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# Colonization with *Staphylococcus aureus* in Swedish Nursing Homes: A Cross-Sectional Study

Key Words: *Staphylococcus aureus*; Methicillin-Resistant *Staphylococcus aureus*; Colonization; Nursing Homes; Drug Resistance, Bacterial

Running Headline: Colonization with *S. aureus* in Nursing Homes

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# Abstract

Background: Screening for bacterial colonization among risk populations could provide better estimates of (1) the volume of the bacteria-related disease reservoir, and (2) the level of antimicrobial resistance, than do conventional laboratory reports.

Methods: 201 participants at ten Swedish nursing homes were screened for colonization with *Staphylococcus aureus* between January and October 2009. 61/201 participants (30 %) were males. The median age was 86 years. Everyone was systematically sampled from (1) the nasal mucosa, (2) the pharyngeal mucosa, (3) the groin, and (4) active skin lesions, if any.

Results: 99/199 participants (50 %) were colonized with *Staphylococcus aureus*. The colonization rate was 34 % (nose), 35 % (throat), 10 % (groin), and 54 % (active skin lesion). An antibiotic-resistant *Staphylococcus aureus* isolate was identified in 8.5 % of all participants regardless of colonization status. All together, 24 resistant isolates were detected, and 21 of these were resistant to fluoroquinolones. There was no case of colonization with methicillin-resistant *Staphylococcus aureus* (MRSA).

Conclusions: The presence of resistant isolates was generally low, and the greater part of the resistant cases was fluoroquinolone-related. To achieve reasonable precision, screening programmes of this kind must include samples from both the nose and throat, and--although low--the prevalence of antimicrobial resistance in Swedish nursing homes still calls for reflection on how to use the fluoroquinolones wisely.

# Introduction

The increasing antimicrobial resistance of bacteria (AMR) is of major concern to health authorities worldwide. Increased AMR results in a decrease in available treatment options, thereby limiting medicine's potential for successfully treating infectious diseases in the future [1]. A number of resistant bacteria are of particular interest in this respect, given their capacity to cause more serious disease than their non-resistant counterparts [2,3], and their tendency to acquire additional or even multiple resistance mechanisms that may eventually render them untreatable [4].

Together with several other Gram positive bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) is listed by the European Centre for Disease Prevention and Control as a key pathogen connected with AMR [5]. MRSA is also included in the European Antimicrobial Resistance Surveillance System, which collects laboratory data on AMR from the greater part of the countries in the European Union [6].

From a European perspective, the Nordic countries have enjoyed a low prevalence of MRSA in their clinical settings. According to recent Swedish data, less than 1 % of invasive strains of *Staphylococcus aureus* (*S. aureus*) are MRSA [7]. This percentage is very low compared to figures from some of the countries in the central and southern parts of Europe, where the proportion of invasive MRSA versus invasive methicillin-susceptible *S. aureus* (MSSA) may be as high as 50 % [8].

Official reports on resistance development are generally derived from routine laboratory data. Such data, however, is error-prone since the reasons for obtaining a laboratory sample might vary, and the origin of the samples is not always carefully documented. Therefore, screening studies of specified populations are necessary to quantify the volume of the bacteria-related disease reservoir and to quantify any changes in AMR while they occur. To date, relatively few such studies have been done in European nursing homes [9,10].

We decided to screen the participants in the ongoing SHADES programme (*Study of Health and Drugs among the Elderly in Swedish Nursing Homes*) for colonization with *S. aureus*

[11]. The aim was to assess the prevalence of colonization and the occurrence of AMR in connection with this key pathogen and within a specified clinical setting.

## Materials and Methods

We recruited a convenience sample of 201 individuals for bacterial screening among those already eligible for the basic study in the SHADES programme. As a consequence, data on several parameters (including prior antibiotic treatment) was already available at study start.

The participants were living in 10 nursing homes in three Swedish cities--Eslöv in the extreme south of Sweden, and Jönköping and Linköping in the south-central part of the country. We decided to merge the data from Linköping with the data from Jönköping for the sake of convenience, since only a few participants were eligible in Linköping and given both cities are located in the same geographic region.

