

ORIGINAL ARTICLE

## ICU stay promotes enrichment and dissemination of multiresistant coagulase-negative staphylococcal strains

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### Abstract

Patients in the intensive care unit (ICU) are prone to be colonized and infected by multi-resistant bacteria. It is previously known that nosocomial infections are often preceded by cross-transmission events. The aim of the present investigation was to study the impact of the patient's length of ICU stay on the resistance patterns, diversity and dissemination of coagulase-negative staphylococci (CoNS) within and between patients. Two groups of patients were studied, including 20 consecutive patients sampled within 2 h from admission (short-stayers, SS), and all patients treated for at least 5 d in the ICU (long-stayers, LS), available for sampling every second week ( $n = 15$ ). Sampling was performed from 5 sites: oropharynx, nares, neck, axilla and perineum. A total of 868 CoNS isolates deriving from LS patients and 403 isolates from SS patients were analysed for antimicrobial susceptibility, clonal diversity and dissemination within and between patients. The highest resistance rates were seen for oxacillin and ciprofloxacin, being 92% and 83%, respectively. Long-stayers were at significantly higher risk of being colonized with CoNS isolates resistant against oxacillin, clindamycin, ciprofloxacin, gentamicin as well as with multiresistant strains. By genotyping 22 phenotypes that were shared among at least 2 patients, 32 PFGE types of which 16 colonized more than 1 individual were identified. One of the clones was isolated from 10 individuals, including 2 SS patients, indicating an epidemic strain. Prolonged ICU stay was significantly correlated to decreased clonal diversity, increased endogenous dissemination of resistant strains and cross-transmission. The results emphasize the importance of good infection control practice, especially in this vulnerable group of patients.

### Introduction

Nosocomial infections are by far the most common complication affecting hospitalized patients, and the risk is especially high in intensive care units (ICUs), where about 30% of the patients are affected [1]. In a recent nationwide surveillance of infections in Swedish ICUs, coagulase-negative staphylococci (CoNS) were the most frequently encountered bacteria, representing 17% of all clinical isolates and 32% of the blood culture isolates [2]. The most important types of infections caused by CoNS, predominantly *Staphylococcus epidermidis*, are catheter-related infections and bacteraemia [3,4]. The main reasons why CoNS have become important nosocomial pathogens are their ability to form biofilms, to adhere to plastic materials used in

hospitals, including catheters, their robust characteristics and ability to become multiresistant to antimicrobial agents [5]. Antibiotic resistance among CoNS is high and correlated with antibiotic consumption [6,7]. Thus, methicillin resistance is more common among CoNS isolates from ICU patients than from non-ICU patients, and least common among outpatients [8,9]. In terms of antibiotic resistance and microbial colonization of the patients, large discrepancies have been demonstrated between countries, as well as between different hospitals within the same country [10].

In most hospital-acquired infections, the microorganisms have probably first colonized the patient or medical device, and subsequently caused an infection [11]. CoNS are normally found on the

human skin and mucous membranes and resistant strains may be selected by antibiotic treatment and be spread endogenously, thus colonizing other areas of the body. These bacteria may also disseminate to other patients indirectly via contaminated or colonized staff [12,13]. Data from Scandinavia regarding antibiotic resistance and microbial colonization of patients, particularly in the ICU setting, are sparse and scattered.

The aim of the present investigation was to study the impact of the patient's length of stay on the diversity and dissemination within and between patients, and resistance patterns of CoNS colonizing patients treated at a Swedish ICU.

