Antimicrobial susceptibility and body site distribution of community isolates of Coagulase Negative Staphylococci

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Abstract

The primary aim of this study was to determine antimicrobial resistance in coagulase negative staphylococci (CoNS) from healthy adults in the community. Healthy adults (n=114) were swabbed on six body sites; both armpits, both knee pits and both sides of the groin. Species determination was performed using MALDI-TOF and susceptibility testing for eleven relevant antimicrobials was performed by the disc diffusion method and minimal inhibitory concentration gradient test.

In total, 693 CoNS-isolates were identified. Susceptibility testing was done on 386 isolates; one CoNS from each species found on each participant from the different body sites. The prevalence of antimicrobial resistance in the CoNS isolates were; erythromycin (24.6%), fusidic acid (19.9%), tetracycline (11.4%),clindamycin (7.8%), gentamicin (6.2%) and cefoxitin (4.1%). Multidrug resistance was observed in 5.7% of the isolates. *Staphylococcus epidermidis* and *S. hominis* were the first and second most prevalent species on all three body sites. We conclude that CoNS isolates from healthy adults in the community have a much lower prevalence of antimicrobial resistance than reported in nosocomial CoNS isolates. Still, we believe that levels of resistance in community CoNS should be monitored as the consumption of antimicrobials in primary care in Norway is increasing.

Running head: Antimicrobial susceptibility of community coagulase negative staphylococci .

Keywords: Coagulase negative staphylococci, commensals, body site distribution, antimicrobial resistance, body site distribution, community CoNS

INTRODUCTION

The development and global spread of antimicrobial resistance is a threat to modern medicine. The commensal skin flora, dominated by coagulase negative staphylococci (CoNS), may act as a reservoir of antimicrobial resistance, and transfer resistance genes to more virulent staphylococci such as *Staphylococcus aureus* (1-4). Over the last decades, CoNS have received increased interest as important opportunistic nosocomial pathogens frequently involved in medical implant infections and infections in immunocompromised patients, e.g. patients with haematological diseases and very preterm infants (5). Studies on antimicrobial resistance in CoNS have mainly focused on invasive isolates, commonly from hospitalized patients (6-8). Multidrug-resistant hospital adapted clones have been identified in both *S. epidermidis* and *S. haemolyticus* (6, 9). However, only limited data exist regarding antimicrobial resistance among community CoNS isolates (10-12). Furthermore, CoNS species with different resistance and virulence traits may have different niches on the human body (2).

The primary aim of this study was to determine susceptibility to commonly used antimicrobial agents in a selection of CoNS isolates from healthy adults in the community. Secondly, we report the body site distribution of CoNS on three body sites screened in this study. This may increase our understanding of the role CoNS play as reservoirs of antimicrobial resistance.

MATERIAL AND METHODS

Healthy adult volunteers (age 18-49 years, mean age; 25.5 years) were recruited, primarily from different sport teams (basketball, n= 14, ice hockey, n= 9, four different football teams n=55 and members of a student's sports centre n=19) and office employees (n= 17). All participants filled in a questionnaire regarding antimicrobial consumption, hospitalization and travel abroad during the last three months. Health care workers and volunteers who reported antimicrobial consumption and/or contact with health care institutions during the last three months were not included in the study.

All participants were swabbed with Amies charcoal transport swabs (Sarstedt, Nümbrecht, Germany) on six body sites; both armpits, both knee pits and both sides of the groin. Swabs were streaked on blood agar plates (Oxoid, Basingstoke, England) and incubated overnight (16-20 hours) at 37°C. All visible CoNS with different morphotypes on blood agar plates were selected for further analyses (5-36 colonies from each participant). The phenotypes were characterized by colonies of different diameter with white, grey, creamy or yellow pigmentation and moderately heavy, weak or absent haemolysis.

