Trends in antimicrobial non-susceptibility in methicillin-resistant *Staphylococcus aureus* from Germany (2004–2011)

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Abstract

We analysed trends in antimicrobial non-susceptibility in methicillin-resistant *Staphylococcus aureus* (MSRA) from Germany to assess the impact of the changing population structure of MRSA on antimicrobial resistance rates. During two large nationwide multicentre studies in 2004–2005 and 2010–2011, we collected consecutively *spa*-genotyped MRSA isolates. The increase in non-susceptibility rates for tetracycline and trimethoprim–sulphamethoxazole was associated with the spread of livestock-associated MRSA. A decrease in non-susceptibility rates for aminoglycosides and quinolones affected all major lineages (*spa*-clonal complexes 003, 008, and 032). All isolated remained susceptible to glycopeptides and linezolid.

Keywords: Genotype, Germany, LA-MRSA, methicillin-resistant *Staphylococcus aureus*, resistance

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a challenge for healthcare systems around the globe. The advent of novel clonal lineages belonging to community-associated MRSA and livestock-associated (LA)-MRSA may have jeopardized much of the success of the past years in combating healthcare-related MRSA [1,2].

Many antimicrobial agents have been developed with either bacteriostatic or bactericidal activity, but reduced susceptibility against anti-MRSA compounds is emerging [3]. For instance, the increase in vancomycin MICs within the susceptibility range (≤2 mg/L, vancomycin creep) is worrying, as it could be associated with poorer outcome of infections [3,4]. Thus, knowledge of the prevalence and trends of resistance of circulating MRSA strains is needed to support the best possible use of the remaining antibiotic armamentarium in empirical chemotherapy.

The aim of this study was to systematically assess trends in rates of resistance to antimicrobial agents in MRSA isolates from Germany that have been genotypically characterized in a previous survey [5]. Briefly, 36 laboratories from different regions in Germany were invited to participate in this survey, and 33 laboratories contributed up to 50 consecutively collected MRSA isolates during two studies (1 February 2004 to 31 January 2005, and 1 February 2010 to 31 January 2011). Only one isolate per patient was included, and no exclusion criteria were applied. Isolates were stored between −20°C and −80°C in the participating laboratory, and were sent in a batch after each sampling period to the Institute of Medical Microbiology, University Hospital Münster, Germany. There, isolates were stored at −80°C in bacterial storage systems (Cryobank; Mast Diagnostica, Bootle, UK) and were subjected to susceptibility testing after the sampling period of each study with the Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) and the antimicrobial susceptibility cards AST P549 (in 2004–2005) and AST P580 (in 2010–2011). Both susceptibility test cards (bioMérieux) include the same range of concentrations for the same antibiotics, with the exception of an additional dilution step for vancomycin (0.5 mg/L) in AST P580. No changes were made during the two studies (hardware or software) that would affect the measurement of MICs. The MICs of the tested antimicrobial agents were interpreted according to EUCAST clinical breakpoints (Version 3.1, 11 February 2013). As the lowest MIC of the antimicrobial susceptibility cards for rifampicin is above the EUCAST breakpoint for susceptibility, we used the CLSI breakpoints to differentiate between intermediate and susceptible isolates [6].

All isolates were genotyped by the use of *S. aureus* protein A typing (*spa* typing) [7]. Related *spa* types were clustered into *spa* clonal complexes (*spa*-CCs) with the BURP algorithm as implemented in the software Ridom StaphType (2.2.1), with preset parameters as previously published [8].

The 50th and 90th percentiles of MIC (MIC_{50} and MIC_{90}) and MIC ranges were calculated with R software, version 2.13.1 (http://cran.r-project.org) and the package 'epicalc'.

Non-susceptibility rates, which include both resistant and intermediate categories, were compared by use of the chi-squared test.

In total, 3207 MRSA isolates were included, and 3205 were tested (2004–2005, $n = 1603$; 2010–2011, $n = 1602$). We failed to reculture one isolate of each sampling period for susceptibility testing after cryo-conservation.

We detected a significant decrease in non-susceptibility from 2004–2005 to 2010–2011 for gentamicin (OR 0.33, 95% CI 0.3–0.4, p <0.005) and tobramycin (OR 0.4, 95% CI 0.4–0.5, p <0.005; Table 1). This decrease in aminoglycoside non-susceptibility was not restricted to a single *spa*-CC but occurred in several lineages, including *spa*-CC 003 (associated with multilocus sequence typing sequence type (ST)5, ST225, 'Rhine-Hesse' clone), *spa*-CC 032 (associated with ST22, 'Barnim' clone), and *spa*-CC 008 (associated with ST8, ST247, ST250, and ST254), but not in *spa*-CC 004 (ST45; Table S1). All other rates of non-susceptibility of the tested antibiotics remained unchanged in isolates belonging to the three major *spa* types (t003, t008, and t032). This may suggest that general changes in antimicrobial susceptibility are explained by a general change in the MRSA population structure (e.g. spread of the 'Rhine-Hesse' and 'Barnim' clones) rather than by the acquisition or loss of resistance genes. However, the decrease in aminoglycoside non-susceptibility might also be associated with the reduction in aminoglycoside use from approximately 9 (2001) to 4 (2009) defined daily doses per 100 bed-days in Germany [9].

