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ORIGINAL ARTICLE

## Staphylococcus aureus colonization among nursing home residents in a large Finnish nursing home

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### Abstract

We studied colonization with methicillin-resistant and -sensitive *Staphylococcus aureus* (MRSA, MSSA) in the second largest nursing home in Finland, in which the residents volunteered had their nostrils, throats, perineums, skin lesions, and catheter exit sites swabbed, and catheter urines cultured. The specimens were cultured onto non-selective and selective agar, with or without enrichment in salt-containing trypticase soy broth (TSB). *S. aureus* was identified by routine methods, methicillin resistance was detected by oxacillin and cefoxitin disk diffusion and MIC E-tests, and GenoType MRSA<sup>®</sup> -test was used for *mecA* gene confirmation. A total of 663 cultures were obtained from 213 residents. Of those, 165 specimens (25%) from 94 residents (44%) were positive for *S. aureus*, and 3 specimens (0.4%) from 2 (0.9%) residents were positive for MRSA. Of the 165 *S. aureus* isolates, 31 (19%) from 25 (27%) residents were found only from sites other than nostrils (30 MSSA and 1 MRSA). TSB enrichment detected additional 33 (5%) *S. aureus* isolates (32 MSSA and 1 MRSA), resulting in 8 (5%) additional residents. None of the MRSA strains would have been found if only nostrils and throat had been screened, and no enrichment broth had been used.

### Introduction

Approximately 20% of individuals carry *Staphylococcus aureus* persistently, 60% of population harbour *S. aureus* intermittently, and 20% are non-carriers [1]. Colonization with *S. aureus* occurs predominantly at nostrils, skin, rectum, and perineum [2]. The highest colonization rates in individuals living in long-term care facilities (LTCF) have been obtained from samples taken from nostrils and wounds [3]. Differences in colonization rates may depend on a variety of factors – the presence of an outbreak of *S. aureus*/methicillin-resistant *S. aureus* (MRSA) infections, the presence of invasive devices and skin lesions, the type and severity of underlying conditions of residents, and the infection control practices at the LTCF. In addition to hospitals, residents in LTCFs have been suggested to serve as reservoirs of MRSA.

Surveillance cultures for MRSA should always include samples at least from nostrils, and from skin lesions, if present [4]. Besides the number of different screening sites, reliable microbiological diagnostics of MRSA are essential for treatment, surveillance and control. Specific screening methods for MRSA cultures have been introduced [5], and the Clinical and Laboratory Standard Institute (CLSI) recommends medium containing salt and oxacillin additions [6]. It has also been reported that the use of an enrichment broth cannot be avoided without a loss of sensitivity on MRSA screening [5,7].

In Finland, the increasing trend of MRSA cases especially among the elderly [8], and most recently, in Helsinki metropolitan area [9] triggered us to perform a study on carriage of methicillin-sensitive *S. aureus* (MSSA) and MRSA in the largest nursing home (NH) in Helsinki metropolitan area, the

Kustaankartano Centre for the aged. The objectives of this study were to assess the rate of *S. aureus* colonization, to evaluate different body sites for ability to detect *S. aureus* carriage, to assess the necessity of performing broth enrichment to increase sensitivity, and to assess the usability of oxacillin resistance screening agar (ORSAB), which is widely used in routine diagnostics in Finland [8].

## Material and methods

### Setting

The study was performed in the largest NH (25 wards with a total of 584 beds) in the Helsinki metropolitan area. During 2004, a total of 685 long-term residents (mean length of stay, 3.1 y), and 420 short-term residents (approximately 1060 short-term nursing periods) were treated in the NH (annual occupancy, 99.3%). Of the residents, 76% were females, their mean age was 83 y, and 62% suffered from dementia.

Among the 25 wards, 9 were chosen with the following criteria: 1) at least 1 of the residents had either an indwelling catheter or open skin lesions, and 2) there were no previously known MRSA residents. In the study wards, among the long-term residents the average annual turnover was 5, and among the short-term residents 100. Of the study residents, 4% were bedridden. The study was carried out in each ward within 1 d during the week 27 September to 1 October 2004.

