Short Communication

Nasal Colonization and Antibiotic Resistance of Staphylococcus Species Isolated from Healthy Veterinary Personnel at Veterinary Medical Care Facilities in Tripoli

Mohamed Omar Ahmed, Aesha A. Othman, Mohamed Salah Abbassi[1], Hiam R. Elnageh, Nariman F. Almshawt, Yousef M. Abouzeed, Murad A. Hiblu[2]

Departments of Microbiology and Parasitology and [2]Internal Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya, [1]Institute of Veterinary Research of Tunisia, University of Tunis El Manar, Bab Saadoun, Tunis, Tunisia

Abstract

Background/Aim: Veterinary medical personnel may carry important antibiotic-resistant organisms playing important role in their dissemination and emergence. The purpose of the study was to investigate nasal colonization and antibiotic resistance of *Staphylococcus* species isolated from veterinary personnel (VP). **Methods:** A total of 47 VP were sampled, whereby nasal samples were subjected to selective and typical laboratory protocols. Presumptive isolates were further confirmed and fully characterized by the Phoenix automated microbiological system then further tested by polymase chain reactions for *mecA* and panton-valentine leukocidin (*pvl)* genes. **Results:** A total of 34 (72%) VP were colonized with various species, mostly coagulase-negative staphylococci. A collection of 34 staphylococci isolates were collected of which 21% and 6% were, respectively, positive for *mecA* and *pvl* genes expressed exclusively by *Staphylococcus aureus* and *S. epidermidis*. **Conclusion:** VP may carry various staphylococci species of public health importance expressing multidrug resistant and virulent traits. Preventative measures and continuous monitoring are required to control the spread of methicillin-resistant staphylococci in veterinary clinics.

Keywords: Libya, methicillin-resistant staphylococci, *mecA*, panton-valentine leukocidin, *staphylococcus*, veterinary personnel

Introduction

Staphylococcus species are commensal bacteria of mammals birds and frequently responsible for opportunistic and hospital-acquired infections in humans.[1] Staphylococci species are frequently associated with animal skin and soft-tissue infections and have been mainly associated with pathogenic coagulase-positive staphylococci (CoPS), with less attention to the coagulase-negative staphylococci (CoNS).[2] Similar to human health-care settings, veterinary clinics have created similar conditions contributing to the emergence and spread of hospital-acquired and antibiotic-resistant pathogens.[3] These settings may also play a role in the circulation and further evolution of pathogenic and multidrug-resistant organisms in humans, animals, and the environment.[4]

Methicillin-resistant staphylococci (MRS) are known public health pathogens and are involved in the community and health-care settings.[5,6] These are also reported from companion and food animals and veterinary personnel (VP) showing distinctive epidemiological characteristics causing serious outbreaks and persistent infections.[7,8] However, the available epidemiological and clinical information on such dynamics are limited particularly during the nonoutbreak periods. The objective of the current study was to investigate the colonization rate and antimicrobial resistance of *Staphylococcus* species isolated from VP working at veterinary clinics in Tripoli. The involved clinics provide different medical and health services for pet and companion animals.

Address for correspondence: Dr. Mohamed Omar Ahmed, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, P.O. Box 13662, Tripoli, Libya.
E-mail: a.mo@live.com

Access this article online

Quick Response Code:

Website: www.ljmsonline.com

DOI: 10.4103/ljms.ljms_53_21

How to cite this article: Ahmed MO, Othman AA, Abbassi MS, Elnageh HR, Almshawt NF, Abouzeed YM, *et al*. Nasal colonization and antibiotic resistance of staphylococcus species isolated from healthy veterinary personnel at veterinary medical care facilities in Tripoli. Libyan J Med Sci 2021;5:128-31.

