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Antimicrobial Susceptibility Patterns of Coagulase-Negative staphylococci Isolated from Infected Wounds in Rural Tanzania

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Introduction

Staphylococci are one of the major groups of Gram-positive bacteria, which are commonly categorized and differentiated in the laboratory based on their ability to produce coagulase as either coagulase-positive or coagulase-negative staphylococci (CoNS).A comprehensive review published in 2014 enumerated a totalof 47staphylococcal species, of which 38 (81%) belonged to CoNS [1]. With the advent of more sophisticated diagnostic techniques, additional species of elevated clinical importance have also been described [2]. CoNS such as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus hominis* commonly occur as commensal bacteria on the skin and mucous membranes of humans and many other mammals, in particular onwarm and moist body areas.In contrast to the clinically highly relevant coagulase-positive species *Staphylococcus aureus*, the pathogenic potential of CoNS is more dependent on the susceptibility of the host and CoNS are generally considered less virulent. Indeed, infections predominantly occur in pre-term newborns, immunocompromised patients and individuals with implanted medical devices and/or foreign bodies [3, 4].

While the medical relevance of CoNS may usually be lower than that of*S. aureus*, the opposite is true for antibiotic resistance patterns. Indeed, while *S. aureus* remains susceptible to many beta-lactam antibiotics outside areas of high endemicity for methicillin-resistant *S. aureus* (MRSA) strains, up to 90% of CoNS may be resistant to Methicillin and most beta-Lactams as well as other classes of anti-infective drugs [1,5,6,7]. Treatment options for MRSA and infections caused by multi-resistant CoNS are limited and include glycopeptides (e.g. Vancomycin), oxazolidinones (e.g. Linezolid), lipopeptides (e.g. daptomycin) and glycyclcyclines (e.g. tigecycline), many of which are not available in resource-constrained settings. While specific research initiatives have elucidated the clinical importance of *S. aureus* infections in Africa [8], far less attention has been addressed to the species distribution, antimicrobial susceptibility patterns and clinical relevance of CoNS in sub-Saharan Africa and other tropical and subtropical settings. Here, we present a microbiological assessment of such staphylococcal species detected in swabs obtained from patients with skin and soft tissue infections in the Bagamoyo area in Tanzania.

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The Study

This research was part of a larger study, which was financially supported by the Deutsche For schungsgemeinschaft (DFG) within the frame of a research grant entitled “Infection biology and epidemiology of staphylococci and staphylococcal diseases in sub-Saharan Africa” (grant no.: PAK 296). Ethical approval (Ref: NIMR/HQ/R.8a/Vol. II/316) for the conduct of this study was obtained from the Tanzanian national ethics committee prior to study initiation. Further details on the study area, recruitment and laboratory procedures as well as study findings other than for CoNS have been described elsewhere [9].

In brief, the study was conducted between February and October 2012 in Bagamoyo in the Coast Region of Tanzania. Outpatients presenting to six healthcare facilities with clinical features of skin or soft tissue infections were included and clinical data were recorded in a short questionnaire. Wound swabs were taken and sent to a regional microbiology laboratory for further processing. Samples were plated on agar cultures and examined for bacterial growth. Preliminary identification as CoNS was done using colony morphology, Gram staining and biochemical characteristics (i.e. positive catalase and negative coagulase reactions). CoNS isolates were then frozen at -20°C in trypticase soy broth medium, which was supplemented with 20% glycerol. Next, samples were transferred to the Institute of Medical Microbiology and Hygiene at Saarland University Medical Center in Homburg, Germany for confirmatory identification via matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics GmbH; Bremen, Germany). Antimicrobial susceptibility testing (AST)was carried out using the VITEK 2 system (BioMérieux; Marcy l'Etoile, France). Clinical breakpoints put forth by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied (www.eucast.org).

Among a total of 185 clinical swabs taken from 185 individuals, CoNS were isolated in 37 individuals, owing to a prevalence of 20%. More CoNS were detected in male than female patients (27 *vs.* 10 isolates) and 81% (30/37) samples stemmed from infected wounds. Most CoNS were found in the age group 19-35 years (n=15), followed by 36-55 years (n=8) and 5-18 years (n=6).