### Microbiology

All residents in attendance and volunteered at the 9 selected wards had their nostrils, throats, perineal sites, skin lesions, and catheter exit sites swabbed, and catheter urines cultured. Sampling was performed by staff members of NH under the guidance of NA and A-MK. On d 1, the screening swabs (Probact Transport Swab; Schofield St-Heywood, UK) were cultured onto the salt-containing (6.5% NaCl) enrichment trypticase soy broth (TSB) without selective drug for MRSA because the rate of MSSA was also assessed and the non-selective sheep-blood agar (SBA) as a control plate for TSB at the National Reference Laboratory (KTL). Urines were cultured with a 1- $\mu$ l loop onto agar plates at NH; after transfer to KTL they were also cultured onto TSB broth. Perineal and catheter exit site swabs, and catheter urines were also cultured onto the non-selective cystine lactose electrolyte deficient (CLED) agar to prevent growth of other colonizing flora. The agar plates and TSB tubes were incubated aerobically at +36 ( $\pm$ 1 $^{\circ}$ C) for 24 h, and on d 2, recultured from TSB

onto the SBA, and oxacillin resistance screening agar (ORSAB; CM1008, Oxoid, UK) plates were selectively supplemented with 2 mg/l of oxacillin, and 100,000 U/l of polymyxin B (Oxoid), which was used only after the enrichment step. The SBA plates were incubated aerobically at +36 ( $\pm$ 1 $^{\circ}$ C) for 48 h and ORSAB for 96 h, and inspected daily. The agar plates cultured on d 1 (SBA and CLED) were incubated aerobically at +36 ( $\pm$ 1 $^{\circ}$ C) for an additional 24 h. *S. aureus*-like colonies from SBA and CLED and blue colonies indicating oxacillin-resistant *S. aureus* from ORSAB were selected, subcultured onto the SBA (in case of swarming *Proteus* sp., subcultures were also carried out onto CLED), and the pure cultures incubated at +36 ( $\pm$ 1 $^{\circ}$ C) overnight. Standard microbiological methods for identification of *S. aureus* were used [10].

Oxacillin resistance was determined by oxacillin 1  $\mu$ g and cefoxitin 30  $\mu$ g disk tests (Oxoid, Hampshire, England) according to Clinical and Laboratory Standard Institute (CLSI) guidelines [6]. Minimum inhibitory concentration (MIC) test of oxacillin was determined to the isolates expressing disk diameters of oxacillin <13 mm or cefoxitin <20 mm by the E-test, according to the manufacturer's instructions (AB Biodisk, Solna, Sweden).

Biochemically identified *S. aureus* strains expressing oxacillin MIC >2 were determined for *mecA* and *S. aureus* specific fragments in duplicates by multiplex PCR-reaction with GenoType<sup>®</sup> MRSA-test (Hain Lifesciences, Germany) according to the manufacturer's instructions using the reagents supplied. Briefly, the protocol consisted of DNA isolation, PCR amplification, and reverse hybridization including chemical denaturation of the PCR products, hybridization of the biotinylated PCR products to membrane-bound probes, stringent washing, adding of streptavidin/alkaline phosphatase (AP) conjugate, and an AP mediated staining reaction.

In the case of MRSA, other antimicrobials were tested as elsewhere described [8], and MRSA isolates were genotyped with pulsed-field gel electrophoresis (PFGE) as elsewhere described [11].

### Ethical aspects

We were at liberty to collect the samples from the residents with approval from the Ministry of Social Affairs and Health and the data protection authority. In addition, permission for sampling was asked individually from each patient.

## Results

Of the 217 residents present, 4 refused sampling completely, and of the remaining 213, 3 (1.4%)

refused nostril swabbing, 15 (7.0%) throat swabbing, and 8 (3.8%) perineal swabbing.