Species determination was performed with MALDI-TOF MS using a Microflex LT instrument (Bruker Daltonics, Massachusetts, USA), Flexcontrol software and the Biotyper database (Bruker Daltonics, Massachusetts, USA) (13). A simple extraction method with 70 % formic acid (Sigma-Aldrich, St. Louis, MO, USA) was used on the isolates before adding HCCA matrix solution (Bruker Daltonics, Massachusetts, USA / Sigma-Aldrich, St. Louis, MO, USA). Both positive (ATCC 9144 *S. aureus*) and negative (matrix

solution) controls were applied on each test plate run on the MALDI-TOF MS. All samples were run in parallel. Processing of samples were done according to the user manual (13) Only samples that obtained a log (score) value of ≥ 2 were used further, as these results are considered to give a high probability of identification at the species level (14).

After species determination, one CoNS-isolate of each species was randomly selected from each participant, and underwent testing for antimicrobial susceptibility. Antimicrobial susceptibility testing and interpretation was performed according to EUCAST guidelines (15). Oxoid MH agar plates were used (Oxoid, Basingstoke, England).The disk diffusion test was used for cefoxitin (as a marker for methicillin resistance), trimethoprim-sulfamethoxazole (TMS), clindamycin, erythromycin, fusidic acid, gentamicin, linezolid, tetracycline, ciprofloxacin and rifampicin (Oxoid, Basingstoke, England). A minimal inhibitory concentration (MIC) gradient test was used for vancomycin susceptibility testing of all isolates, and for selected isolates with linezolid inhibition zones around defined breakpoints. (Liofilchem, Roseto degli Abruzzi, Italy). ATCC 29213 *S. aureus* was used as reference strain. All isolates were also tested for inducible resistance to clindamycin (15). Multidrug resistance (MDR) was defined as resistance to at least three classes of antimicrobial agents.

Ethical approval

The Regional Committee (REC) for Medical Research Ethics approved the collection of CoNS isolates (REC number 2013/974/REK). Informed written consent was obtained from all participants.

RESULTS

In total, 114 participants (57 male and 57 female) were included in the study. None of the participants had consumed any antimicrobial agents, worked at, or been admitted to a health care institution 3 months prior to the swabbing. A total of 693 CoNS were identified from the different body sites of the 114 volunteers (Figure 1). Eleven potential *Staphylococcus species* were not included because of a log (score) value < 2 on MALDI-TOF MS. *S. epidermidis* and *S. hominis* were the first and second most prevalent species at all three body sites, body site distribution and prevalence is listed in Figure 1.

We performed antimicrobial susceptibility testing on 386 isolates; one CoNS from each species found on each participant (Table 1). Different CoNS species per person included in the antimicrobial susceptibility testing varied from one to seven (mean=3). In total 110 *S. epidermidis*, 93 *S. hominis*, 59 *S. capitis*, 48 *S. haemolyticus*, 38 *S. lugdunensis*, 13 *S. saprophyticus*, and 25 other CoNS were tested. The highest prevalence of resistance was towards erythromycin 95/386 (24.6 %), fusidic acid 77/386 (19.9 %) and tetracycline 44/386 (11.4 %). There was a very low prevalence (< 2%) of resistance towards rifampicin, ciprofloxacin and TMS. Overall, 16/386 (4.1 %) of CoNS isolates were methicillin resistant. Resistance to vancomycin or linezolid was not detected in any isolates. MDR was observed in 5.2% of the isolates. *S. hominis* displayed the highest prevalence of MDR (10.8%), followed by *S. epidermidis* (6.4%) and *S. haemolyticus* (6.3%). In 16.6% of the participants, all of the tested strains were susceptible to all antimicrobial agents. In 13.5 % of the participants, all strains tested displayed resistance to one or more antimicrobial agents. Resistant isolates were not associated with any specific body sites.

There was no correlation between the prevalence of antimicrobial resistance and participants belonging to different sports teams, nor was there any differences observed in prevalence of antimicrobial resistance in the different age groups or between the male and female participants (data not shown).