In total, twelve different CoNS species were found, with *S. haemolyticus* being the predominant species (n=10), followed by *S. epidermidis* (n=8) and *S. sciuri* (n=5). Methicillin resistance was detected in 11/37 strains (30%) using a cefoxitin screen, and was commonly observed in *S. haemolyticus* (n=8). Antimicrobial susceptibility patterns are shown in Table 1. Among the non-beta-lactams, antibiotic resistance rates werehighfor fosfomycin (68%), fusidic acid (49%), trimethoprim-sulfamethoxazole(43%),tetracycline (43%) and erythromycin (30%). Of note, all CoNS remained susceptible to daptomycin, linezolid, rifampicin,tigecycline and vancomycin. Methicillin-resistant CoNS showed higher resistance rates to different classes of non-beta-lactam antibiotics than methicillin-sensitive CoNS (Table 1).

Table 1. Antimicrobial resistance rates of 37 coagulase-negative staphylococci (CoNS) detected in swabs taken from outpatients with skin and soft tissue infections in Bagamoyo, Tanzania. All identified CoNS species remained susceptible to vancomycin, rifampicin, linezolid, daptomycin, teicoplanin and tigecycline (not included in the table).

Bacterial species	N	Benzyl penicillin	Cefoxitin	Gentamicin	Ciprofloxacin	Moxif loxacin	Erythro mycin	Clinda mycin	Tetra cycline	Fosfo mycin	Fusidic acid	Trimhoprim-sulfamethoxazole
S. haemolyticus	10	8 (40%)	8 (80%)	5 (50%)	2 (20%)	2 (20%)	4 (40%)	5 (50%)	7 (70%)	9 (90%)	4 (40%)	9 (90%)
S. epidermidis	8	2 (25%)	2 (25%)	1 (13%)	1 (12,5%)	1 (13%)	4 (40%)	1 (13%)	3 (38%)	5 (63%)	1 (13%)	4 (50%)
S. sciuri	5	0	0	0	1 (20%)	5 (100%)	1 (20%)	2 (40%)	1 (20%)	1 (20%)	5 (100%)	0
S. warneri	3	3 (100%)	1 (33%)	0	0	0	0	0	0	2 (67%)	1 (33%)	0
S. simulans	2	0	0	0	0	0	0	0	0	1 (100%)	0	0
S. hominis	2	1 (50%)	0	0	1 (50%)	0	2 (100%)	0	2 (100%)	1 (50%)	1 (50%)	2 (100%)
S. saprophyticus	2	0	0	0	0	0	0	0	1 (100%)	2 (100%	2 (100%	1 (50%)
S. kloosii	1	0	0	0	0	0	0	0	1 (100%)	1 (100%)	1 (100%)	0
S. lugdunensis	1	1 (100%)	0	0	0	0	0	0	0	0	0	0
S. pasteuri	1	0	0	0	0	0	0	0	1 (100%)	1 (100%)	1 (100%)	0
S. xylosus	1	0	0	0	0	0	0	0	0	1 (100%)	1 (100%)	0
S. caprae	1	0	0	0	0	0	0	0	0	1 (100%)	1 (100%)	0
N	**37**	**15**	11	**6**	**5**	**8**	**11**	**8**	**16**	**25**	**18**	**16**
%	**100%**	**40.5%**	**30%**	**16%**	**14%**	**22%**	**30%**	**22%**	**43%**	**68%**	**49%**	**43%**

Conclusion

We identified a number of different CoNS species in skin and soft tissue infections of Tanzanian outpatients, with a considerable proportion being resistant to methicillin, and thus, most beta-lactam antibiotics. The predominance of *S. haemolyticus* and *S. epidermidis* found in our work is in agreement with a recent review on CoNS of medical and veterinary importance in Africa [10]. A similar species distribution and antimicrobial susceptibility pattern was also reported in this journal when analysing swabs taken from the anterior nares of hospitalised individuals in Jordan [11]. While CoNS have received relatively little attention in the past, they are nowadays increasingly being recognised as reservoirs of genes that may facilitate MRSA infections [12]. Our study is limited by the small sample size, the absence of further molecular tests to elucidate CoNS resistance patterns and the use of a semi-automated susceptibility testing method instead of e.g. micro dilution. Yet, this study is the first to provide data on the characteristics of CoNS in clinical isolates stemming from skin and wound infections in rural Tanzania, and further research to improve our understanding of the epidemiology of these bacteria is warranted.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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