A total of 663 specimens (median, 3; range 1–7) were obtained from 213 residents: 165 specimens (25%) from 94 residents (44%) were positive for *S. aureus*, and 3 specimens (0.4%) from 2 (0.9%) residents in the same ward were positive for MRSA (Table I). By PFGE typing, 2 MRSA strain types were identified: 1 isolate of a non-multidrug-resistant strain FIN-7 from 1 patient, and 2 isolates of a multidrug-resistant strain FIN-21 from another patient (Table I). The highest proportion of *S. aureus* growth was obtained from the catheter exit sites (56%, 5/9), and lowest from the perineum (13%, 2/207) and the catheter urine (19%, 3/16). Of the 165 *S. aureus* isolates, 32 (19%) were found only from sites other than nostrils from a total of 25 residents: 11 specimens were from throat, 7 from perineum (6 MSSA and 1 MRSA), 2 from skin lesions, 1 from catheter exit site, 6 from combination of throat and perineum from 3 residents, 2 from combination of perineum and skin lesion from 1 patient, and 3 from combination of throat, perineum, and skin lesion from 1 patient (Table II).

TSB enrichment detected an additional 33 (5%) *S. aureus* isolates (32 MSSA and 1 MRSA; Table I) from a total of 28 residents compared to both selective and non-selective agar plating, which resulted in finding 8 (4%) additional *S. aureus*-positive residents. Of those 8 residents, isolates from 3 residents were from nostrils, 1 from throat, 1 from perineum, 1 from skin lesion, 1 from combination of nostrils and perineum, and 1 from combination of nostrils, throat and perineum. Four *S. aureus* isolates (0.6%), 2 from nostrils and 2 from throat, were found only from SBA used as a control plate for TSB in primary culture (Table I). Four *S. aureus* isolates were isolated only from CLED, of which 2 were from primary cultures of perineum, and 2 from subcultures of nostrils because of swarming *Proteus* sp.

Within 96 h, 187 of 663 (28%) specimens grew in ORSAB, and 2 of these (1%) were MRSA. Of the 187 cultures, 46 (25%) expressed blue colonies within 24 h, 132 (71%) within 48 h, 180 (96%) within 72 h, and 187 within 96 h. The 2 MRSA strains were detected in ORSAB within 24 h. Half (94/187, 50%) of the cultures were from perineum, 44 (24%) from nostrils, 39 (21%) from throat, and 10 (5%) from skin lesions, urine, or catheter exit sites. One of the 3 MRSA strains, isolated from perineum, did not grow in ORSAB. Sensitivity of ORSAB was 67% compared to both SBA and CLED, and specificity after 24 h was 93%, and it decreased to 72% after 96 h.

Table I. Prevalence of *S. aureus* (MSSA and MRSA) isolations among 663 specimens from 213 residents in a Finnish nursing home.

Screening site	Total no. of specimens	No. of specimens positive for <i>S. aureus</i> (%)	Growth of <i>S. aureus</i> with both of TSB enrichment and SBA and/or CLED (%)	Growth of <i>S. aureus</i> only with TSB enrichment (%)	No. of <i>S. aureus</i> found only from control plates: SBA and/or CLED (%)	No. of specimens positive for MRSA	PFGE name of MRSA (International clones)
Nostrils	210	69 (33)	55 (80)	10 (14)	4 (6)	1	FIN-21 (Southern Germany, Italian clone, MLST-228, SCCmec I) <sup>d</sup>
Throat	198	53 (27)	44 (83)	7 (13)	2 (4)	–	–
Perineum	207	27 (13)	12 (44)	13 (48)	2 (7)	1	FIN-7 (Brazilian clone, MLST-8, SCCmec IV) <sup>e</sup>
Skin lesion	23 <sup>a</sup>	8 <sup>b</sup> (35)	5 (63)	3 (38)	–	–	–
Catheter exit site	9	5 (56)	5 (100)	–	–	1	FIN-21 (Southern Germany, Italian clone, MLST-228, SCCmec I) <sup>d</sup>
Catheter urine	16	3 (19)	3 (100)	–	–	–	–
Total	663	165 (25)	124 (75)	33 (20)	8 (5)	3 <sup>c</sup>	–

<sup>a</sup> Taken from 16 residents. <sup>b</sup> Found from 7 residents. <sup>c</sup> Found from 2 residents. <sup>d</sup> Resistant to gentamicin, tobramycin, erythromycin, clindamycin, and ciprofloxacin in addition to beta-lactams. <sup>e</sup> Resistant only to beta-lactams.

