staphylococcus strains. Of these strains, 37.1% (26/70; 24 CoNS and 2 coagulase-positive staphylococci; CoPS) were identified as methicillin-resistant staphylococci (MRS) expressing significant resistance to important antimicrobial classes represented mainly by subspecies of CoNS. This is the first study reporting a high prevalence of various Staphylococcus species, particularly strains of CoNS expressing multidrug resistance patterns of public health concern, colonizing healthy horses in Libya.

Key words: *antimicrobial resistance, coagulase-negative staphylococci, healthy horse, nasal colonization, Libya*

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Methicillin-resistant staphylococci (MRS) of clinical and public health concern have been increasingly reported in horses [17]. They have been reported in the skin and nasal passages of horses infected with diverse staphylococci causing opportunistic and zoonotic infections, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) [18, 19, 22]. These pathogens are widely reported to have variable geographic distributions worldwide in clinical and healthy horses [16].

The current study investigated the prevalence and antimicrobial susceptibility of *Staphylococcus* species isolated from the nostrils of 92 healthy horses from four locations in Tripoli in January–February 2018. The inclusion criteria for the horses were no signs of any illnesses and no treatment with any medications, including antimicrobials, for at least three months prior to this study. The ages of the included horses ranged from 0.3 to 24 years (mean, 7.5 years), and the sex distribution was 77.2% (n=71) female and 22.8% (n=21) male. The breeds of the horses included Thoroughbreds (n=81, 88.0%), English (n=3, 3.3%), Arabians (n=1, 1.1%), and half-breeds (n=1, 1.1%). The remaining 6 (6.5%) horses were of unspecified breeds. The study was approved and registered by the Postgraduate Studies Department of the Ministry of Education, Libya (Reference number, 14144). The purpose of the study and benefits of participation were explained to all owners before the study, and informed consent was obtained.

A total of two nasal specimens were obtained from the nostrils of each horse using moist sterile cotton-tipped (in-house) swabs. Each swab was inserted approximately 10 cm, pressed slightly against the mucosa, and then transferred to the laboratory and processed within 4 hr.

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Samples were streaked onto mannitol-salt agar and blood agar, respectively, and then incubated for 24–48 hr at 35°C. A typical colony was selected from each plate and further examined with a Gram stain and catalase test. Presumptive staphylococci isolates were further tested with a BD Phoenix automated identification and susceptibility testing system (PAMS, MSBD Biosciences, Sparks, MD, U.S.A.) for definite characterization at the genus and species levels and to determine the susceptibility against antimicrobial agents.

The antimicrobial susceptibility profiles of confirmed staphylococci and the decision of MRS were carryed out based on the interpretation of the Phoenix system according to the CLSI guidelines [10]. The Fisher's exact test was used to analyse data and compare the proportions of colonization rates of *Staphylococcus* species. *P*-values ≤0.05 were considered statistically significant.

Staphylococcus species were recovered from 48.9% (45/92) of the horses (mean age, 7 years; range, 0.4 to 23 years). The rate of colonization in male horses was 33.3% (7/21), whereas it was 53.5% (38/71) in female horses (*P*>0.05). In total, 27.2% (25/92) of the horses tested positive in both nostrils, whereas 21.7% (20/92) of the horses tested positive carrier in a single nostril. Among the total positive horses, 91.1% (41/45) were colonized with a single type of staphylococci; 84.4% (38/45) of the horses were only colonized with CoNS, whereas 6.7% (3/45) of the horses were only colonized with CoPS. The remaining 8.9% (4/45) of the horses were colonized with different *Staphylococcus* species.