DISCUSSION

This is, to our knowledge, the largest, recent study focussing on antimicrobial susceptibility in community CoNS. The commensal CoNS isolates displayed resistance to all antimicrobial classes tested apart from vancomycin and linezolid. MDR was detected in 5.2% of the isolates. However, in around 1 of 6 participants no antimicrobial resistant CoNS-isolates were found. The highest prevalence of antimicrobial resistance was towards erythromycin, fusidic acid, tetracycline and clindamycin, all antimicrobial agents commonly prescribed in primary health care to treat respiratory tract and skin infections (16). A recent Portuguese study on community CoNS reported overall higher prevalence of resistance than in our Norwegian isolates, and showed a higher prevalence of resistance towards agents commonly prescribed antibiotics in primary care (12). Similar rates of antibiotic resistance have also been reported in community isolates of *S. epidermidis*, (11, 17, 7, 18). It has previously been demonstrated that CoNS skin commensals easily develop resistance towards ciprofloxacin and betalactams, due to secretion of these antimicrobial agents in sweat, reflecting the ability to

rapidly adapt to changing external pressure (19, 20). However, the rates of resistance to cefoxitin and ciprofoxacin was low in our study.

In Norway, around 85% of the total human consumption of antimicrobial agents is in the primary care setting. The three most commonly prescribed groups of antibiotics are penicillins, tetracyclins and macrolides (21, 22), the consumption in Troms county is marginally lower that at the national level (personal communication, Hege Blix, Norwegian Institute of Public Health). The high consumption of macrolides in Norway may explain the relatively high prevalence of macrolide resistance among community CoNS isolates (22). In the Norwegian national guidelines for antibiotic use in primary care, macrolides are not recommended as first-line therapy for any other conditions than pneumonia caused by mycoplasma and/or chlamydophilia (23), but the relatively high consumption indicates that guidelines are not universally followed. Overuse of macrolides may contribute to increased antibiotic resistance (24), and the current macrolide use in Norway is higher than wanted by the regulatory authorities.

Among hospital CoNS isolates the resistance pattern is markedly different (8, 10, 25). A Norwegian study on antimicrobial resistance patterns of clinical CoNS isolates from total hip arthroplasty infections during 1993-2007 reported an increase in methicillin resistance rates from 57 to 84%, as well as increasing rates of resistance to most other antimicrobials tested (26). Antimicrobial resistance is, however, not routinely monitored in commensal CoNS and we do not know if the prevalence of resistance in the community has increased. Compared to community isolates, clinical isolates have a much higher prevalence of antimicrobial resistance, most likely

reflecting that hospital adapted resistant clones seem to outcompete the commensal flora (9). Only 5.2 % of the community CoNS in our study displayed MDR, but these isolates may also have a competitive advantage if entering the hospital and being exposed to the increased antimicrobial pressure in the hospital setting. Interestingly we observed that 13.5% of the participants were colonised with isolates that were resistant to one or more antimicrobial agents. These individuals might act as a reservoir of antimicrobial resistance genes in the community to other CoNS or *S. aureus*. Acquisition of antimicrobial resistance genes by horizontal gene transfer between closely related staphylococcal species has been hypothesised as the main cause for the successful spread of the community associated USA 300 methicillin-resistant *S. aureus* clone (27, 28).

Selection of swab sites for collection of strains was based on previously reported body sites frequently colonised with CoNS; the axillae, the groin and a the more dry extremities such as the knee (29, 30). As expected, *S. epidermidis* was the dominant species on all body sites. The second most common species was *S. hominis,* previously reported to commonly colonize the axillae, arms and legs and areas with apocrine glands such as the inguinal and perineal areas (31, 32, 29). Of note is that *S. capitis*, previously thought to be most prevalent on the head, was frequently found in the samples from the groin and the knee pit, whereas *S. saprophyticus*, a urinary tract pathogen, was rarely found in the groin (2).