Table II. Distribution of screening sites and site combinations of *S. aureus* (MSSA and MRSA) isolations among 94 residents.

Screening site	<i>S. aureus</i> (MSSA and MRSA) findings
nostrils	23
nostrils + throat	27
nostrils + throat + perineum	7
nostrils + perineum	3
nostrils + skin lesion	2
nostrils + throat + perineum + catheter	2
urine + catheter exit site	
nostrils + throat + catheter exit site	1
nostrils + catheter exit site	1
nostrils + throat + perineum + skin lesion <sup>1</sup>	1
nostrils + throat + catheter urine + catheter exit site	1
throat	11
throat + perineum	3
throat + perineum + skin lesion	1
perineum	7
perineum + skin lesion	1
skin lesion	2
catheter exit site	1
Total number of residents positive for <i>S. aureus</i>	94

<sup>1</sup> *S. aureus* was isolated from 2 skin lesions of the patient.

## Discussion

Our study showed that the colonization of *S. aureus* in the largest NH in Helsinki metropolitan area, the Kustaankartano Centre for the aged, was high (44%), but the prevalence of MRSA was low (0.9%). With the agreement of residents, we swabbed different sites extensively and used the enrichment broth in addition to selective and non-selective agar plates to acquire the best possible sensitivity.

In Finland, a rise in the number of MRSA cases outside Helsinki metropolitan area was detected in the late 1990s, and an emerging problem was suggested in LTCFs [8]. Since 2003, MRSA has also increased in Helsinki metropolitan area [9]. The FIN-21 strain, a sequence type (ST) 228 by multi-locus sequence typing and staphylococcal cassette chromosome *mec* I (SCC*mec*) by multiplex-PCR, has caused many epidemics in secondary and tertiary care hospitals in Helsinki metropolitan area since autumn 2002, and it was the most often found MRSA genotype in Finland in 2004 (Kerttula et al., unpublished). The same strain type has also caused hospital epidemics in other European countries, such as German and Italy [11,12]. Thus, it was assumed that FIN-21 strain would have been found in the largest NH in Helsinki metropolitan area. Surprisingly, only 1 patient was colonized with this strain. The other MRSA genotype found in our

present study, FIN-7 (ST8:IV), has spread in almost all hospital districts in Finland (Kerttula et al., unpublished).

The prevalence of MRSA colonization in this study was lower than published earlier in Finland [13,14]. In contrast to this study, those surveillance studies were performed in an outbreak situation. The residents in Kustaankartano Centre for the aged represent typical Finnish NH residents with regard to their activities in daily life (ADL) – skills, degree of cognitive decline or prevalence of pressure ulcers, which means high prevalence of dementia and need of other persons' physical assistance in some or all of the ADL performance. The wards chosen for the current study covered all types of residents, including residents with either indwelling catheter or open skin lesions. However, only one-third of the residents were included in this study, and so we cannot generalize the results to other Finnish nursing homes.