### Sample Collection

Each participant underwent sampling between January and October, 2009. The greater part of the samples was collected in the spring months (78 %). The samples were collected systematically from (1) the nasal mucosa, (2) the pharyngeal mucosa, (3) the groin, and (4) active skin lesions, if any. The specimens were obtained using a rayon-tipped swab (155C Plastic Rayon White, Copan Italia S.p.A.; Italy). The sampling was performed by a nurse on location.

The samples were incubated for 16 to 20 hours in a modified MAMSA broth (Proteose Peptone LP0085B, Liver Digest Neutralised LP0027B and Yeast Extract Powder LP0021B [Oxoid Ltd; U.K.]; sodium chloride 2.5 % and mannitol 1.0 % with the addition of aztreonam 8.0 mg/mL) for enrichment of *S. aureus* [12], and subsequently cultured on blood agar base plates. The presence of *S. aureus* was determined by testing for DNase activity.

Tests for antibiotic susceptibility were performed according to the disc diffusion method calibrated to *NordicAST* MIC breakpoints on iso-tryptophan plates (Iso-Sensitest Agar CM0471B [Oxoid] and L-tryptophan) [13]. The test antibiotics were: ciprofloxacin, fusidic acid, tetracycline, sulphamethoxazole/trimethoprim (SMZ/TMP), vancomycin, gentamycin, clindamycin, erythromycin, linezolid, and rifampicin (all testing discs Oxoid Ltd; U.K.). The presence of MRSA was determined by testing for resistance to ceftiofur.

## Data Analysis

The data was entered into an SPSS™ (SPSS Inc., Chicago, Illinois, U.S.A.) database. We calculated primarily (1) the prevalence of colonization with *S. aureus* at the respective sampling sites, (2) the proportion of antibiotic-resistant *S. aureus* isolates, and (3) the odds ratio for the comparison between these parameters and various individual characteristics such as sex and age.

## Ethical Considerations

This study was approved by the Regional Ethical Review Board at Linköping University (date: October 18, 2007; case number: M150-07).

## Results

All together, 201 participants were included in the study. Of these, 99/201 (49 %) were living in the Linköping/Jönköping area, and 102/201 (51 %) in Eslöv. 61/201 participants (30 %) were males. The participants' ages ranged from 61 to 101 years with a median of 86 years. Their length of stay in the nursing homes ranged from zero to 14 years with a median of 1.8 years.

## Colonization

A total of 576 bacterial samples were collected from (1) the nasal mucosa, (2) the pharyngeal mucosa, (3) the groin, and (4) active skin lesions, if any. Bacterial colonization with *S. aureus* occurred in 99/199 participants (50 %). The category 'nose' registered the highest bacterial count with 67/197 cases (34 %), followed by 'throat' with 58/164 cases (35 %), 'groin' with 20/197 cases (10 %), and 'active skin lesion' with 7/13 cases (54 %). A sizeable percentage of cases were lost in the category 'throat' due to sampling problems. The lost cases differed from the cases at hand with regard to area of residence [Linköping or Jönköping] (OR 2.95, 95 % CI 1.37 to 6.36), but there was no difference with regard to age, sex, length of stay, recent hospital admissions, or antibiotic treatment during the 180 day period that preceded microbiological sampling (data not shown).

The expected increase in accumulated prevalence of colonization as body site categories were added together in sequence (beginning with the highest individual count and ending with the combination of all four categories) occurred as follows: Nose, 34 %; nose + throat, 47 %; nose + throat + groin, 48 %; and nose + throat + groin + active skin lesion, 50 %.

Men were colonized in the nose to a greater extent than women. Those who had stayed in the nursing homes longer than the median (> 1.8 years) were colonized in the throat to a higher degree than those below the median (Table I). There was no difference in colonization status when the material was subdivided into groups by age (above median), residence (in Jönköping or Linköping), recent hospital admissions, or antibiotic treatment during the 180 day period that preceded microbiological sampling (data not shown).

## Antibiotic Consumption

Ninety-four courses of antibiotics were prescribed during the 180 day period that preceded each participant's date of microbiological sampling. This frequency corresponds to approximately one course of antibiotics per individual per year. The most commonly used antibiotics were pivmecillinam and phenoxymethylpenicillin (Figure 1).



The 94 courses were prescribed to 60/201 participants (30 %), 25/201 of whom (12 %) received more than one course. The participants living in Jönköping or Linköping received less antibiotics than those living in Eslöv (OR 0.39, 95 % CI 0.21 to 0.74). There was no difference in treatment status when the material was subdivided into groups by age [above median] (OR 1.00, 95 % CI 0.55 to 1.83), length of stay [above median] (OR 0.69, 95 % CI 0.37 to 1.27), or sex [male] (OR 1.09, 95 % CI 0.57 to 2.10).