This study has strengths and limitations. We took care to ensure that the isolates were truly community isolates by not including volunteers who recently had been treated with antimicrobial agents or were working in health care facilities. Due to a large number of isolates we decided to restrict susceptibility testing to one isolate of each species from each participant. Spread of community acquired methicillin resistant *S. aureus* between members of sports teams in close contact sports, such as football has been demonstrated (33, 34). As we have swabbed groups of participants belonging to the same sports teams, we might have introduced a potential bias due to a possible spread of strains between members of the same sports teams, carrying specific antimicrobial resistance genes. This could artificially increase the prevalence of antimicrobial resistance in our collection, compared to the general population. However, we believe that the large number of isolates included to a large extent reflect the antimicrobial susceptibility pattern of CoNS outside hospitals in Norway. Our data on body site distribution clearly show that different CoNS species may have other body niches than previously reported (29, 30) We did not perform susceptibility testing on all 693 isolates detected from all body sites. Thus, we cannot specify resistance pattern to each body site.

There is a paucity of information regarding antimicrobial resistance in commensal CoNS. We conclude that the prevalence of antimicrobial resistance among community CoNS in Norway is relatively low. However, MDR is present and these isolates may be more adaptable when introduced in a hospital setting. With the increase in antimicrobial prescriptions in primary care in Norway (22), prevalence of resistance in community CoNS should be monitored. Further comparative studies should be conducted in order to understand which factors are involved in hospital adaption of community isolates resulting in the high prevalence of MDR-CoNS in hospitals.

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TRANSPARENCY DECLARATIONS

The authors have no interests to declare.

AUTHOR'S CONTRIBUTION

JPC participated in conception and design, collection of strains, antimicrobial susceptibility testing, interpretation of data and writing of the manuscript.

RW participated in collection of strains, antimicrobial susceptibility testing, MALDI-TOF MS, interpretation of data and manuscript writing.

PH participated in antimicrobial susceptibility testing and manuscript writing.

EE participated in antimicrobial susceptibility testing and manuscript writing.

CK participated in conception, design and writing the manuscript.

EGAF participated in conception and design, collection of strains, writing of the manuscript and given final approval of the manuscript to be published.

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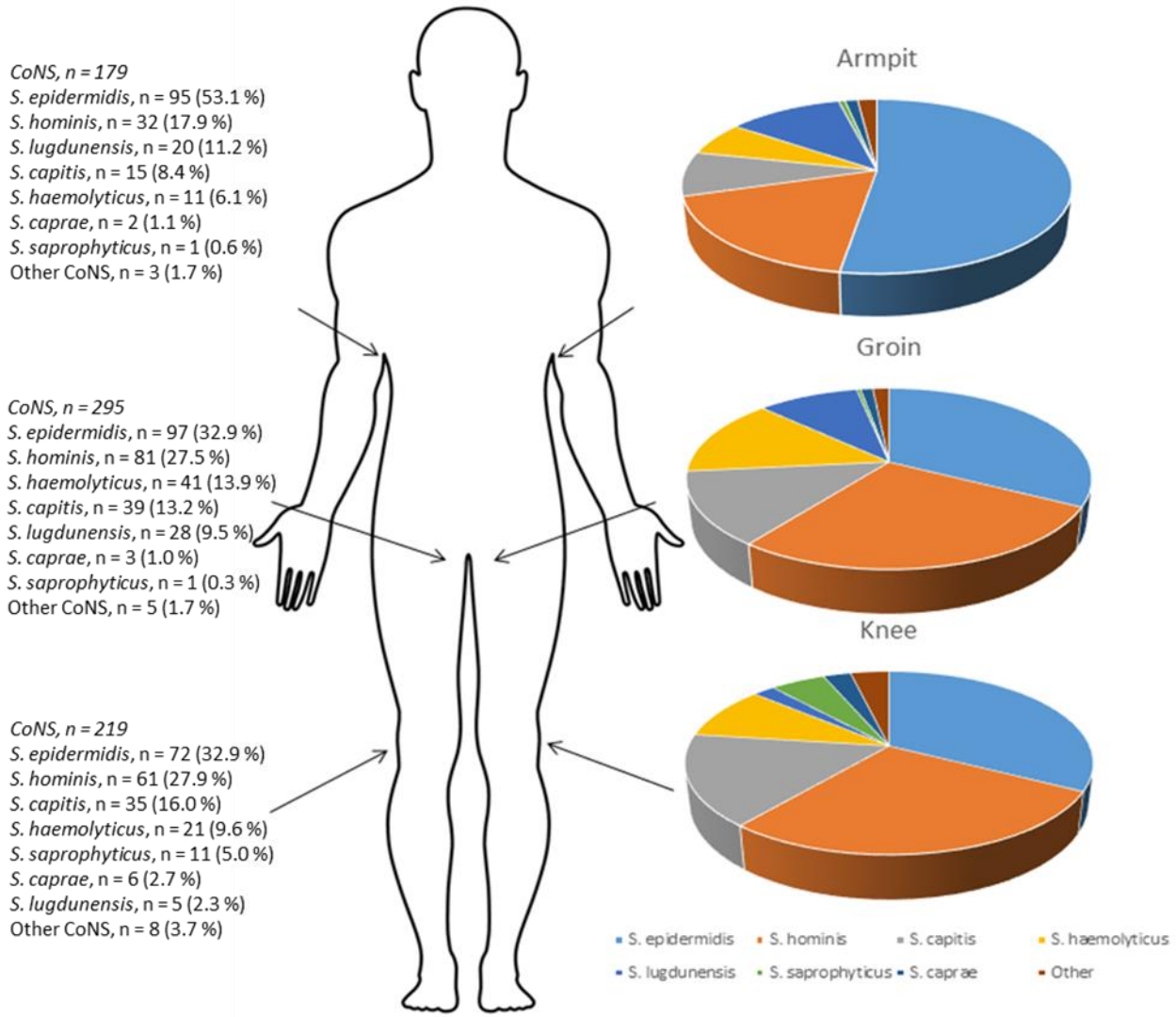

