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Original Article

Characterization and Antibiotic Resistance of Staphylococci Strains Isolated from Brack Hospital in the Southern Region of Libya

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Abstract

Background: *Staphylococcus* species are one of the most important healthcare-associated pathogens able to acquire and spread multidrug-resistant determinants. **Objectives:** The present study investigated and analyzed 110 potential *Staphylococcus* species isolated from various clinical and nonclinical samples at Brack hospital. **Materials and Methods:** A nonselective laboratory approach was applied, using cultural characteristics, Gram stain, and catalase reactivity followed by confirmation at the species levels and determination the susceptibility against antimicrobial agents using the Phoenix automated microbiological system. **Results:** In total, 57.5% were confirmed as species and subspecies of *Staphylococcus* represented by ten different species: nine subspecies of coagulase-negative staphylococci (CoNS) (76.2%) and one coagulase-positive staphylococci (CoNS) subspecies (23.8%). Of these strains, 16.6% were identified as methicillin-resistant staphylococci (MRS) mostly of the CoNS group expressing significant resistance to important antimicrobial classes. **Conclusion:** This study reports a high prevalence of various staphylococci species, particularly of CoNS group expressing multidrug resistance patterns of public health concern, from a healthcare setting in the south region of Libya. The identification of higher rate of MRCoNS underlines the importance of monitoring all multidrug-resistant staphylococci species requiring further epidemiological investigations.

Keywords: Antimicrobial susceptibility testing, Brack hospital, coagulase-negative staphylococci, Libya, multidrug-resistant staphylococci

Introduction

Staphylococcus species are major healthcare-associated pathogens responsible for critical and opportunistic infections among humans associated with increased morbidity and mortality rates.[1] Methicillin-resistant staphylococci (MRS) are bacterial pathogens expressed by variable *Staphylococcus* species showing significant multidrug resistance to important antimicrobial classes including drugs of last resort such as glycopeptides.[1] MRS represent serious medical and public health concerns that rapidly spread with variable epidemiological distribution worldwide.[2]

In Libya, methicillin-resistant *Staphylococcus aureus* (MRSA) is the most reported nosocomial pathogen exclusively isolated from human healthcare settings; however, most of the available data are inadequate, and mainly originated from urban areas (i.e., cities from the north coastal region of Libya) with a paucity of information from suburban and underdeveloped areas.[3,4] From the south region of Libya, only two studies have documented healthcare-associated organisms (i.e., MRSA and *Pseudomonas aeruginosa*) isolated at Sebha medical center.[5,6] Recently, MRS have been reported from humans and companion animals, mainly belonging to the species of coagulase-negative staphylococci (CoNS) presenting public health and zoonotic concerns.[7,8] The current study investigated

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a collection of 110 identified staphylococci strains isolated from humans from various clinical and nonclinical sources at Brack hospital in the southern region of Libya between August and December 2018. The collected isolates were further analyzed to identify and confirm the genus and species of the isolates and further characterize the antimicrobial susceptibility profiling. Brack hospital is a major healthcare setting that provides various medical and health services to the community in the southern region of Libya.

Materials and Methods

Source of the collection

The collection was isolated from patients (n = 63) and healthcare works (n = 47) and obtained from nasal swabs (n = 23), hand swaps (n = 73), and clinical samples from urine catheters (n = 14). The collection originated from 79% (n = 87) of females and 21% (n = 23) of males, and the age of the involved individuals ranged from 1.5 to 63 years (mean = 28.5 years). Isolates were identified based on standard laboratory procedures using direct culturing onto blood and MacConkey agar plates and further identified based on the typical criteria of staphylococci species including Gram-positive cocci, clustering, and catalase reactivity. Isolates were stored at −20°C until further analysis.

Laboratory identification and biochemical characterization

Each isolate was enriched in brain heart enrichment broth and incubated aerobically for 24–48h at 37°C. A loopful from each broth was streaked onto both mannitol salt and blood agar and incubated for 24h at 35°C. Plates were then checked for typical growing colonies featuring staphylococci as yellow, circular, and shiny colonies. A typical colony was selected from each plate and further examined with a Gram stain and catalase test and identified as presumptive staphylococci. Isolates were further tested with a BD Phoenix automated identification and susceptibility testing system (PAMS, MSBD Biosciences, Sparks, MD, USA) for definite characterization at the genus and species levels and to determine the susceptibility against antimicrobial agents. The antimicrobial susceptibility profile was identified based on the interpretation of the Phoenix system and by the criteria of CLSI guidelines.[9] The detection of MRS was based on the interpreted criteria of minimum inhibitory concentration (MIC) for oxacillin and cefoxitin as follows: Susceptible, MIC ≤2 µg/ml, and resistant, MIC ≥4 µg/ml. The MIC breakpoints for CoNS (excluding *S. lugdunensis*) and *S. pseudintermedius* were ≤0.25 mcg/ml for susceptibility and ≥0.5 mcg/ml for resistance.