### Antibiotic Susceptibility

Out of 576 bacterial samples, 152/576 (27 %) were positive *S. aureus* cultures. Of these 152 positive cultures, 24/152 (16 %) were resistant to at least one of the tested antibiotics. These 24 resistant isolates were obtained from 17 participants, which corresponded to a prevalence of 17/94 (18 %) in the cluster of colonized participants, and 17/201 (8.5 %) among all participants regardless of colonization status.

The prevalence of AMR under the specific body site categories ‘nose’, ‘throat’, ‘groin’ and ‘skin lesion’ was approximately 20 % (Table II). There was no difference in susceptibility status when the material was subdivided into groups by age (above median), sex (male), residence (in Jönköping or Linköping), length of stay (above median), recent hospital admissions, or antibiotic treatment during the 180 day period that preceded microbiological sampling (data not shown).

The antibiotics that some *S. aureus* isolates were resistant to were: ciprofloxacin (21 isolates), fusidic acid (1 isolate), tetracycline (1 isolate), and sulphamethoxazole/trimethoprim [SMZ/TMP] (1 isolate). There were no isolates resistant to vancomycin, gentamycin, clindamycin, erythromycin, linezolid, rifampicin, or cefoxitin. No isolate was found to be methicillin-resistant.

None of the twelve participants who received treatment with fluoroquinolones during the 180 day period that preceded bacterial sampling was colonized with an *S. aureus* strain resistant to this class of antibiotics.

## Discussion

### Main Findings

Microbiological screening of 201 Swedish nursing home residents showed that about one in every two individuals was colonized with *S. aureus* at any body site. At the specific level, the body site category 'nose' registered the highest bacterial count, followed by 'throat'. AMR was present in 16 % of all positive cultures, 18 % of all colonized participants, and 8.5 % of all participants regardless of colonization status.

We did not find any case of colonization with methicillin-resistant *S. aureus* (MRSA). Resistance to fluoroquinolones was found in 21/24 (88 %) of all resistant isolates.

### Comments on Method

The strength of our study lies in the fact that comprehensive screening programmes are necessary to quantify the volume of the bacteria-related disease reservoir in a specific clinical setting and to quantify any changes in AMR while they occur. At the same time, the drawback of screening studies may be the low general occurrence of positive findings--especially with regard to rare elements of AMR.

The decision to include participants who were eligible for the SHADES programme may raise concerns about a possible selection bias favouring nursing homes that were better managed than others--possibly due to fewer turnovers of staff and a lower antibiotic consumption rate. However, the antibiotic consumption in our study was similar to the consumption reported in a previous study performed at Swedish nursing homes [14].

Our screening period was somewhat protracted due to logistic reasons and we have not been able to compensate for a possible seasonal variability in the colonization rates. We did not

include rectal samples in this screening study. The isolates were not tested for resistance to penicillins (in accordance with Swedish standard practice) due to the high expected frequency of penicillinase-producing isolates (> 80 %) [13].

## Comments on Results

The prevalence of MRSA in our study was low as opposed to the situation in the United Kingdom and some of the countries in the central and southern parts of Europe. In these areas, the proportion of invasive MRSA versus invasive MSSA may be as high as one in every two laboratory samples [6], and the parallel proportion of colonization-related MRSA versus colonization-related MSSA as high as one in every four isolates [15].

Since no MRSA was found in our material, the only AMR of any significance lay in the fluoroquinolones. Moreover, studies have implicated the fluoroquinolones as ‘drivers’ for resistance in *S. aureus* at the population level [16]. Even so, we did not find any association between the presence of resistant isolates and possible contributing factors such as length of stay, recent admittance to hospital, or current use of fluoroquinolones in the nursing homes. Our conclusion is that the cases of resistance in our material came out by chance and might simply reflect the level of resistance in the population from which the participants were recruited.

The prevalence of colonization in our material was somewhat higher than expected. The overlapping within the body site categories resulted in an accumulated prevalence of 50 %-- that is to say almost twice as high as the mean prevalence noted in a similar study by Hoefnagels-Schuermans [9]. However, in that study, only the nose and perineum were used as test sites.