## Material and methods

### *Patients and sampling procedures*

35 consecutive patients were included in the study performed in a multidisciplinary ICU in April–July 2001. Informed consent was obtained from relatives before patients entered the trial. The study was approved by the local ethics committee at Karolinska University Hospital Huddinge, Sweden. Two categories of patients were included. The first group, which will be referred to as short-stayers (SS), consisted of 20 consecutive patients, 13 men and 7 women, mean age 62 y (range 3–86 y), in whom sampling was performed within 2 h of admittance to the ICU. Eight of these patients had not received any antimicrobial treatment during at least 7 d before sampling, while 8 patients had received a single dose of cefuroxime and metronidazole as preoperative prophylaxis, and 4 were treated with at least 2 antibiotic agents, including a carbapenem. The second group, referred to as long-stayers (LS), included patients who had stayed in the ICU for at least 5 d (median 14 d, range 5–102 d). Sampling of LS was performed consecutively on 4 occasions with 2-week intervals and included all patients who fulfilled the inclusion criteria on the sampling day. Due to repeated sampling of all current LS every second week, one individual could be included more than once. This approach was chosen since a particular patient could, at each sampling period, have a significant impact on the colonization pattern on the other patients in the ward. The aim was to include at least 20 sampling occasions in this group and at the fourth sampling period, 6 weeks after the start of the study, a total of 23 LS subjects, 10 men and 13 women, mean age 58 y (range 26–78 y), were included. Due to repeated sampling every second week, 5 LS patients were included twice or more meaning that 15 individuals were included as LS (8 men and 7 women), mean age 57 y (range

26–78 y), although each individual was statistically regarded as a 'new' subject when analysing a particular sampling occasion. All patients in the LS group were treated with at least 2 antimicrobials, of which 1 was a carbapenem, during their ICU stay. None of the sampled SS patients were included in the LS group.

Sampling sites were vestibulum nasi, the oropharynx, the axilla, the perineum and the skin area on the right side of the neck (corresponding to the insertion site for a central venous catheter in the internal jugular vein). The samples were collected with a sterile cotton swab which was rolled and pressed to an area of 1–2 cm<sup>2</sup>, depending on sampling site, during 5–10 s. The cotton swab was then placed in a transport medium and delivered within 30 min to the laboratory where it was stored at –70°C until analysis.

### *Microbiological analyses*

The samples were inoculated on Mannitol salt phenol-red agar and blood-agar plates and incubated at 37°C for 24 h and cultured semi-quantitatively. From each patient and site, at least 1 colony of each morphotype was isolated in pure culture and identified by colony morphology, Gram staining and biochemical tests [14]. A total of 1271 isolates, 1–14 from each site, were identified as CoNS and stored in glycerine-containing broth at –70°C until further analysed by phenotyping and genotyping.

### *Antimicrobial susceptibility testing*

The minimum inhibitory concentrations (MICs) of oxacillin (Sigma-Aldrich, Stockholm, Sweden), ciprofloxacin (Bayer, Leverkusen, Germany), vancomycin (Eli Lilly, Stockholm, Sweden), gentamicin (Biochrom, Berlin, Germany), fusidic acid (Sigma-Aldrich, Stockholm, Sweden), clindamycin and linezolid (Pharmacia-Upjohn, Kalamazoo, MI, USA) were determined against the 1271 CoNS isolates using the microdilution broth method performed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [15]. *Staphylococcus aureus* ATCC 29213 was used as reference strain. The breakpoints for resistance were set according to the NCCLS (oxacillin R ≥ 0.5 mg/l; clindamycin R ≥ 4.0 mg/l; ciprofloxacin R ≥ 4.0 mg/l; gentamicin R ≥ 16 mg/l; linezolid R ≥ 8 mg/l; and vancomycin R ≥ 32 mg/l). The breakpoint used for fusidic acid was set to R ≥ 1.0 µg/ml, according to the Swedish Reference Group for Antibiotics (SRGA) [<http://www.srga.org/>].

*Phenotyping with PhenePlate™ system*

All 1271 CoNS isolates were sub-typed to the clonal level according to their phenotype with the PhenePlate™ (PhP) system using PhP-CS plates (PhPlate Microplate Techniques AB, Stockholm, Sweden) designed for typing CoNS [16,17]. The PhP system is based on repeated measurements of 23 biochemical tests which render as diverse results as possible within a group of closely related species. The results of different isolates were compared pairwise, and the similarities were calculated as correlation coefficients. Dendrograms were created by clustering, using the unweighted pair group method of arithmetic mean (UPGMA). By running duplicate identical reference strains in each round of analysis and subsequently clustering reference strains from different assays, an identity-level (ID-level) of 99% was chosen. In context, isolates with identical or very similar biochemical profiles i.e. with a correlation coefficient  $\geq 0.99$  were regarded as belonging to the same PhP type. All data processing was performed with the PhP software (PhPlate AB, Stockholm, Sweden). Phenotypes originating from individuals who had been included twice or more were also clustered separately in order to study stability over time within these patients.

