

# Staphylococcus aureus carriage among GPs in the Netherlands

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## ABSTRACT

### Background

The extent to which GPs serve as a reservoir for antibiotic-resistant *Staphylococcus aureus* is unknown and not well studied.

### Aim

To determine the prevalence of nasal *S. aureus* carriage among GPs in the Netherlands, as well as the antimicrobial resistance and the genotypes of isolated *S. aureus*.

### Design of study

Observational, point-prevalence, and cross-sectional study.

### Setting

GPs attending the annual conference of the Dutch College of General Practitioners in 2006.

### Method

Nasal swabs were randomly taken from 395 GPs and analysed for the presence of *S. aureus*. Antimicrobial susceptibility was determined by a microbroth dilution method and the genotypes by spa typing, which was associated with multilocus sequence typing.

### Results

Of the GPs, 129/395 (33%; 95% confidence interval [CI] = 28 to 37%) were carriers of *S. aureus*. No methicillin-resistant *S. aureus* (MRSA) was found. Resistance was observed to penicillin (71%; 95% CI = 63 to 79%), fusidic acid (7%; 95% CI = 3 to 13%), and clarithromycin (6%; 95% CI = 3 to 12%). In 72% of the isolates, an MRSA-related genotype of *S. aureus* was found.

### Conclusion

The low antibiotic resistance found among *S. aureus* of GPs suggests that GPs are not a reservoir of antibiotic-resistant *S. aureus* strains. The relatively high resistance to fusidic acid, which has not previously been described in the Netherlands and is mostly because of antibiotic use, suggests that patients infect GPs and not the other way round. GPs may be at risk for nasal carriage of *S. aureus* with an MRSA-related genotype.

### Keywords

antibiotic resistance; general practitioners; *Staphylococcus aureus*.

## INTRODUCTION

The indigenous microflora of patients and healthy individuals form a large reservoir for antibiotic-resistant micro-organisms and antibiotic-resistant genes, which are transferable to potential pathogens.<sup>1</sup> From all observed infections in general practices, respiratory tract infections are the most prevalent and the most common reason to consult a GP. About two-thirds of the antibiotics prescribed in general practices are for these infections,<sup>2</sup> which results in a selection of antibiotic-resistant microflora in patients. After an influenza virus infection, *Staphylococcus aureus* (*S. aureus*) is the most common cause of a severe pneumonia,<sup>2</sup> and the most common causative agent of skin infections.<sup>3</sup>

The contact of GPs with patients who might be infected or colonised with antibiotic-resistant bacteria<sup>4</sup> can lead to transmission of resistant bacteria to GPs. Consequently, GPs may become carriers or develop infections with these bacteria, which can be further transmitted to other patients. Healthcare workers are therefore considered to be

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the intermediary in the transmission of bacteria between healthcare centres (hospitals and long-term care facilities) and the community.<sup>5</sup>

Transmission of methicillin-resistant *S. aureus* (MRSA) from healthcare workers to patients has been the subject of more than 100 studies.<sup>5</sup> Clear evidence about transmission was found in only one-quarter of studies. Some studies considered the possibility of transmission; others could not prove any transmission between healthcare workers and patients.<sup>5</sup> The role of GPs as a reservoir for antibiotic-resistant *S. aureus* is not known.

Therefore, the aim of this study was to assess the prevalence of nasal *S. aureus* carriers among GPs and to determine the antimicrobial-resistance pattern as well as the genetic background of the isolated *S. aureus*.

## METHOD

### Study population

At an annual conference of the Dutch College of General Practitioners (Nederlands Huisartsen Genootschap) in 2006, nasal swabs were taken from volunteering GPs.

### Isolation of *S. aureus*

The swabs were analysed for the presence of *S. aureus*, using inoculation on colistin-nalidixic acid agar plates (BD Diagnostics, Breda, the Netherlands) and enrichment in Nutrient Broth No. 2 (Oxoid, the Netherlands) containing 6.5% sodium chloride. After overnight incubation, 10 µl of the broth was inoculated on oxacillin-resistant screening agar (Oxoid, the Netherlands). Putative *S. aureus* colonies were identified as *S. aureus* using Gram-stain, catalase, and coagulase testing as described previously.<sup>6</sup>

### Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern was determined using a microbroth dilution method with Mueller Hinton II Broth cation-adjusted (Becton, Dickinson and Company, Breda, the Netherlands), according to the guidelines of the Clinical Laboratory Standards Institute (CLSI).<sup>7</sup> The microtitre plates containing freeze-dried antibiotics were provided by MCS Diagnostics BV, Swalmen, the Netherlands.