Submission Date: 20-08-2020, **Revision Date:** 28-08-2021,
Acceptance Date: 31-08-2021, **Publication Date:** 11-10-2021

Methods

During April 2018, 47 VP volunteered from 13 veterinary clinics (mean age was 38.8 years; ages ranged from 22 to 56 years). VP were involved on the basis that they were neither suffering from any clinical conditions nor under therapeutic treatments, including antibiotic drugs, at least 3 months before sampling. A nasal sample was obtained from each participant using a moist cotton swab and processed in the laboratory within 2h. Each swab was streaked directly onto mannitol salt agar and incubated aerobically at 35°C for 48h. A typical colony was selected from each plate and grown overnight on Columbia blood agar at 35°C for 24h; afterward, presumptive laboratory identification was performed by Gram stain, catalase, and coagulase tests. Presumptive isolates (regardless of coagulase productivity) were further tested by the BD Phoenix automated microbiology system (BD Diagnostic Systems, Sparks, MD, US) for definitive confirmation at the species level and for the determination of susceptibility to antimicrobial agents. Staphylococci species were subjected to polymerase chain reaction (PCR) adapted protocols and screened for *mecA* and Panton-valentine Leukocidin (*pvl)* genes.[9,10] The significant difference of colonization between the methicillin resistance staphylococci was carried out using Epi Info™ of the Center for Disease Control and Prevention ($P \leq 0.05$).

Results

Staphylococcus species were recovered from 72% ($n = 34/47$) of VP yielding 34 staphylococci isolates represented by five subspecies of coagulase-negative staphylococci (62%; $n = 21$ of 34) and one subspecies of coagulase-positive staphylococci (38%; $n = 13$ of 34). These respectively included *S. epidermidis* (47%; $n = 16$), *S. aureus* (38%; $n = 13$), *S. warneri* (6%; $n = 2$), *S. lugdunensis* (3%; $n = 1$), *S. cohnii* (3%; $n = 1$) and *S. auricularis* (3%; $n = 1$). PCRs confirmed 15% (7 of 47) of VP were carriers of *mecA*-MRS with no significant difference between MRCoPS and MRCoNS carriage ($P > 0.05$). In total, 21% ($n = 7/34$) of the isolates were positive for *mecA*; four *S. aureus* and three *S. epidermidis*. Furthermore, 6% ($n = 2/34$) of isolates expressed the *pvl* gene expressed exclusively by *S. aureus*. The multidrug resistance phenotype (>4 antibiotic classes) was expressed exclusively by *S. epidermidis* and *S. aureus*. A *mecA*-*S. epidermidis* isolates expressed extended MDR resistance including to trimethoprim-sulfamethoxazole, nitrofurantoin, and ciprofloxacin [Table 1]. Two *S. aureus* expressed the MLS_B resistance phenotype but were negative for PCRs. No resistance was detected against mupirocin, fusidic acid, linezolid, rifampin, and daptomycin.

Discussion

Colonization with *Staphylococcus* species is considered an early step in the pathogenesis of infections.[11] The colonization rate of *S. aureus* and *pvl*-*S. aureus* among VP was reported at 39% and 28%, respectively, mainly expressing the *mecA* gene among the characterized MRS strains.[12] Recent studies have estimated that between 7% and 15% of VP may carry MRSA but vary significantly between regions and countries.[8,13] In the current study, 72% of VP were found to be positive carriers for different staphylococci, mostly of the CoN group. Also, 15% and 4% of VP carried MRS-*mecA* and MRSA *pvl*-isolates exclusively carried by *S. aureus* and *S. epidermidis* species.

MRSA may circulate simultaneously between the community and health-care settings leading to the emergence of evolved and more virulent clones.[14,15] In veterinary hospitals, MRSA can be introduced continuously and circulate for up to 9 months, causing postsurgical outbreaks and occupational transmission.[16-18] The identification of VP carrying *pvl*-MRSA strains in the current study is worrisome due to their documented virulent role and association with severe clinical complications.[14,19] In addition, the frequency rate of *mecA*-and *pvl*-MRSA among VP may reflect the level of exposure to pets and companion animals[20] but also raises serious concerns regarding the spread of MRS and *pvl* positive staphylococci within human populations. In Libya, MRSA has been exclusively isolated from human clinical samples carrying *mecA* and *pvl* genes belonging to the frequently reported global clones (i.e., CC5 and CC80).[19] In addition, the recent emergence of critical hospital-acquired pathogens (i.e., vancomycin-resistant *Enterococcus faecium* and colistin-resistant carbapenemase-producing Gram-negative isolates) raises alarming concerns about the status of the health-care system in Libya.[21,22]