Results

In total, only 57.5% (n = 42 of 73) were confirmed as species and subspecies represented by ten different staphylococci species: nine species of a space after CoNS. (n = 32; 76.2%) and one subspecies of (CoPS) (n = 10; 23.8%) [Table 1]. The identified species were *S. aureus* (n = 10), *S. gallinarum* (n = 6), *S. xylosus* (n = 6), *S. saprophyticus* (n = 4), *2; S. epidermidis* (n = 4), *S. warneri* (n = 4), *S. equorum* (n = 2), *S. simulans* (n = 2), *S. kloosii* (n = 2), and *S. hominis* (n = 2), [Table 1]. Furthermore, 16.6% (n = 7 out of 42; 6 CoNS and 1 CoPS) of the strains expressing MRS phenotypes were found to have similar antibiogram profiles [Table 2]. The remaining isolates were susceptible to all antimicrobial classes.

Table 1: Characterization of *Staphylococcus* species (n = 42)

Number of identified staphylococci species	Number of MRS by automated system
CoP=10	CoP=1
10; *S. aureus*	1; *S. aureus*
CoN=32	CoN=6
6; *S. gallinarum*	3; *S. gallinarum*
6; *S. xylosus*	2; *S. xylosus* (MLSB)
4; *S. saprophyticus*	1; *S. eqourum*
4; *S. epidermidis*	
4; *S. warneri*	
2; *S. eqourum*	
2; *S. simulans*	
2; *S. kloosii*	
2; *S. hominis*	

MRS: Methicillin-resistant staplylococci, CoPS: coagulase positive, CoNS: Coagulase negative, MLSBi: Inducible Macrolide-Lincosamide-Streptogramin B resistance, *S. aureus*: *S. aureus*, *S. xylosus*: *S. xylosus*, *S. saprophyticus*: *S. saprophyticus*, *S. epidermidis*: *S. epidermidis*, *S. warneri*: *S. warneri*, *S. eqourum*: *S. equorum*, *S. simulans*: *S. simulans*, *S. kloosii*: *S. kloosii*, *S. hominis*: *S. hominis*, *S. gallinarum*: *S. gallinarum*

The seven MRS isolates were respectively distributed between *S. gallinarum* (n = 3), *S. xylosus* (n = 2), *S. aureus* (n = 1), and *S. equorum* (n = 1). Of these, five MRS isolates expressed typical resistance to penicillin, oxacillin, ampicillin, amoxicillin–clavulanate, cefoxitin, and cefotaxime but were susceptible to gentamicin, tetracycline (except one), trimethoprim–sulfamethoxazole, nitrofurantoin, moxifloxacin, rifampin, ciprofloxacin, linezolid, daptomycin, teicoplanin, vancomycin, fusidic acid, erythromycin, clindamycin, and mupirocin. Two MRS *S. xylosus* isolates expressed MLSBi phenotype expressing further resistance to erythromycin, clindamycin, trimethoprim, and tetracycline [Table 2].

Discussion

In general, most of the available information on *Staphylococcus* from Libya has focused on *S. aureus* of clinical sources with little information on other species. A previous study investigated a collection of 218 isolates of staphylococci originated from clinical samples collected at Tripoli hospital reported MRSA in 28.4% (n = 62/218) of the collection followed by MRCoNS (21.5%; n = 47/218).[10] Another study involving different hospitals in Benghazi reported an MRSA prevalence at 8% of samples collected from various surfaces and environmental sources and identified 32 out of 100 *S. aureus* strains expressing the MRS phenotype but susceptible to vancomycin and mupirocin.[11]

Table 2: Antimicrobial susceptibility profiling of methicillin-resistant staphylococci expressing strains (*n*=7)