*Confirming phenotyping with genotyping*

The clonal relationship between CoNS isolates clustered as 1 phenotype isolated from at least 2 patients, was further confirmed by pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared as described by De Lencastre et al. [18]. The DNA containing disks were restricted overnight with SmaI (Promega Corporation, Madison, WI, USA) at  $+37^{\circ}\text{C}$  and loaded in a gel run for 20 h at  $+14^{\circ}\text{C}$  in a contour-clamped homogeneous electric field (CHEF) apparatus (Bio-Rad GenePath™ system, Bio-Rad Laboratories, Hercules, CA, USA). The DNA fragment patterns were analysed visually and by the Molecular Analyst Software program (Bio-Rad Laboratories, Hercules, Calif., USA) and interpreted according to Tenover et al. [19]. The concordance between the PhP method and PFGE was calculated by pairwise cross-classifying of the results from the 2 methods of the analysed isolates as match or mismatch. The matching was based on a similarity level of  $\geq 99\%$  for PhP, and a difference of  $\leq 3$  bands for PFGE.

*Statistical methods*

Prolonged treatment at the ICU ward as a risk factor for becoming colonized by single- and multiple-

resistant organisms, colonization of CoNS in the oropharynx, decreased diversity of CoNS and multiple-site colonization with single phenotypes, was calculated by the odds ratio (OR) and 95% confidence interval (CI), or by using Student's t-test. A p-value of  $<0.05$  was considered as significant.

**Results***Antibiotic susceptibility of CoNS isolates*

A total of 868 CoNS isolates deriving from LS patients and 403 isolates from SS patients were analysed for antimicrobial susceptibility, and the distribution of MIC values among LS and SS isolates is shown in Table I. None of the tested CoNS isolates was resistant to vancomycin or linezolid. The highest resistance rates were seen for oxacillin and ciprofloxacin, being 92% and 83% respectively. LS subjects were at significantly higher risk of being colonized with CoNS isolates resistant to oxacillin, clindamycin, ciprofloxacin and gentamicin, while resistance for fusidic acid was more common among SS patients.

*Multiresistance*

The majority of the isolates (69%) was considered multiresistant, i.e. expressed resistance to at least 4 of the tested antimicrobial agents. The risk for being colonized by a multiresistant isolate was significantly higher for the LS group (Table II).

*Diversity and dissemination of CoNS phenotypes within patients*

The phenotypic analysis revealed a high diversity of phenotype groups within the patients with a range from 1 to 14 phenotypes per patient according to the PhenePlate system. The number of phenotypes from the different sampling sites ranged from zero to 8, with highest diversity in the perineum, followed by the skin, axilla, nostril and oropharynx. Prolonged stay in the ICU setting increased the risk of oropharyngeal colonization of CoNS, OR 5.8 (95% CI 1.4–23.4). The SS group harboured a significantly greater diversity of PhP types (median 9.5, range 5–14) per patient, while the numbers of phenotypes ranged between 1 and 9 (median 8) among the LS subjects ( $p < 0.05$ ). The frequency of subjects in each group who were colonized by specific phenotypes in at least 3, 4 or 5 sites was 45%, 30% and 5% among SS; and 100%, 83% and 61% among LS, respectively ( $p < 0.001$ ) (Figure 1). Colonization patterns within the 5 individuals who were included twice or more varied markedly over

Table I. Activities of various antimicrobial agents against CoNS isolated from LS or from SS. Susceptibility is expressed as MIC<sub>50</sub> and MIC<sub>90</sub> for each drug, at which 50 and 90% of the isolates are inhibited, respectively.

