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Is living in a border region a risk for a high prevalence of resistance?

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ABSTRACT

This study assessed the antimicrobial resistance and population structure of Staphylococcus aureus isolated from general practice (GP) patients and nursing home (NH) residents in the province of Limburg (near the border with Germany and Belgium) in comparison with those obtained in the remaining provinces of the Netherlands. A total of 617 and 418 S. aureus isolates were isolated from 2,691 to 1,351 nasal swabs from GP patients and NH residents, respectively. Quantitative antibiotic susceptibility testing was performed using a microbroth dilution method. Putative methicillin-resistant S. aureus (MRSA) isolates were tested for the presence of the mecA gene and spa typing was performed on all S. aureus isolates. No significant differences in the prevalence of resistance were found between the two groups of GP isolates, but the isolates from the NH residents showed a lower resistance for trimethoprim–sulfamethoxazole ($p = 0.003$) in Limburg province compared with the remaining provinces in the Netherlands. Among the isolates from NH residents in Limburg province, the prevalence of spa-CC 084 was higher ($p = 0.003$) and that of spa-CC 002 was lower ($p = 0.01$) compared with isolates from NHs in the remaining provinces of the Netherlands. We observed no differences in resistance and population

structure between S. aureus isolates from GP patients in Limburg and the remaining provinces of the Netherlands, and only a few differences were observed between the NH populations. There was no higher prevalence of resistance among the GP and NH isolates from Limburg compared with the remaining provinces.

INTRODUCTION

Staphylococcus aureus is a frequent causative agent of community- and hospital-acquired infections, varying from minor skin and soft tissue infections to invasive infections like bacteremia and endocarditis [1]. In many European countries, the antibiotic resistance of S. aureus is increasing [2] and, consequently, an optimal empiric antibiotic choice for the treatment of S. aureus infections becomes more challenging. In particular, the prevalence of methicillin-resistant S. aureus (MRSA) is a point of concern. In the Netherlands, the prevalence of MRSA is still low [3], which can, at least in part, be attributed to the national antibiotic policy and the infection control guidelines (including the "search and destroy" protocol). However, in recent years, several reports have mentioned MRSA outbreaks in Dutch nursing homes (NHs) [4–7].
The prevalence of antibiotic resistance and MRSA is higher in Belgium and Germany than in the Netherlands [2], with a prevalence of MRSA in hospitals of 20.5 %, 20.9 %, and 1.2 %, respectively [2]. The province of Limburg has a border with Belgium and Germany of 351 km and a border with two other Dutch provinces (i.e., Noord-Brabant and Gelderland) of only 113 km [8], and is a region with intensive cross-border traffic (4 % of all jobs in Limburg is fulfilled by Belgian and German citizens) and cross-border patient mobility due to the (free) access to health care facilities on both sides of the border [9–11].
There is no information available as to whether residents of the province of Limburg, due to its location, have a higher prevalence of resistance among S. aureus isolates and whether these isolates have a different population structure than those in the remaining provinces of the Netherlands. Therefore, we evaluated the antibiotic resistance and population structure of S. aureus isolates from general practice (GP) patients and NH residents in the province of Limburg in comparison with those of the remaining provinces of the Netherlands.

METHODS

Study population and isolation of S. aureus

In 2005, a total of 2,691 nasal swabs (Amies agar gel swabs without charcoal 108C, Copan Diagnostics, Brescia, Italy) were taken from the anterior nostrils from GP patients with no apparent signs of infection from ten GPs in Limburg province (GP-L) and 19 GPs in the remaining provinces in the Netherlands (GP-NL). The nasal swabs were analyzed as described previously [12]. In 2009 and 2010, a total of 1,351 nasal swabs were collected from NH residents from six NHs in the province of Limburg (NH-L) and from 24 NHs in other provinces of the Netherlands (NH-NL, Table 1). The swabs were analyzed with the same methods as the GP swabs [12]. Informed consent was obtained from all participants.