Figure 1: Body site distribution of CoNS, the prevalence represent the proportion of CoNS species found at each body site for 114 volunteers.

282 Table 1: Prevalence of antimicrobial resistance (%) in 386 community CoNS isolates from healthy adults.

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	N	Cefoxitin	TMS	Clindamycin	Erythromycin	Fusidic acid	Gentamicin	Tetracycline	Ciprofloxacin	Rifampicin	MDR*
S. epidermidis	110	2 (1.8)	2 (1.8)	8 (7.3)	29 (26.4)	29 (26.4)	11 (10.0)	6 (5.5)	0	1 (0.9)	5 (4.5)
S. hominis	93	6 (6.5)	2 (2.2)	11 (11.8)	32 (34.4)	29 (31.2)	7 (7.5)	28 (30.1)	1 (1.1)	0	10 (10.8)
S. capitis	59	3 (5.1)	0	4 (6.8)	2 (3.4)	6 (10.2)	3 (5.1)	4 (6.8)	2 (3.4)	0	2 (3.4)
S. haemolyticus	48	3 (6.3)	0	7 (14.6)	29 (60.4)	6 (12.5)	1 (2.1)	4 (8.3)	2 (4.2)	0	3 (6.3)
S. lugdunensis	38	0	0	0	1 (2.6)	2 (5.3)	0	1 (2.6)	0	0	0
S.saprophyticus	13	2 (15.4)	0	0	0	5 (38.5)	0	0	0	0	0
Other CoNS*	25	0	0	0	2 (8)	0	2 (8)	1 (4)	0	0	0
Total* **	386	16 (4.1)	4 (1.0)	30 (7.8)	95 (24.6)	77 (19.9)	24 (6.2)	44 (11.4)	5 (1.3)	1 (0.3)	20 (5.2)

284 * MDR: Multi Drug Resistant, resistant to three or more classes of antimicrobial drugs.

285 ** Other CoNS-species *S. caprae, S. warneri, S. condimenti, S. equorum, S. pasteuri, S. salivarius, S. simulans, S. pettenkoferi*

286 *** 6 additional isolates (two *S. epidermidis*, two *S. hominis*, one *S. lugdunensis* and one *S. pettenkoferi* isolate) were omitted from

287 susceptibility testing due to poor growth.