In our study, the highest rates of *S. aureus* colonization were detected in catheter exit sites, followed by nostrils and skin lesions. However, only few study residents had catheters. Omission of sampling sites other than nostrils would have missed 12% (24 MSSA and 1 MRSA case) of *S. aureus* positive residents. The results are consistent with the earlier LTCF studies [2]. A combination of 3 body sites (nose, throat, and wounds/skin lesions) has earlier been reported necessary to detect nearly all MRSA [4]. Moreover, perirectal-perianal cultures have been shown to detect MRSA with high sensitivity in certain patient populations [4], albeit the cost effectiveness has not shown to support perianal screening in some cases [15]. On the other hand, Coello et al. reported that in non-infected asymptomatic carriers, the perineum swab alone or in combination with nose swab was more sensitive than throat swab alone or in combination with nose swab [16]. Collection of throat swabs in elderly persons suffering from dementia can be troublesome, and in our study the refusal rate was the highest with throat swabbing. Thus, swabbing perineum while being washed could be a more comfortable way of sampling.

We were able to detect an additional 5% of *S. aureus* isolates by enrichment broth, which is less than in other studies [5,7]. However, we would have missed 1 of the MRSA isolates (33%) without enrichment broth. In our study, using enrichment broth was most effective in finding skin lesion isolates, followed by perineum isolates. Some of the screening sites were highly colonized with Gram-negative rods, especially the perineal swabs with the swarming *Proteus* sp. (data not shown). We overcame this problem by primary plating or

subculturing the specimens to CLED. Addition of antimicrobials, e.g. aztreonam or nalidixic acid, to broth would also have prevented this contaminating growth.

Almost one-third of our screening specimens grew in ORSAB expressing blue colonies, but only 1% of those were MRSA. ORSAB is a selective medium containing mannitol and aniline blue for the detection of mannitol fermentation that indicates for *S. aureus* growth. A high salt concentration and lithium chloride should suppress non-staphylococcal growth; oxacillin inhibits MSSA, and polymyxin B inhibits other bacteria able to grow at such a high salt concentration, e.g. *Proteus* sp. Half of those blue-colony growing bacteria were isolated from perineal swabs, and they were practically all coagulase-negative staphylococci (data not shown). Others have also reported problems with specificity of ORSAB [7,17]. The specificity of ORSAB decreased after 24 h of incubation, but to detect also the MRSA strains expressing very low-level oxacillin resistance, it is advisable to incubate the plate as long as 96 h. The 2 multi-drug resistant MRSA strains found in this study grew in ORSAB within 24 h, but the non-multi-drug resistant MRSA strain expressing MIC of 6 mg/l did not. Merlino et al. [18] have reported that low-level oxacillin-resistant MRSA strains do not necessarily grow in ORSAB. Because only 3 MRSA strains were isolated, we could not assess the usability of ORSAB for screening of MRSA. Other commercial MRSA-selective media have been introduced recently, and they have been shown to perform with good sensitivity and specificity [19,20], and 1 of those especially with perineal swabs [19].

The prevalence of MRSA in LTCF settings in other European countries has recently been reported to be 0.7–10.1% [21–25]. In 3 studies, an enrichment broth has been used for culturing MRSA [21,22,25], but the effect of the enrichment broth compared to solid media has not been studied. The sampling sites have varied, in addition to nostrils, from throat, hairline, axillae, groin or perineum, and skin lesions [21], to only nostrils and skin lesions.

In conclusion, *S. aureus* colonization rate was high in the largest NH home in Helsinki metropolitan area, and based on our study, using enrichment broth improved the findings by 5%, and was most useful in finding skin lesion isolates, followed by perineum isolates. Although MRSA colonization rate was low, none of those MRSA strains would have been found if only nostrils, throat and skin lesions had been screened, and no enrichment broth had been used. As a practical suggestion based on this, perineal swabbing might be an alternative for

throat swabs, especially when residents are not cooperative, and enrichment broth is used. In Kustaankartano centre for the aged, the residents colonized with MRSA were assigned a room and a toilet of their own, and hand disinfection was instructed. In addition, hand hygiene was intensified in wards with the colonized residents, and active information was given for casual visitors. Although the prevalence of MRSA was low in this particular NH, performing surveys like this is a useful tool to increase awareness of MRSA among staff members, and to help them to understand the importance of active screening and prevention measures of multi-resistant bacteria.

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