[TABLE 1]

Quantitative susceptibility testing

Quantitative susceptibility testing was performed using microbroth dilution with Mueller Hinton II cation-adjusted broth (Becton-Dickinson, Sparks, MD, USA) and microtiter plates with freeze-dried antibiotics (MCS Diagnostics BV, Swalmen, the Netherlands). S. aureus ATCC 29213 was used as a control strain. The breakpoints for resistance were in accordance to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [13]. Antibiotic susceptibility testing for fusidic acid and mupirocin was performed with a disk diffusion test with Mueller Hinton II agar plates (BD) and antibiotics tablets (fusidic acid 100 µg and mupirocin 10 µg, Rosco, Taastrup, Denmark). Zone diameters of >27mm for fusidic acid and >15mm for mupirocin were considered to be susceptible [14, 15]. Oxacillin-resistant isolates were analyzed for the presence of the mecA gene using a polymerase chain reaction (PCR) assay, as described previously [12].

Typing of the spa locus

Amplification of the spa locus, followed by sequencing, was performed as described previously [16, 17]. The spa types were clustered into spa-clonal complexes (spa-CCs) using the Based Upon Repeat Pattern (BURP) algorithm with Ridom StaphType software version 2.2.1 (http://www.ridom.de). The spa types with <6 repeats were excluded from the analysis and spa types were clustered if the cost was <5, to prevent the formation of too large and non-specific spa clusters. After applying the BURP algorithm, the associated multilocus sequence typing (MLST) clonal complexes (CCs) were allocated through the Ridom SpaServer (http://spaserver.ridom.de).

Statistical analysis

Significant statistical differences in antibiotic resistance were calculated using the Pearson Chi-square test or a Fisher's exact test (PASW software version 18.0, IBM, Armonk, NY, USA). A modified false discovery rate (FDR) method developed by Benjamini and Yekutieli was used as the method of correction for multiple testing [18]. A p-value <0.05 was considered to be statistically significant. The diversity of the groups of isolates regarding the spa types was determined with Simpson's index of diversity [19].

RESULTS

Prevalence of S. aureus carriage

The prevalence of S. aureus nasal carriage was 23 %, 247 isolates out of 1,096 swabs and 370 isolates out of 1,595 swabs, among both GP-L and GP-NL, respectively (Table 1). The prevalence among the NH residents was 31 %, 100 isolates out of 291 swabs in NH-L, and 29 %, 318 isolates out of 1,060 swabs in NH-NL.

Prevalence of antibiotic resistance

The antibiotic susceptibility patterns of the isolates are shown in Table 2. Unfortunately, for further analysis, 22 of the S. aureus isolates could not be cultured from the frozen stock.

[TABLE 2]

No significant differences in resistance were demonstrated between GP-L and GP-NL. Resistance to linezolid, trimethoprim–sulfamethoxazole, vancomycin, gentamicin, and mupirocin was either not observed or less than 1 %. Four isolates were resistant to oxacillin [minimum inhibitory concentration (MIC) 4 or 8 mg/L], but none of these isolates harbored the mecA gene. These isolates had the following spa types: t062, t127, t224, and t1702.
Among the NH isolates, the prevalence of resistance was lower for trimethoprim–sulfamethoxazole (p = 0.003) and clarithromycin (p = 0.093) from NH-L (0 % and 2 %, respectively) compared with NH-NL (9 % and 8 %, respectively). All but one of the trimethoprim–sulfamethoxazole-resistant isolates harbored spa t064. Six isolates (two in NH-L and four in NH-NL) were resistant to oxacillin (MIC 8 mg/L to >64 mg/L), carried the mecA gene, and had the following spa types: t002, t037, t091, t223, t740, and t2164. Resistance to vancomycin, gentamicin, mupirocin, and linezolid was either not demonstrated or less than 1 %.

Distribution of spa types and BURP analysis
A total of 329 spa types were found. The most prevalent spa types among all isolates were t008 (6.6 %), t002 (5.8 %), and t091 (5.1 %). The other spa types accounted for 0.1 % to 4.1 % each. Among the GP-L isolates, spa types t012 (6.5 %), t091 (5.7 %), and t002 (4.0 %) were the most prevalent, and among the GP-NL isolates, spa types t091 (5.5 %), t008 (4.9 %), and t012 (4.9 %) were the most prevalent. Among the NH-L isolates, spa types t008 (22 %), t091 (10 %), and t026 (7 %) were the most prevalent, and among the NH-NL isolates, spa types t002 (9.8 %), t064 (8.2 %), and t008 (6.6 %) were the most prevalent.
The spa types were clustered into 16 spa-CCs (Table 3). Three clusters had no founder and 33 spa types (3.3 %) could not be clustered into an spa-CC and were classified as singletons. Seventy isolates (6.9 %) were excluded from the analysis, since these spa types consisted of less than five repeats and 11 isolates were not typable.