The accumulated prevalence of colonization rose sharply when the body site category ‘throat’ was combined with the category ‘nose’. Adding further body sites to the model, however, resulted in only a marginal rise. This helps to suggest that screening for colonization with *S. aureus* in the elderly must include both the nose and the throat to accurately gauge the prevalence of colonization [17]. The exact impact of the body site ‘throat’ might be difficult

to further assess employing the same methodology, since a sizeable percentage of the participants (18 %) were unable to comply to throat sampling.

## Clinical Implications

It has been shown in this study that screening programmes focusing on elderly individuals must include bacterial sampling from both the nose and throat to give an accurate estimate of the prevalence of colonization with *S. aureus*. At the same time, screening studies may be an inadequate method of measuring the extent of any AMR that is allegedly driven by a specific antibiotic pressure due to an insufficient number of positive cases.

We did not identify any MRSA strains. However, the level of resistance to fluoroquinolones was unsettling when considering the fact that all other elements of resistance were quite rare. Consequently, clinicians should restrict the use of fluoroquinolones to cases of severe infection in accordance with local recommendations [18]. Health authorities should give priority to screening risk populations on a regular basis.

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## Declaration of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

## Authorship/Contribution

Magnus Olofsson collected the data, analyzed the data, and wrote the manuscript. Sigvard Mölsted, Carl Johan Östgren and Patrik Midlöv designed the study. Per-Eric Lindgren was responsible for the microbiological test procedures. All authors participated in analyzing the data and in editing the manuscript at various stages of the writing process.

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TABLE I

Colonization with *Staphylococcus aureus* in 201 Participants Living in Nursing Homes

		BODY SITE			
	All Body Sites (N = 199)	Nose (N = 197)	Throat (N = 164)	Groin (N = 197)	Skin Lesion (N = 13)
<b>Frequency of participants with any positive culture</b>					
N (95 % CI)	99 (85 to 113)	67 (54 to 81)	58 (46 to 71)	20 (13 to 30)	7 (3 to 10)
<b>Prevalence</b>					
% (95 % CI)	50 (42 to 57)	34 (27 to 41)	35 (28 to 43)	10 (6 to 16)	54 (29 to 77)
<b>Accumulated frequency</b>					
ΣN (95 % CI)	-- --	67 (54 to 81)	93 (79 to 107)	95 (81 to 109)	99 (85 to 113)
<b>Accumulated prevalence</b>					
Σ% (95 % CI)	-- --	34 (27 to 41)	47 (40 to 55)	48 (40 to 55)	50 (42 to 57)
DEMOGRAPHICS					
<b>Sex (male)</b>					
Odds ratio (95 % CI)	1.4 (0.7 to 2.7)	1.9 (1.0 to 3.7)*	0.9 (0.4 to 1.9)	2.1 (0.8 to 5.5)	1.5 (0.0 to 17)
<b>Age (above median)</b>					
Odds ratio (95 % CI)	1.3 <sup>a</sup> (0.7 to 2.3)	1.6 <sup>b</sup> (0.9 to 3.0)	1.1 <sup>b</sup> (0.6 to 2.2)	0.6 <sup>a</sup> (0.2 to 1.7)	0.7 (0.0 to 6.5)
<b>Length of stay (above median)</b>					
Odds ratio (95 % CI)	1.5 <sup>c</sup> (0.8 to 2.6)	1.6 <sup>d</sup> (0.9 to 3.0)	2.0 <sup>d</sup> (1.0 to 4.0)*	2.0 <sup>c</sup> (0.7 to 5.3)	3.7 (0.2 to 55)

CI = Confidence Interval.

<sup>a</sup>Three observations missing.<sup>b</sup>One observation missing.<sup>c</sup>Four observations missing.<sup>d</sup>Two observations missing.