CoNS are emerging opportunistic bacteria with a high rate of reported methicillin resistance from companion animals.[23] In Africa, CoNS are increasingly reported ranging from 6% to 68% in susceptible human infections and from 3% to 61.7% in suspected animal infections.[24] In Africa, CoNS of animal origins were mainly reported from cattle expressing varying antibiotic resistance patterns, high MRS phenotypes, and cross-resistance against many antibiotics.[24] Of these, *S. epidermidis*, *S. haemolyticus, S. capitis*, *S. lugdunensis* and *S. xylosus* are clinically the most significant species.[25] Furthermore, CoNS are considered hidden reservoirs for antibiotic resistance and virulence traits, including methicillin-resistant with reduced susceptibility to important antibiotic classes (e.g., glycopeptides). *S. epidermidis* is especially important as a reservoir of antimicrobial-resistant genes that may be transferred to other staphylococci, mainly *S. aureus*.[26] This particular staphylococci species may also possess the secretion of serine protease Esp inhibiting and preventing the colonization of *S. aureus* and biofilm formation.[27] Such characteristics may play a significant role in the evolving and continuous emergence of MRS spreading into environmental reservoirs, including companion and food animals.[15]

Staphylococci species of veterinary relevance are difficult to differentiate due to the under-developed diagnostic protocols in veterinary medicine. The phoenix automated

Table 1: Number of *staphylococci* species resistant to antimicrobial classes ($n=34$)

Species	Number of isolates	Number of MRS by automated system	Number of *mecA* - MRS	Number of *pvl* - MRS	Antimicrobial resistance profiling by automated system											
					AMP	AMC	FOX	CEF	OX	ERY	CLI	TET	STX	GEN	CIP	NIT
S.aureus	13	8	4	2	12	8	7	7	7	5	2	6	0	3	0	0
S.epidermidis	16	8	3	0	16	8	8	8	8	9	0	6	1	5	1	1
S.warneri	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.lugdunensis	1	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0
S.cohnii	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.auricularis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

AMP: Ampicillin, AMC: *Amoxicillin and clavulanic acid,* FOX: Cefoxitin, CEF: Cefotaxime, OX: Oxacillin, ERY: Erythromycin, CLI: Clindamycin, TET: Tetracycline, STX: Trimethoprim - sulfamethoxazole, GEN: Gentamicin, CIP: Ciprofloxacin, NIT: *Nitrofurazone*, MRS: Methicillin-resistant staphylococci

microbiology system has been widely reported as an effective tool for the identifications and antimicrobial susceptibility of staphylococci, including CoNS; however, a few species are unable to be differentiated using this molecular method.[28] Of these, *S. pseudintermedius* (formerly known as *S. intermedius*) are known animal pathogens and are increasingly responsible for clinical complications in humans.[29] *S. pseudintermedius* is easily misdiagnosed with other staphylococci, particularly *S. aureus*, due to similar phenotypic characteristics requiring advanced molecular methods for definite identification such as PCR or MALDI-TOF MS.[2] Furthermore, only the most significant genes associated with clinical complications were screened (i.e., *mecA* and *pvl* genes) in the present study due to limited available resources, and further in-depth molecular and epidemiological analysis are required.

Conclusion

This is the first study that investigated veterinary medical settings for important hospital-acquired bacteria in Libya. The concerning rate of colonization with different staphylococci and MRS strains among the studied VP, highlights the necessity to introduce infection control measures in veterinary clinics. The application of decolonization using topical antimicrobials (e.g., intranasal mupirocin) and antiseptics are needed to reduce the incidence of recurrent infections. Monitoring of MRS in veterinary medical settings is required and further studies are needed to investigate the duration of colonization in VP and the associated risk factors.

Acknowledgments

The authors would like to thank all participants and veterinary colleagues at the veterinary clinics in Tripoli.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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