The antibiotics tested were (range in mg/l): cefaclor (0.06 to 128); cefuroxime (0.06 to 128); clindamycin (0.03 to 64); ciprofloxacin (0.128 to 32); clarithromycin (0.03 to 64); gentamicin (0.06 to 64); imipenem (0.03 to 64); linezolid (0.03 to 64); meropenem (0.06 to 64); moxifloxacin (0.12 to 4); oxacillin (0.03 to 64); penicillin (0.004 to 8); rifampicin (0.008 to 16); teicoplanin (0.06 to 128); tetracycline (0.03 to 64); trimethoprim-sulfamethoxazole (0.015

## How this fits in

The role of GPs as a potential reservoir of antibiotic-resistant micro-organisms is not well known, and very few data are available on antimicrobial resistance of *S. aureus* of GPs. One study among GPs from west Ireland described the prevalence of methicillin-resistant *S. aureus* (MRSA). The present study aimed to provide more insight into the carriage of (antibiotic-resistant) *S. aureus* strains among GPs in the Netherlands. No MRSA was observed among GPs in a low endemic MRSA country. The higher percentage of *S. aureus* with a MRSA-related genetic background shows that surveillance of antibiotic resistance among GPs and patients remains important. Because of the relatively high fusidic acid resistance among GP isolates, the results suggest that GPs do not infect their patients, but rather the other way round.

to 32); and vancomycin (0.06 to 128).

Susceptibility to fusidic acid (100 µg) and mupirocin (10 µg; NeoSensitabs, Rosco, Denmark) was determined using a disk-diffusion method on Mueller-Hinton agar plates (BD Diagnostics, 254081, Breda, the Netherlands).<sup>8,9</sup> The MIC<sub>90</sub> was defined as the minimum inhibitory concentration required to inhibit the growth of 90% of organisms. All isolates that were resistant to clarithromycin and susceptible to clindamycin were tested for inducible clindamycin resistance using the D-test according to CLSI guidelines.<sup>10</sup>

### Genotypic determinations

The genetic background of the *S. aureus* isolates was determined using *spa* typing as previously described.<sup>11</sup> The *spa* types were clustered into *spa* clonal complexes (*spa*-CC) using the algorithm 'based upon repeat pattern' (BURP) with Ridom StaphType software (version 1.5; <http://www.ridom.de/staphtype/>). The default settings recommended by the manufacturer were used. It has been shown that *spa* typing, together with BURP, yields results that are concordant with typing results obtained by multilocus sequence typing (MLST).<sup>11</sup> The associated MLST-CCs were allocated through the Ridom SpaServer. (<http://spaserver.ridom.de>).

### Statistical analysis

This study aims to assess the carrier rate of *S. aureus* in GPs at an annual conference of Dutch GPs. Assuming a 30% prevalence of *S. aureus* (between 25% and 35% as shown in earlier studies), with a power of 0.8 and  $\alpha = 0.05$  (two-sided), a sample size of 323 was required to demonstrate that the carriage rate of GPs did not differ from the rate in other populations.

Confidence intervals were determined with the exact method to guarantee at least 95% coverage, and were placed between brackets (95% confidence

**Table 1. Minimum inhibitory concentration distribution of *Staphylococcus aureus* isolates for antibiotics commonly used by GPs, n = 129.<sup>a</sup>**

	MIC distribution (mg/L)													% resistance				
	<0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.50	1.00	2.00	4.00	8.00	16.0	32.0	64.0	128	MIC90	
Ciprofloxacin							73	26	1								0	0.5
Clarithromycin					0.8	2.3	14	69.8	7.8			0.8				5.4	6.4	1.00
Clindamycin					16.2	80.8	3.1						0.3			0.3	0.6	0.12
Penicillin	2.3	2.3	10.9	6.2	7.0	2.3	3.1	17.8	10.1	11.6	17.8	7.8	2.3				71	4.00
Oxacillin				3.8	4.6	32.3	47.7	11.5									0	0.5
Tetracycline				0.8	6.2	36.9	50.8	3.1					0.8	0.8	0.8		2	0.5

<sup>a</sup>No resistance was found against ciprofloxacin, moxifloxacin, cefaclor, imipenem, meropenem, vancomycin, teicoplanin, gentamicin, cefuroxime, linezolid, or trimethoprim-sulfamethoxazole. Furthermore, all isolates were susceptible to mupirocin. Bold lines indicate the boundary where the cut-off point for *S. aureus* susceptibility is on the left, and the cut-off point for *S. aureus* resistance to a specific antibiotic on the right. Dotted line represents the cut-off below which the MIC value is susceptible. MIC90 = minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

interval [CI] = lowest value to highest value). Statistical analyses were performed using SPSS (version 16.0).