Antimicrobial agent	LS (n = 868)				SS (n = 403)				<sup>a</sup> OR (95%CI)
	MIC <sub>50</sub> (mg/l)	MIC <sub>90</sub> (mg/l)	Range (mg/l)	Percent resistance	MIC <sub>50</sub> (mg/l)	MIC <sub>90</sub> (mg/l)	Range (mg/l)	Percent resistance	
Oxacillin	≥ 16	≥ 16	≤ 0.125 – ≥ 16	98	≥ 16	≥ 16	≤ 0.125 – ≥ 16	78	13.0 (7.7–22)
Clindamycin	≥ 16	≥ 16	≤ 0.125 – ≥ 16	76	≥ 16	≥ 16	≤ 0.125 – ≥ 16	52	2.8 (2.2–3.6)
Ciprofloxacin	≥ 16	≥ 16	≤ 0.125 – ≥ 16	92	≥ 16	≥ 16	≤ 0.125 – ≥ 16	64	6.5 (4.7–8.9)
Gentamicin	≥ 64	≥ 64	≤ 0.5 – ≥ 64	85	32	≥ 64	≤ 0.5 – ≥ 64	54	4.4 (3.3–5.7)
Fusidic acid	≥ 16	≥ 16	≤ 0.125 – ≥ 16	67	≥ 16	≥ 16	≤ 0.125 – ≥ 16	78	0.6 (0.4–0.7)
Vancomycin	≤ 0.125	2.0	≤ 0.125 – 2.0)	0	≤ 0.125	2.0	≤ 0.125 – 8.0	0	
Linezolid	2.0	4.0	1.0 – 4.0	0	2.0	4.0	≤ 0.25 – 4.0	0	

<sup>a</sup>Relative risk for LS patients of being colonized with strains resistant against each antibiotic, compared to SS patients.

Table II. Rate of multiresistance against antimicrobial agents among CoNS isolated from LS or SS. Odds ratio (OR) expressed as relative risk of prolonged ICU stay for being colonized by a multiresistant strain.

Multiresistance	LS	SS	Total	OR (95% CI)
≥ 4 antibiotics	77%	52%	69%	3.1 (2.6–4.3)
≥ 5 antibiotics	50%	30%	43%	2.3 (1.8–3.0)

time. Each patient harboured 1–6 phenotypes (median 2) that remained stable over time, and 5–17 (median 11.5) transient phenotypes. Both persistent and transient clones from all these 5 individuals were involved in dissemination events.

*Dissemination of CoNS phenotypes and genotypes between patients*

A total of 22 separate phenotypes were isolated from at least 2 individuals, indicating a spread of CoNS among the patients. 61 isolates, presumably involved in transmission events, were subjected to genotyping by PFGE in order to confirm or dismiss clonal relations within these clusters. Genotyping revealed a total of 32 clones of which 16 colonized more than 1 individual. 12 of the 15 individuals in the LS group, including 4 at repeated occasions, and 6 SS patients, were involved in at least 1 transmission event. One of the clones was isolated from 10 individuals, including 2 SS patients, indicating an epidemic strain. There was generally a very good agreement between the 2 typing methods. Six of the 22 phenotypes did not generate identical or closely related PFGE patterns. Isolates within 4 of these were genetically related, i.e. exposed >3 but ≤6 bands difference. The concordance between the 2 methods was 82%. Gel images of selected CoNS strains isolated from more than one individual and their corresponding dendrogram based on the phenotype are shown in Figure 2.

**Discussion**

Coagulase-negative staphylococci are prone to cause catheter-related infections, which are one of the leading causes of nosocomial infections in the ICU setting, and associated with increased morbidity, mortality and additional costs [13,20,21]. They are often difficult to treat since catheters cannot be routinely removed and most CoNS strains express multiresistance. In the present study, the resistance rate to oxacillin was very high (92%), especially compared to previous reports of clinical isolates of CoNS from different parts of Europe, the USA, Canada and Latin America, where rates between 70 and 80% are reported [1,10,22]. The discrepancies