[TABLE 3]

Overall, the percentage of isolates belonging to each spa-CC was similar for GP-L and GP-NL and for NH-L and NH-NL. The only difference observed was for the NH isolates: the prevalence of spa-CC 084 (7 %) was higher (p = 0.003) and that of spa-CC 002 (17 %) was lower (p = 0.01) among isolates collected from NH-L compared with those from NH-NL.
There was also variation in the diversity of the spa types among the NH isolates: 0.929 [95 % confidence interval (CI) 0.896–0.962] and 0.971 (95 % CI 0.963–0.978) for the NH-L and NH-NL isolates, respectively.
The spa types were associated to an MLST ST or CC via the Ridom SpaServer (Table 4). No significant differences were found between the GP-L and GP-NL isolates (56 % and 61 %, respectively) and the NH-L and NH-NL isolates (73 % and 79 %, respectively)

[TABLE 4]

DISCUSSION

In this study, we observed no difference in the prevalence of resistance between the S. aureus isolates from GP-L and GP-NL, but there was a significantly lower prevalence of resistance to trimethoprim–sulfamethoxazole for the isolates from NH-L compared with those from NH-NL. Significant differences in the prevalence of spa-CCs 084 and 002 were also found for the NH isolates.

The strength of the study is that all samples were analyzed at the same laboratory with the same methods. Bias due to differences in methods can, therefore, be excluded. The study also has some weaknesses: the number of NH-L isolates was lower compared to the other groups of isolates. Also, clinical data was not available and, although there was a difference in the sampling period between the GP and NH isolates, we do not expect that this could explain the differences in the prevalence of resistance observed [20].

The 23 % prevalence of nasal colonization with S. aureus among both groups of GP patients was in agreement with previous reports [21], which also applies for the 29 % and 31 % prevalences among the NH resident populations. Previous studies reported prevalences in the range 23.9–43 % [22–24].

No differences in antibiotic resistance were found between isolates collected from the two groups of GP patients, but between the two groups of NH residents, a difference in trimethoprim–sulfamethoxazole resistance was observed. The difference observed might be due to differences in antibiotic use. NH residents are a frail population, with a high use of antibiotics and more risk factors for acquiring and retaining a more resistant S. aureus isolate [22, 25]. However, among GP patients and NH residents, resistance to trimethoprim–sulfamethoxazole was rare, despite their relatively high use [26], and further studies seem warranted. The 27 resistant isolates (out of 1,013) were found in 13 different NHs and all but one harbored spa type 064, which is an spa type associated with MLST CC 8, an MRSA-associated MLST CC.

Quinolone resistance was higher among the NH residents compared with the GP patients. Higher use of quinolones in NHs compared to GP patients is probably the main reason for the higher prevalence of resistance [27, 28]. However, also, differences in living environment, higher age, comorbidities, and use of indwelling devices could play a role [23].

Among the isolates from the GP patients, the four oxacillin-resistant isolates were classified as borderline oxacillin-resistant S. aureus (BORSA). Isolates like these have been described previously, but mostly in a clinical setting [29–31]. The clinical relevance of these isolates remains questionable.

With BURP, all spa types were allocated in spa-CCs. The only significant difference was found for spa-CC 084 and spa-CC 002 between the NH groups. Overall, spa-CC 012 was quite large and could be associated to two very different MLST CCs, i.e., CC 30 and 45. With more strict BURP settings (cost <4), this spa-CC could be divided into a few smaller clusters, of which spa-CC 012, associated to MLST CC 30, and spa-CC 015, associated to MLST CC 45, were the largest (74 % of all isolates in this group). In line with the differences in spa-CCs, differences in the prevalence of isolates associated with an MRSA-associated MLST CC were observed.