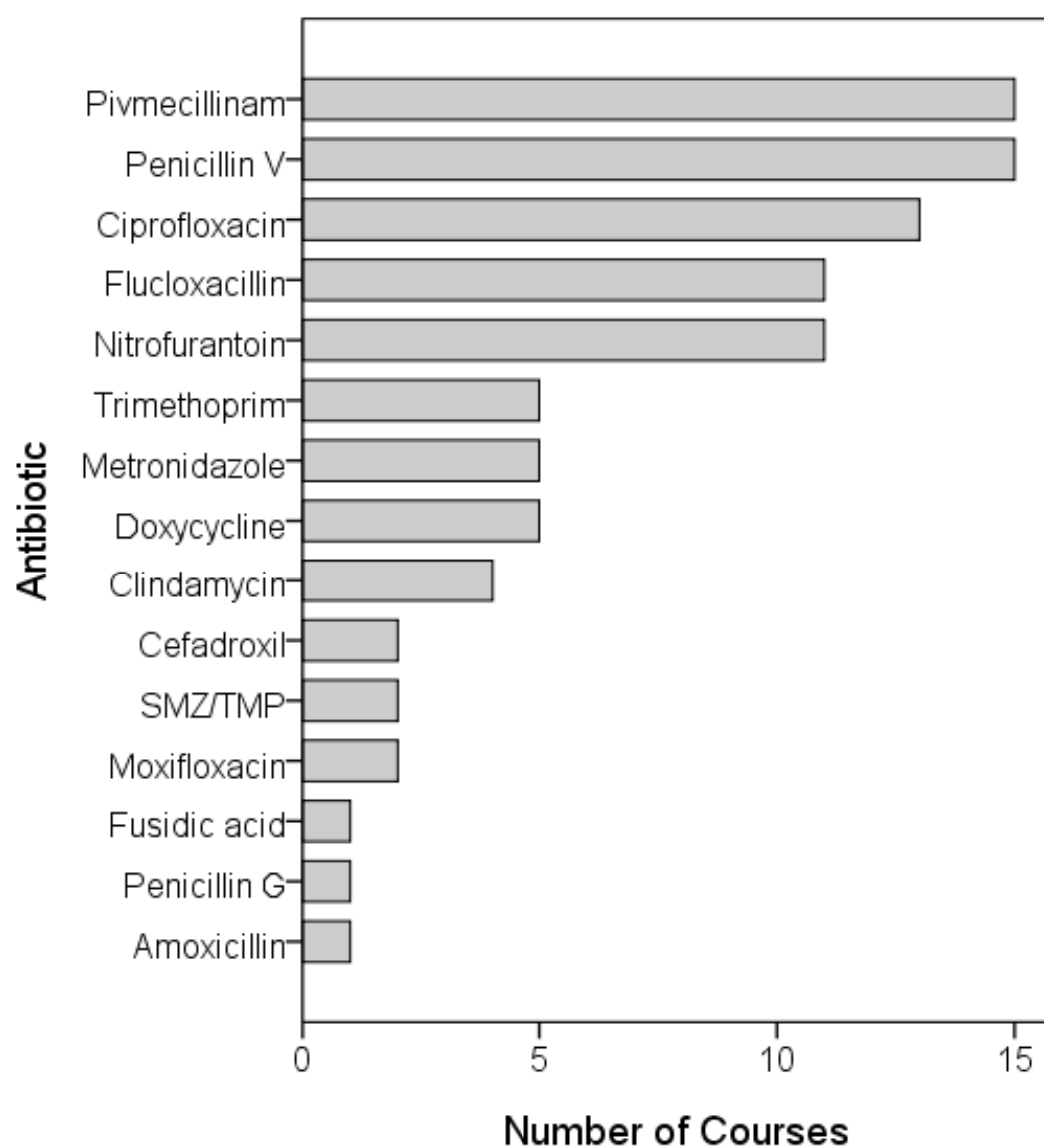


**TABLE II****Antimicrobial Resistance in 99 Participants Colonized with *Staphylococcus aureus***

	All Body Sites (N = 94)	BODY SITE			
		Nose (N = 63)	Throat (N = 57)	Groin (N = 20)	Skin Lesion (N = 6)
<b>Frequency of participants with any antimicrobial resistance in positive cultures</b>					
N	17	11	8	4	1
(95 % CI)	(10 to 26)	(6 to 19)	(4 to 15)	(1 to 9)	(0 to 4)
<b>Prevalence</b>					
%	18	17	14	20	17
(95 % CI)	(11 to 28)	(10 to 29)	(7 to 26)	(8 to 42)	(3 to 57)

CI = Confidence Interval.

Figure 1 (available as TIF-file: Number\_of\_Courses.TIF)



## Captions

Figure 1. The frequency distribution of antibiotics prescribed during the 180 day period that preceded each subject's date of microbiological sampling.