Furthermore, 46.7% (21/45) of the colonized horses carried two similar species of staphylococci (20 horses carried two different CoNS species, and one horse carried two different CoPS species), whereas 44.4% (20/45) carried a single species of staphylococci only (18 horses with a CoNS species and 2 horses with a CoPS species; *P*>0.05). The process yielded 70 *Staphylococcus* strains, of which 37.1% (26/70) expressed typical MRS phenotypes representing 21.7% (20/92) of the total horses; 18 of the 20 horses were CoNS carriers (8 carrying a single species; 10 carrying two species), and the other two (n=2) comprised a CoPS carrier and a horse carrying a different species. The 70 *Staphylococcus* species were represented by 17 different species of staphylococci, including 15 species of CoNS (n=62) and two subspecies of CoPS (n=8; Table 1). Furthermore, the 37.1% (26/70; 24 CoNS and 2 CoPS) of the strains expressing MRS phenotypes were found to have similar antibiogram profiles (Table 2).

The present study revealed high colonization rate of staphylococci compared with regional data [12]; 27.2% of the horses were colonized in both nostrils, and 21.7% of the horses were colonized in a single nostril, mostly by CoNS, which accounted for 84.4% of the horses testing positive. Strains of the *S. sciuri* group and *S. xylosus* are commensal bacteria of the skin and mucous membranes of different animal species, particularly horses, causing opportunistic infections in animals (e.g., mastitis or dermatitis) and zoonotic infections in humans in direct contact with them [14, 25]. *S. xylosus* is frequently isolated from animal products (e.g., cheese, milk, and meat products) and further used in the development of flavour and food processing [11].

Table1. Proportion and features of isolated *Staphylococcus* species

Staphylococcus spp.	No. of isolates	No. of MRS
S. xylosus	12	2
S. sciuri	8	2
S. equorum	8	2
S. lentus	5	5
S. simulans	5	0
S. gallinarum	5	0
S. chromogenes	4	4
S. saprophyticus	3	2
S. felis	2	2
S. warneri	2	0
S. pasteuri	2	2
S. haemolyticus	2	2
S. schleiferi	2	0
S. carnosus	1	1
S. kloosii	1	0
Total of CoNS	62	24
S. aureus	5	2
S. intermedius	3	0
Total of CoPS	8	2

MRS, methicillin-resistant staphylococci; CoPS, coagulase-positive staphylococci; CoNS, coagulase-negative staphylococci.

In Africa, reports of MRS and MRSA in equine populations are very rare, and the reported colonization rate in the current study is higher compared with that in a regional report [12]. In the current study, most of the horses colonized with MRS were colonized with the CoNS group, mainly represented by the *S. sciuri* group. Such finding has reportedly been linked to antibiotic selection pressure as well as a previous history of prolonged antibiotic treatments, hospitalization, and transportation stress [20–22].

Horses are frequently colonized with diverse CoNS strains found in healthy and clinical animals showing concerning multidrug resistance phenotypes with variable epidemiological distribution [1]. For instance, higher prevalences of CoNS and MRCoNS and no/low prevalence of MRSA have been reported in healthy horses in the Netherlands [7, 9]. In Africa, 6 to 68% of suspected human infections and 3% to 61.7% of suspected animal infections are reported to be caused by CoNS (e.g., *S. epidermidis*, *S. haemolyticus, S. capitis*, *S. lugdunensis*, and *S. xylosus*)

Table 2. Antimicrobial susceptibility profiling of 26 methicillin-resistant staphylococci (MRS) strains

Staphylococcus spp.	Resistant	Intermediate	Susceptible
S. lentus (MLSB)	IPM, FOX, CTX, AMP, PenG, OXA, AMX	ERY, CLI	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET
S. saprophyticus	IPM, FOX, CTX, AMP, PenG, OXA, AMX, FUS	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. saprophyticus	IPM, FOX, CTX, AMP, PenG, OXA, AMX, FUS	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. xylosus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. chromogenes	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. lentus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. chromogenes	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. chromogenes	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. pasteuri (MLSB)	IPM, FOX, CTX, AMP, PenG, OXA, AMX, CLI, ERY	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET
S. lentus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. lentus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. schleiferi	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. equorum	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. carnosus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. pasteuri	IPM, FOX, CTX, AMP, PenG, OXA, AMX	ERY	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, CLIN
S. sciuri	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. lentus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. xylosus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. aureus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. schleiferi	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. aureus	IPM, FOX, CTX, AMP, PenG, OXA, AMX	CLI	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY
S. equorum	IPM, FOX, CTX, AMP, PenG, OXA, AMX	CLI	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY
S. felis	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. sciuri	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. felis	IPM, FOX, CTX, AMP, PenG	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN
S. chromogenes	IPM, FOX, CTX, AMP, PenG, OXA, AMX	N	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, ERY, CLN