**RESULTS**

A total of 395 of 1236 GPs participated (a response rate of 32%). *S. aureus* was isolated in 129 participants, resulting in a *S. aureus* carriage rate of 33% (95% CI = 28 to 37%). Oxacillin-resistant isolates were not observed. Resistance to penicillin, clarithromycin, clindamycin, and fusidic acid was found in 71% (95% CI = 63 to 79%), 6% (95% CI = 3 to 12%), 1% (95% CI = 0 to 4%), and 7% (95% CI = 3 to 13%) of the isolates respectively. One strain was rifampicin resistant. No resistance was observed against ciprofloxacin, moxifloxacin, cefaclor, imipenem, meropenem, vancomycin, teicoplanin, gentamicin, cefuroxime, linezolid, or trimethoprim-sulfamethoxazole. All isolates were

susceptible to mupirocin. MIC distributions of the most common antibiotics used in general practices are shown (Table 1). Inducible clindamycin resistance was observed in six of the seven clarithromycin-resistant isolates.

A total of 74 different spa types were observed. The spa types were clustered into 14 spa-CCs, of which five spa-CCs had a no founder cluster. Twenty-three singletons and six spa types were excluded from the analysis, because the number of repeats was less than five. Two isolates were not type-able with spa typing, because a repeat of 25 base pairs was found. The main spa-CC was spa-CC012, which harboured 17% of the *S. aureus* isolates and was associated with MLST CC30, followed by spa-CC015 (MLST CC45) which comprised 13%. Other spa-CCs were present in 10% or less than 10% of the strains (Table 2). More than 72% (95% CI = 67 to 83%; n = 97) of the isolates had a genetic background common to MRSA clones, such as CC1, CC5, CC8, CC22, CC30, CC45, CC59, or CC398.

**Table 2. Spa typing results of *Staphylococcus aureus* from GPs.**

Associated MLST-CC	GP prevalence, n (%)	Spa-CC
<b>MRSA-associated</b>		
CC45	29 (22.8)	015; 065/004; singleton; excluded
CC30	28 (22.0)	012, 369
CC5	13 (10.2)	002, no founder 1
CC8	10 (7.9)	024, singleton
CC22	5 (3.9)	005
CC1	4 (3.1)	No founder 2; no founder 5
CC59	2 (1.6)	No founder 3
CC398	1 (0.8)	Singleton
<b>MSSA-associated</b>		
CC7/15	14 (11.0)	346; no founder 4
CC121	6 (4.7)	659/171
CC182	3 (2.4)	Singleton
CC25	2 (1.6)	Singleton
CC20	1 (0.8)	Singleton
Unknown MLST	9 (7.1)	Singleton; excluded

spa-CC = spa clonal complexes. MLST = multilocus sequence typing. MLST-CC = multilocus sequence typing clonal complexes. MRSA = methicillin-resistant *S. aureus*. MSSA = methicillin-sensitive *S. aureus*.

**DISCUSSION**

**Summary of main findings**

This study showed that the prevalence of nasal carriage of *S. aureus* among GPs was 33% (95% CI = 28 to 37%). The low antibiotic resistance observed among the isolates suggests that the participating GPs are not an important reservoir for antibiotic-resistant *S. aureus*. The resistance to fusidic acid was 7% (95% CI = 3 to 13%) and has not been described before in the Netherlands. Although the nasal *S. aureus* carrier rate among GPs was within the normal range, 72% (95% CI = 67 to 83%) of the isolates had a MRSA-related genetic background, suggesting that GPs may have a potential high risk for future MRSA carriage.<sup>12-13</sup>

**Strengths and limitations of the study**

Statistical analysis showed that a sample size of 323

was sufficient for screening so the 395 tested provided sufficient power. The present study shows, to the best of the authors' knowledge, the first analysis of carriage of (antibiotic-resistant) *S. aureus* among GPs. Unfortunately, demographic details about the participants were not available, and a selection bias between GPs attending and not attending at the conference could have occurred. Furthermore, earlier studies showed that multiple-site screening will result in a higher yield.<sup>14,15</sup> However, in practice, multiple-site screening is feasible in a hospital situation but not in a survey like the present one. The most practical solution was chosen.