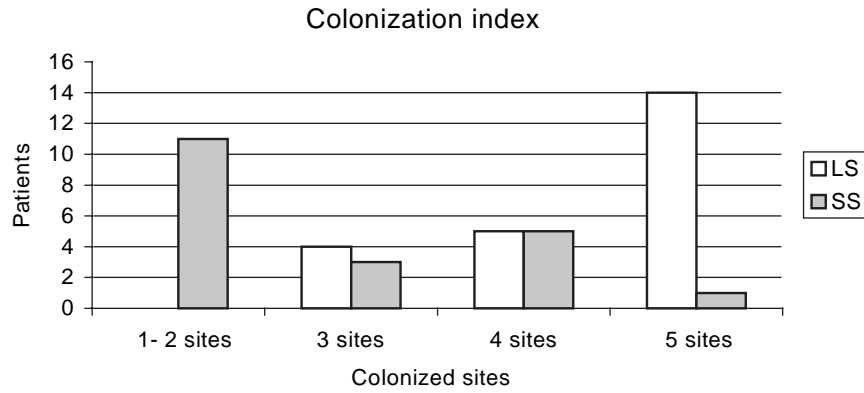


Figure 1. Colonization index: number of patients with specific CoNS phenotypes colonizing multiple sites, indicating endogenous spread. (LS: patients with  $\geq 5$  d ICU stay when sampled; SS: Patients sampled within 2 h after ICU admittance).

in rates of resistance may partly be a result of the different sites of isolation and the fact that multiple isolates from each subject were analysed. The risk of being colonized with multiresistant CoNS was significantly higher for LS, which clearly indicates that prolonged stay at the ICU increases the probability for acquisition of multiresistant isolates. The validity

of the PhP system as a screening method for phenotyping large numbers of environmental and/or clinical isolates has previously mainly been documented for enterococci and Enterobacteriaceae. The outcome of the present study indicates that the concordance of this method with PFGE, which is considered as the 'gold standard', was quite