The 26 typical MRS isolates originated from 20 horses; 18 were CoNS carriers, and two were carriers of CoPS and a different species, respectively. N, not available; IPM, Imipenem; FOX, cefoxitin; CTX, cefotaxime; AMP, ampicillin; PenG, penicillin G; AMX, amoxicillin and clavulanic acid; OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; STX, trimethoprim-sulfamethoxazole; GEN, gentamicin; CIP, ciprofloxacin; NFZ, nitrofurazone; MUP, mupirocin; FUS, fusidic acid; LZD, linezolid; RIF, rifampin; DAP, daptomycin; VAN, vancomycin; TEC, teicoplanin; MXF, moxifloxacin.

mainly from food-producing animals, with limited available information on companion and pet animals [5]. In Libya, MRSA is the most reported nosocomial pathogen exclusively isolated from human healthcare settings; however, critical multidrug-resistant Gram-negative rods and vancomycin-resistant enterococci (VRE) have recently emerged [2–4]. A novel recent study from Libya involving healthy and clinical cats and dogs revealed high colonization rates of various *Staphylococcus* species showing high multidrug (i.e., methicillin) resistance patterns and belong mainly to CoNS species (MRCoNS) [13].

CoNS are recognized as a reservoir of virulent and antibiotic resistance genes that can be acquired by other staphylococci mainly through the transconjugant transfer of the staphylococcal cassette chromosome *mec*(SCC*mec*) transposon containing the *mecA* gene, as in the case of transfer between *S. aureus* and *S. epidermidis* [24]. Another *mec* gene homolog, which is currently designated as *mec*C and has about 70% comparability with the *mec*A gene, was identified in 2011, and it carried by SCC*mec* elements isolated from animals, human clinical specimens, and the environment [8]. Unfortunately, due to the limitations of the current study, these important and widely reported genes were not investigated within the studied collection.

Antimicrobial susceptibility testing of *Staphylococcus* species could provide empirical data to guide therapy and overcome recurrent infections; however, other factors should be taken into consideration, such as the infection site, infection type, age, and health status [1]. For instance, the response to fluoroquinolone therapy for MRS is unpredictable despite *in vitro* susceptibility, and resistance may develop during antibiotic therapy [23]. Although suitable antimicrobials, such as chloramphenicol and trimethoprim-sulfonamide can be used, the use of other critically important drugs, such vancomycin, linezolid, mupirocin, rifampin, and fusidic acid, should be limited due to the controversial nature of their use in horses and importance to human medicine. In addition, controlling the colonization of MRS in horses is problematic because transient colonization can be normal in horses, and thus decolonization with an antimicrobial therapy is not recommended [23].

Staphylococcus species of veterinary origin are difficult to characterize due to the lack of developed diagnostic protocols [15]. The automated Phoenix system has been widely used as an effective tool to identify species of staphylococci and determine antimicrobial susceptibility; however, a few species are not easy to identify [6]. For instance, *S. pseudintermedius* is frequently misdiagnosed as *S. aureus* due to their close phenotypic characteristics, which require advanced molecular protocols for a definite identification, such as PCR and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technology [15]. Furthermore, the current findings reveal the need to follow therapeutic guidelines and control and prevention measures to minimize the spread of *Staphylococcus* species with antimicrobial resistance. Further analyses of MRS colonization and transmission and the associated risk factors are required in equine medicine adopting the One Health concept.

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