### Comparison with existing literature

Very few data on antimicrobial resistance among *S. aureus* isolates of GPs are available. One review, by Albrich and Harbarth, described the prevalence of *S. aureus* carriage among healthcare workers in hospitals as ranging from 0 to 40%, with a median of 24% in hospitals.<sup>5</sup> An earlier study among hospital workers in the Netherlands showed a carrier rate of methicillin-sensitive *S. aureus* (MSSA) of 35%, and an MRSA prevalence of less than 5%.<sup>16</sup> The carrier rate of MSSA in healthcare workers was comparable with the results found in the present study, but the MRSA prevalence among healthcare workers in the Netherlands was higher than that observed among the tested GPs.<sup>16</sup>

The only study among GPs was a study in west Ireland. This study found an MRSA prevalence of 7.7%.<sup>4</sup> This was higher in comparison with that of the Irish community, but the same as the prevalence of MRSA among hospital staff (6.6%).<sup>4</sup> In the present study, no MRSA was observed, which is in line with the low prevalence of MRSA among hospitalised patients (1 to 2%) and outpatients (0 to 2%) in the Netherlands.<sup>17</sup> The observed *S. aureus* carriage among GPs was comparable with the *S. aureus* carriage prevalence in other populations in the Netherlands.<sup>18,19</sup>

Fusidic acid is used as a topical agent for local infections, especially for the eyes and the skin. Several studies have described that the topical use of this drug has led to an increase of resistance.<sup>20-22</sup> Some suggest that a *S. aureus* clone responsible for the resistance to fusidic acid is spreading,<sup>22,23</sup> and that this is resulting in increased resistance. An earlier study in the Netherlands from 2002 showed no resistance to fusidic acid among impetigo isolates.<sup>21</sup> Therefore, according to the guidelines of the Dutch College of General Practitioners, fusidic acid remained the first choice for topical use in skin infections, especially impetigo.<sup>20,21</sup>

The 7% resistance to fusidic acid is, to the best of our knowledge, the first observation of such a high

resistance percentage among commensal isolates in the Netherlands. The *S. aureus* of GPs are probably not exposed to fusidic acid through the GPs. Therefore, these results suggest that the GPs might become infected with *S. aureus* isolates by patients. There is no clear explanation for the relatively high resistance prevalence.

Further studies, investigating the prevalence of fusidic acid resistance in other populations in the Netherlands, especially among patients with impetigo infections, and the cause of increasing fusidic acid resistance, are warranted. Depending on these results, recommendations as to the first drug of choice in the case of impetigo infections in the Netherlands could be established.

MLST CC30, the main associated MLST CC found in this study, was also found in other studies among outpatients and hospitalised patients in the Netherlands and worldwide.<sup>24-27</sup> Another common MLST CC worldwide is MLST CC45, which was also highly prevalent in the present study.<sup>25-27</sup> As the Ridom SpaServer does not detect repeats longer than 24 bases, two isolates with a repeat of 25 base pairs were not type-able, in accordance with European rules for typing justified by SeqNet.org.<sup>28</sup>

In previous studies in the Netherlands, approximately 50% of the *S. aureus* isolated from several patient populations had a genetic background common to major MRSA clones.<sup>11,26</sup> The high percentage (72%) of *S. aureus* with an MRSA genotype observed in this study was unexpected, and no explanation for this phenomenon could be found. Recently, Nubel *et al* showed that MRSA evolution occurs more often than one might expect.<sup>13</sup> As Staphylococcal Cassette Chromosome mec (SCCmec) is only stable in MSSA isolates with a MRSA related genotype,<sup>13</sup> GPs could be a carrier of MRSA in the (near) future. One of the possible solutions to prevent that might be decolonisation of the nose of GPs.

### Implications for future research and clinical practice

The role of GPs in transmission of MRSA to patients and community dwellers remains unclear and needs further study. In the Netherlands, the MRSA prevalence is still low, but is increasing.<sup>10</sup> The higher percentage of *S. aureus* with a MRSA-related genetic background might result in a higher MRSA carriage rate among GPs, and in transmission of MRSA from GPs to patients.

Furthermore, the increased resistance in fusidic acid among commensal *S. aureus* of GPs is a point of concern and warrants further studies as to the prevalence of resistance among *S. aureus* from skin infections in GPs' patients.



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**Ethics committee**

The study was approved by the ethical committee of the Maastricht University Medical Centre (MUMC).

**Competing interests**

The authors have stated that there are none.

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