The overall non-susceptibility rates of levofloxacin and moxifloxacin also decreased (OR 0.4, 95% CI 0.3–0.5, p <0.005 each; Table 1), despite a continuous increase in general quinolone use from approximately 15 (2001) to 18 (2009) defined daily doses per 100 bed-days [9].

Non-susceptibility rates significantly increased for tetracycline, from 5.0% to 9.0% (OR 1.9, 95% CI 1.4–2.5, p <0.005). Tetracycline non-susceptibility is highly prevalent among LA-MRSA isolates, and stands in strong contrast to the trend of decreasing tetracycline/doxycycline non-susceptibility that has been observed in Germany in the past 30 years [10–12]. LA-MRSA isolates mainly belong to *spa*-CC 011, which is indicative of ST398. Stratification of resistance data in *spa*-CCs showed high non-susceptibility rates for tetracycline (51.2%) and trimethoprim–sulphamethoxazole (23.3%) and low non-susceptibility rates for aminoglycosides (gentamicin, 4.7%; tobramycin, 17.4%) and quinolones (levofloxacin and moxifloxacin, 46.5%) in isolates belonging to *spa*-CC 011 as compared with other major lineages (including spa-CCs 003, 004, 008, and 032; Table S1). When all *spa*-CC 011 isolates were excluded from the analysis, resistance rates in 2004–2005 vs. 2010–2011 were apparently stable for tetracycline (4.7% vs. 6.6%), trimethoprim–sulphamethoxazole (1.3 vs. 2.2), and quinolones (96.2 vs. 92.0), and were reduced for gentamicin (12.4 vs. 4.5) and tobramycin (64.4 vs. 43.4). This finding shows that the recent increase in the proportion of LA-MRSA in Germany from 0.2% (2004–2005) to 5.4% (2010–2011) explains the increase in tetracycline and trimethoprim–sulphamethoxazole non-susceptibility but not the decrease in aminoglycoside non-susceptibility in the German MRSA population [5]. The antimicrobial non-susceptibility rates in LA-MRSA mirrors the proportion of antimicrobial consumption in livestock in Germany (tetracycline, 54.3%; β-lactams, 23.0%, sulphonamides ± trimethoprim, 12.7%) [13].

TABLE 1. Comparison of antibiotic MICs and non-susceptibility rates of MRSA isolates in Germany in 2004–2005 and 2010–2011

	2004–2005 ($n = 1603$)				2010–2011 ($n = 1602$)				
	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range, minimum/ maximum (mg/L)	Non-susceptibility (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	Non-susceptibility (%)	p-value[a]
Gentamicin	≤0.5	≥16	≤0.5/≥16	12.4	≤0.5	≤0.5	≤0.5/≥16	4.5	<0.005
Tobramycin	≥16	≥16	≤1/≥16	64.3	≤1	≥16	≤1/≥16	42.0	<0.005
Levofloxacin	≥8	≥8	≤0.12/≥8	95.5	≥8	≥8	≤0.12/≥8	89.6	<0.005
Moxifloxacin	4	4	≤0.25/≥8	96.0	4	4	≤0.25/≥8	89.6	<0.005
Erythromycin	≥8	≥8	≤0.25/≥8	79.0	≥8	≥8	≤0.25/≥8	74.7	<0.005
Clindamycin	≥8	≥8	≤0.25/≥8	72.9	≥8	≥8	≤0.25/≥8	65.5	<0.005
Linezolid	2	2	≤0.5/4	0	2	2	1/4	0	NA
Teicoplanin	≤0.5	≤0.5	≤0.5/2	0	≤0.5	≤0.5	≤0.5/2	0	NA
Vancomycin	≤1	≤1	≤1/2	0	≤0.5	1	≤0.5/2	0	NA
Tetracycline	≤1	≤1	≤1/≥16	5.0	≤1	≤1	≤1/≥16	9.0	<0.005
Fosfomycin	≤8	≤8	≤8/≥128	2.6	≤8	≤8	≤8/≥128	1.3	<0.005
Fusidic acid	≤0.5	≤0.5	≤0.5/≥32	3.1	≤0.5	≤0.5	≤0.5/≥32	2.6	0.3
Rifampicin	≤0.5	≤0.5	≤0.5/≥32	1.9	≤0.5	≤0.5	≤0.5/≥32	1.4	0.5
Trimethoprim–sulphamethoxazole	≤10	≤10	≤10/≥320	1.3	≤10	≤10	≤10/≥320	3.3	<0.005

NA, not applicable.
[a]Univariate analysis comparing non-susceptibility rates from the two sampling periods.