Staphylococcus spp.	Origins	Source	Coagulase group	Resistant	Intermediate	Susceptible
S. gallinarum	HP	Hand/ female	−	IPM, FOX, CTX, AMP, PenG, OXA, AMX	ERY	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, FUS, ERY, CLI
S. gallinarum	HP	Hand/ male	−	IPM, FOX, CTX, AMP, PenG, OXA, AMX	ERY, CLI, TEC	GEN, DAP, STX, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, FUS
S. gallinarum	HP	Hand/ male	−	IPM, FOX, CTX, AMP, PenG, OXA, AMX	CLI, TEC	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, FUS, ERY, CLI
S. aureus	HP	Hand/ male	+	IPM, FOX, CTX, AMP, PenG, OXA, AMX, TET	--	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, FUS, ERY, CLI
S. xylosus MLSB	HCWs	Nasal/ female	−	IPM, FOX, CTX, AMP, PenG, OXA, AMX, ERY, CLI, TET, STX	--	GEN, DAP, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, FUS
S. xylosus MLSB	HCWs	Nasal/ female	−	IPM, FOX, CTX, AMP, PenG, OXA, AMX, ERY, CLI, TET, STX	--	GEN, DAP, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, FUS
S. equorum	HP	Hand/ female	−	IPM, FOX, CTX, AMP, PenG, OXA, AMX	--	GEN, DAP, STX, TEC, VAN, LZD, MUP, NFZ, CIP, MXF, RIF, TET, FUS, ERY, CLI

HP: Hospitalized patient, HCWs: Healthcare works, MLSB: Macrolide-lincosamide-streptogramin B, --: None, +: Positive, −: Negative, 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, *S. gallinarum: S. gallinarum, S. aureus: S. aureus, S. xylosus: S. xylosus, S. equorum: S. equorum*

In the current study, 42 isolates were confirmed as *Staphylococcus* species of which seven isolates expressed MRS phenotypes predominantly of the CoNS group originating from nasal and hand samples. A previous study from the southern region of Libya investigating 43 strains of *S. aureus* recovered from different departments at Sebha medical center reported MRSA in 16% of the isolates but susceptible to vancomycin.[5] The revelation of the present study showed the variable epidemiological status of *Staphylococcus* species in the studied area but may also indicate an epidemiological shift in the distribution of *Staphylococcus* species within the Libya healthcare system. In fact, a recent molecular investigation on a collection of clinical *S. aureus* collected at the largest Libyan hospital in Tripoli revealed the presence of atypical genotypic strains among MRSA strains showing a dynamic molecular shift in MRSA consistent with global molecular changes.[3] Nevertheless, the available information on the epidemiological distribution of *Staphylococcus* species within the Libyan health system and the community is incomplete and inadequate mainly due to underdeveloped infrastructures and economic resources.

S. aureus and *S. epidermidis* are typical colonizers of the skin and nares, linked to biofilm formation and responsible for serious persistent infections.[12,13] CoNS, on the other hand, are emerging opportunistic organisms able to persist on a variety of environmental surfaces but less involved in community-associated diseases.[14] The majority of the CoNS species identified in the current study are typical human-associated organisms (i.e., the *S. epidermidis*-like group – *S. epidermidis*, *S. haemolyticus S. capitis*, *S. hominis*) and more commonly isolated from opportunistic and bloodstream infections associated with antibiotic use and the use of medical devices.[14,15]

Staphylococci of animal and farms origins are frequently associated with human and bloodstream infections showing multidrug resistant to important antimicrobial classes (e.g., glycopeptides and fluoroquinolones).[16,17] Of these, *S. gallinarum*, known to be widespread in the environment and isolated from skin and respiratory tract of farm animals, is increasingly reported from opportunistic infections associated with the prevention use of antibiotics.[16] *S. xylosus* is a commensal organism of the skin and the mucous membranes of humans and animals responsible for opportunistic and zoonotic infections (e.g., mastitis or dermatitis).[18] This species is frequently isolated from animal products (e.g., cheese, milk and meat) and used in the development of flavor and food processing due to its antioxidant and degradation properties.[19] The identification of such animal- and environmental-associated species raises concerns over zoonotic contamination and contacts.[20]

MRSA and MRCoNS are commonly isolated from humans and animals responsible for severe infections in healthcare facilities and the community.[21,22] MRCoNS are an emerging cause of hospital-acquired infections; however, the available knowledge on their prevalence is very limited from the underdeveloped regions.[14,23] In the current study, six MRCoNS were identified showing similar multidrug-resistance properties (i.e., AMP, Pen, AMX, OXA, FOX, CTX) of which three were *S. gallinarum* and expressed further and variable intermediate susceptibility to erythromycin, clindamycin, and teicoplanin. In addition, two MR *S. xylosus* strains were characterized expressing MLSB phenotype showing further resistance to trimethoprim–sulfamethoxazole and tetracycline which reportedly linked to antibiotics usage.[24]