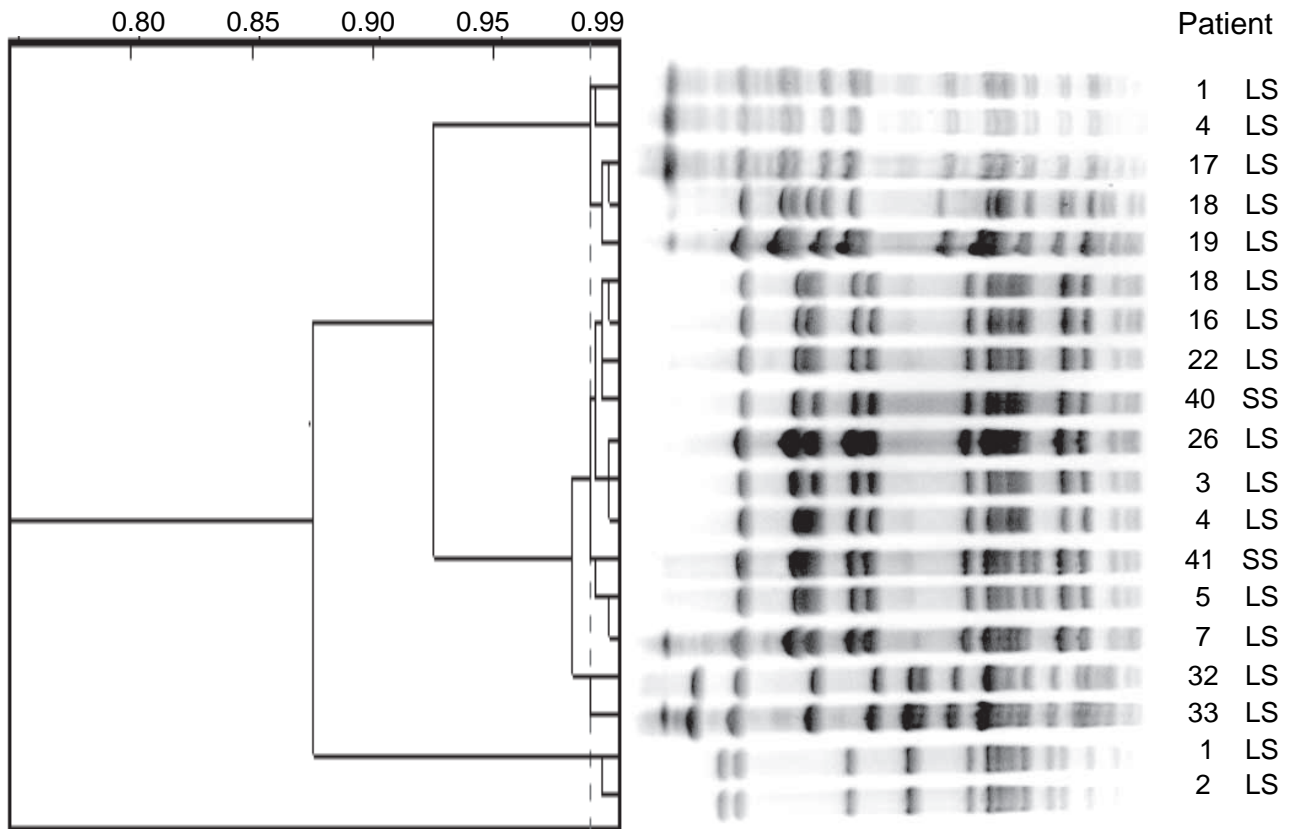


Figure 2. Dendrogram of phenotypically related CoNS isolates from different patients, created with the PhPWIn software. The dotted line at 0.99 (99% similarity) corresponds to the identity level. The corresponding banding pattern, achieved by PFGE analysis, is shown for each isolate. Strains with identical or closely related banding pattern are interpreted as belonging to the same clone and thus illustrating bacterial dissemination between patients.

high, at 82%, and thus the approach to use PhP combined with a genotyping method for studies on transmission of CoNS is feasible.

The diversity of CoNS strains was significantly reduced with patients' length of ICU stay. These findings are in agreement with a previous report, analysing CoNS from the vestibulum nasi of patients sampled at admittance to ICU and approximately 4 d later, when it was shown that the nasal CoNS flora became more resistant and less diverse over time [9]. The results of the present study support the theory that resistant strains become enriched and spread to different sites of the body where they may enter through breaches and cause infections. For example, at the common site for catheter insertion on the neck, at least 1 strain was in 96%, 87%, 78% and 78% of the LS patients also isolated from the axilla, the perineum, the nose and oropharynx, respectively. Corresponding rates among the recently admitted patients were 50%, 35%, 20% and 20%, respectively. These results show that the probability of a unique strain to disseminate from one ecological niche to another within a patient increases with prolonged ICU stay.

Approximately 70% of the LS patients were involved in cross-transmission events. This is in accordance with a previous study [23] performed in ventilated patients at the same ICU ward. Cross-transmission does not always lead to infections, but certainly is a risk factor. According to a study by Weist et al., as much as 37% of all nosocomial infections was due to bacteria involved in cross-transmissions [24]. In another study, methicillin-resistant CoNS colonizing central venous catheters were analysed, and nasal carriage of the same strain was demonstrated in 20 of the 54 patients [25]. A clonal spread of CoNS in adults was reported by Mosen et al., where peritonitis in 6 patients was caused by CoNS with identical PFGE pattern. All these patients were treated at the same dialysis ward during a 5-y period and in most cases by the same team of physicians and staff [6]. Cross-infections due to endemic strains of CoNS have also been described in several other studies [9,26]. These results emphasize the increased risk of endogenous and exogenous colonization of nosocomial strains for acquiring nosocomial infections during prolonged hospital stay. The high rate of bacterial dissemination among the LS group may have several explanations. It has been shown that nurses in the ICU harbour the highest frequency of multiresistant CoNS compared to nurses at other wards in the hospital [27]. Strains of CoNS are known to survive easily in the surroundings for several days or weeks and become epidemic [28]. From 6 patients in the SS group, strains with identical PFGE types isolated

from at least 1 other patient were detected. These strains could either be epidemic clones residing in the community or in other wards in the hospital, or may have been transmitted by ICU staff already at admission. It has previously been shown that oxacillin resistant CoNS have been able to spread clonally from patient to patient in different wards and even between countries [29].

In conclusion, the present study performed at a general ICU in a Swedish university hospital, demonstrated high resistance levels among colonizing coagulase-negative staphylococci. Prolonged ICU stay was significantly correlated to increased resistance, decreased clonal diversity, endogenous dissemination and cross-transmission of multiresistant CoNS. These results emphasize the importance of barrier treatment and other hygiene measures, as well as of prudent use of antibiotic therapy based on daily re-evaluation of the treatment according to microbiological and laboratory results and the patient's condition, especially in this vulnerable group of patients.

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