The diversity of the spa types from the GP isolates was comparable with previous reports [32, 33]. The lower diversity of the spa types among the NH isolates might be due to the enclosed living environment, transmission within the NHs, and higher antibiotic use. These factors, combined with the lower number of participating NHs in Limburg, might explain the lower diversity among the NH isolates in Limburg.
We observed only minor differences in antibiotic resistance and population structure between isolates collected from GP patients and NH residents in Limburg and the remaining parts of the Netherlands.
Although the differences in the prevalence of S. aureus resistance among hospital isolates from Belgium, Germany, and the Netherlands are considerable, the differences were much smaller among isolates from the extramural setting, especially among GP patients. So, living in the border region of the province of Limburg is not a risk for a high prevalence of resistance, but whether this is due to the absence of cross-border spread or only spread mostly via the extramural setting, where resistance is low, remains to be investigated.
Furthermore, this study provides an overview of S. aureus resistance and population structure among GP patients and NH residents in the Netherlands. Surveillance at a local and international level is warranted, in order to keep informed as to changes in the prevalence of resistance over time.

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Competing interests
None to declare.

Ethical approval
Ethical approval for this project was granted by the Medical Ethics Committee of the Maastricht University Medical Centre, reference number: 07-4-012.4/pl.

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TABLES

Table 1 Overview of the collected swabs and *Staphylococcus aureus* isolates

Unit	GP-L	GP-NL	NH-L	NH-NL	Total
No. of swabs	1,096	1,595	291	1,060	4,042
No. of isolates	247	370	100	318	1,035
Prevalence	23 %	23 %	31 %	29 %	26 %
No. of *spa* types	130	163	39	114	329

GP-L general practice patients in the province of Limburg, *GP-NL* general practice patients in the remaining provinces of the Netherlands, *NH-L* nursing home residents in the province of Limburg, *NH-NL* nursing home residents in the remaining provinces of the Netherlands

Table 2 Antibiotic resistance among *S. aureus* isolates collected from general practice (GP) patients and nursing home (NH) residents

Antimicrobial agent	GP-L (247)	GP-NL (348)	NH-L (100)	NH-NL (318)
Oxacillin	1 %	1 %	2 %	1 %
Clarithromycin	7 %	5 %	2 %	8 %
Clindamycin	1 %	0 %	0 %	2 %
Ciprofloxacin	1 %	2 %	24 %	34 %
Fusidic acid	6 %	6 %	6 %	4 %
Tetracycline	3 %	5 %	2 %	2 %
Trimethoprim–sulfamethoxazole	0 %	0 %	0 %*	9 %*

GP-L general practice patients in the province of Limburg, *GP-NL* general practice patients in the remaining provinces of the Netherlands, *NH-L* nursing home residents in the province of Limburg, *NH-NL* nursing home residents in the remaining provinces of the Netherlands

*Significant difference between the two groups of NH isolates

Table 3 Distribution of *spa*-clonal complexes (*spa*-CCs) among isolates collected from GP and NH patients

spa-CC	Isolates (%)	*spa* (%)	GP-L (%)	GP-NL (%)	NH-L (%)	NH-NL (%)
spa-CC 012	299 (30)	100 (30)	73 (30)	110 (32)	29 (29)	87 (27)
spa-CC 084	124 (13)	27 (9)	37 (15)	47 (14)	18 (18)*	22 (7)*
spa-CC 002	105 (10)	26 (8)	19 (8)	26 (7)	5 (5)*	55 (17)*
spa-CC 024	126 (12)	19 (6)	15 (6)	25 (7)	23 (23)	63 (20)
spa-CC 078	36 (4)	19 (6)	17 (7)	14 (4)	1 (1)	4 (1)
spa-CC 127	66 (7)	18 (5)	12 (5)	26 (7)	6 (6)	22 (7)
spa-CC 166	35 (3)	15 (5)	11 (4)	15 (4)	0 (0)	9 (3)
spa-CC 005	30 (3)	13 (4)	5 (2)	14 (4)	2 (2)	9 (3)
spa-CC 159	17 (2)	10 (3)	7 (3)	9 (3)	0 (0)	1 (0)
spa-CC 216	27 (3)	8 (2)	7 (3)	4 (1)	1 (1)	15 (5)
spa-CC 160	12 (1)	6 (2)	6 (2)	5 (1)	0 (0)	1 (0)
spa-CC 364	9 (1)	4 (1)	4 (2)	4 (1)	0 (0)	1 (0)
spa-CC 1045	5 (0)	3 (1)	2 (1)	1 (0)	1 (1)	1 (0)
No founder 1	3 (0)	2 (1)	0 (0)	3 (1)	0 (0)	0 (0)
No founder 2	2 (0)	2 (1)	1 (0)	1 (0)	0 (0)	0 (0)
No founder 3	3 (0)	2 (1)	2 (1)	1 (0)	0 (0)	0 (0)
Singletons	33 (3)	23 (7)	8 (3)	15 (4)	1 (1)	9 (3)
Excluded	70 (7)	32 (10)	19 (8)	25 (7)	11 (11)	15 (5)
Not typable	11 (1)	0 (0)	2 (1)	3 (1)	2 (2)	4 (1)
Total	1,013	329	247	348	100	318