Nasal/nasopharyngeal colonization with MRCoNS is documented as a major risk factor for persistent and drug-resistant infections.[23,25] CoNS are recognized as a major 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*.[26] Another *mec* gene homolog is *mec*C, which has about 70% comparability with the *mec*A gene, and can be carried by SCC*mec* elements and isolated from animals, human clinical specimens, and the environment.[27] Reportedly, the identification of the high rate of MRCoNS, particularly those carrying the *mec* genes among human isolates, may reflect higher exposure to antimicrobial drugs and/or the coexistence of resistance determinants. This may favor the horizontal transfer of mobile genetic elements to other commensal organisms leading to the emergence of more virulent drug-resistant strains such as vancomycin-resistant *S. aureus*.[28,29] Unfortunately, due to the limitations of the current study, these important genes (i.e., *mecA*, *mecC,* and *pvl*) were not investigated.

As the global epidemiological distribution of staphylococci and the associated multidrug resistance phenotypes have dramatically changed, accurate laboratory identification and characterization is important.[3,30] The Phoenix system proved to be efficient for accurate identification and antimicrobial susceptibility of staphylococci including CoNS as well as other major healthcare-associated pathogens such as enterococci and Gram-negative rods.[31] Strains of *S. aureus* may also be misinterpreted using phenotypic testing methods and microbiological automated systems with other CoPS or certain clinically important CoNS strains such as *S. lugdunensis* and *S. schleiferi*.[32] Therefore, reliable, efficient, and advanced molecular laboratory methods such as MALDI-TOF MS or polymerase chain reaction technology are required.[33]

Conclusion

This study revealed novel information on important healthcare-associated pathogens isolated from a healthcare setting in the southern region of Libya. The current investigation revealed a high rate of CoNS among the collection expressing concerning MRS phenotype underlying the importance of monitoring all *Staphylococcus* species. Appropriate and effective prevention strategies in healthcare facilities, including antibiotics stewardship and epidemiological studies, are required to control the dissemination of MRS.

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Conflicts of interest

There are no conflicts of interest.

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ملخص المقال باللغة العربية

توصيف لسلالات المكورات العنقودية المعزولة من مستشفى براك بالمنطقة الجنوبية لليبيا ومقاومتها للمضادات الحيوية

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الخلفية: تعد أنواع المكورات العنقودية واحدة من أهم مسببات الأمراض المرتبطة بالرعاية الصحية والقادرة على اكتساب ونشر محددات مقاومة الأدوية المتعددة.

الأهداف: بحثت الدراسة الحالية وحللت 110 نوعاً محتملاً من المكورات العنقودية المعزولة من مختلف العينات السريرية وغير السريرية في مستشفى براك.

المواد والطرق: تم تطبيق نهج المعملي غير الانتقائي، باستخدام خصائص المزرعة، صبغة غرام، وتفاعل الكاتلاز متبوعاً بالتأكيد على أنواع السلالات، وتحديد القابلية ضد الأدوية المضادة للميكروبات باستخدام نظام فينيكس الميكروبيولوجي الآلي.

النتائج: في المجموع، تم تأكيد 57.5٪ على أنها أنواع ونويع فرعية من المكورات العنقودية ممثلة بعشرة أنواع مختلفة: تسعة أنواع فرعية من المكورات العنقودية سلبية التختير (76.2٪) ونوع فرعي واحد من المكورات العنقودية إيجابية التختير (23.8٪). من بين هذه السلالات، تم تحديد 16.6٪ على أنها مكورات عنقودية مقاومة للميثيسيلين، في الغالب من مجموعة المكورات العنقودية سلبية التختير التي أبدت مقاومة كبيرة لفئات من مضادات الميكروبات المهمة.

الخلاصة: تشير هذه الدراسة إلى ارتفاع معدل انتشار أنواع المكورات العنقودية المختلفة، لا سيما سلالات المكورات العنقودية سلبية التختير التي تعبر عن أنماط مقاومة للأدوية المتعددة تثير قلق الصحة العامة من بيئة الرعاية الصحية في المنطقة الجنوبية من ليبيا. إن تحديد المعدل الأعلى لـ مكورات عنقودية مقاومة للميثيسيلين يؤكد على أهمية مراقبة جميع أنواع المكورات العنقودية المقاومة للأدوية المتعددة التي تتطلب مزيدًا من التحقيقات الوبائية.

الكلمات المفتاحية: اختبار الحساسية لمضادات الميكروبات، مستشفى براك، تجلط الدم، المكورات العنقودية السلبية التختير، ليبيا، المكورات العنقودية المقاومة للأدوية المتعددة.