GP-L general practice patients in the province of Limburg, *GP-NL* general practice patients in the remaining provinces of the Netherlands, *NH-L* nursing home residents in the province of Limburg, *NH-NL* nursing home residents in the remaining provinces of the Netherlands

*Significant difference between the NH-L and NH-NL isolates

Table 4 Composition of the *spa*-CCs

spa-CC	*spa* types	MLST CC (ST)
spa-CC 012	t004, t012, t015, t018, t019, t021, t031, t034, t037, t040, t050, t065, t069, t073, t074, t095, t096, t102, t116, t122, t138, t230, t238, t266, t274, t275, t276, t300, t302, t318, t331, t338, t370, t404, t406, t483, t505, t571, t576, t583, t589, t620, t630, t631, t706, t740, t772, t822, t840, t861, t908, t937, t950, t1130, t1149, t1238, t1239, t1281, t1504, t1510, t1574, t1827, t1932, t2077, t2135, t2143, t2208, t2209, t2210, t2239, t2254, t2269, t2275, t2387, t2479, t2489, t2496, t2539, t2540, t2541, t2544, t2548, t2557, t2561, t2566, t2568, t2572, t2610, t2659, t2674, t2682, t2821, t2864, t4441, t4905, t5834, t7110, t7126, t7143, t7147	30, 45
spa-CC 084	t084, t085, t091, t094, t252, t279, t346, t348, t360, t393, t491, t547, t774, t796, t853, t867, t1243, t1363, t1716, t1943, t2074, t2543, t2556, t2567, t2616, t5875, t7134	7, 15
spa-CC 002	t001, t002, t010, t041, t062, t067, t179, t242, t306, t311, t389, t447, t509, t539, t548, t688, t837, t1215, t1340, t2070, t2164, t2212, t2491, t2542, t2724, t6160	5
spa-CC 024	t008, t024, t064, t104, t190, t197, t334, t377, t530, t648, t701, t711, t846, t1171, t2041, t3060, t3802, t4299, t5279	8
spa-CC 078	t056, t078, t081, t087, t150, t258, t353, t436, t469, t660, t775, t814, t1102, t1312, t1541, t1671, t1898, t2039, t2078	25, 101
spa-CC 127	t114, t127, t177, t189, t224, t267, t286, t359, t376, t591, t1236, t1407, t1787, t2500, t2569, t2612, t2819, t7123	1
spa-CC 166	t089, t136, t153, t166, t240, t369, t884, t1014, t2038, t2071, t2073, t2080, t2547, t2854, t7162	
spa-CC 005	t005, t060, t223, t474, t790, t1433, t1629, t2570, t2618, t2681, t5485, t5926, t7156	22
spa-CC 159	t159, t171, t272, t284, t408, t645, t659, t738, t2213, t2820	121
spa-CC 216	t172, t216, t471, t1293, t2079, t2488, t3527, t4303	59
spa-CC 160	t156, t160, t213, t771, t1702, t3938	12
spa-CC 364	t364, t493, t2492, t2680	
spa-CC 1045	t099, t100, t1045	9
No founder 1	t148, t2016	
No founder 2	t186, t729	(88)
No founder 3	t246, t2495	
Singletons	t106, t344, t587, t818, t878, t1362, t1406, t2050, t2075, t2076, t2490, t2494, t2558, t2559, t2573, t2615, t2617, t5874, t7108, t7132	(20)
	t164	(59)
	t199	(109)
	t209	
Excluded	t059, t118, t287, t362, t502, t524, t535, t605, t643, t808, t929, t1152, t1200, t1209, t1456, t1509, t2176, t2207, t2211, t2246, t2365, t2383, t2493, t2571, t2611, t2613, t2614, t2853, t4386	
	t026	45
	t233	(59)
	t386	(1)

Overview of the *spa* types in every *spa*-CC and the associated MLST clonal complex (CC) or sequence type (ST), associated via the Ridom SpaServer