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State-wide surveillance of antibiotic resistance patterns and *spa* types of methicillin-resistant *Staphylococcus aureus* from blood cultures in North Rhine-Westphalia, 2011–2013

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of bacteraemia. We aimed to obtain a complete picture of severe MRSA infections by characterizing all MRSA isolates from bloodstream infections in the largest German federal state (North Rhine-Westphalia, 18 million inhabitants) using *S. aureus* protein A (*spa*) sequence-typing and antimicrobial susceptibility testing. MRSA isolates (n = 1952) were collected prospectively (2011–2013) and *spa*-typed. Among 181 different *spa* types, t003 (n = 746 isolates; 38.2%) and t032 (n = 594; 30.4%) were predominant. Analysis of the geographical occurrence of *spa* clonal complexes (*spa*-CCs) and *spa* types revealed divergent distribution between federal state districts for *spa*-CCs 003 ($p < 0.001$; including t003, $p < 0.001$ and t264, $p < 0.001$), 008 (p 0.021), 011 (p 0.002), 032 ($p < 0.001$; including t022, p 0.014 and t032, $p < 0.001$) and *spa* type t2807 ($p < 0.001$). MICs of antimicrobial substances were tested using broth microdilution. Of all isolates, 96% were resistant to fluoroquinolones, 78% to erythromycin, 70% to clindamycin, 4% to gentamicin, 2% to rifampicin, 0.4% to daptomycin, 0.1% to linezolid and 0% to vancomycin, respectively. Vancomycin MICs of 2 mg/L involved 0.5% of the isolates. In conclusion, the detection of regional molecular clusters added valuable information for epidemiological case tracing and allowed conclusions to be reached on the importance of newly emerging MRSA reservoirs, such as livestock (*spa*-CC011), for MRSA bacteraemia in some parts of the federal state. Susceptibility testing revealed broad resistance to substances used for oral treatment, but demonstrated that those antibiotics that are mostly applied for treatment of MRSA bacteraemia and important combination partners were highly susceptible.

Keywords: Antimicrobial resistance, bacteraemia, Germany, livestock, methicillin-resistant *Staphylococcus aureus*, *spa*
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Introduction

In a recent prevalence survey it was found that healthcare-associated infections involved 5.7% of patients in Europe [1]. *Staphylococcus aureus* was the second most frequent organism, causing 12.3% of these infections with 41.2% of all isolates being methicillin-resistant (MRSA) [1]. Although the percentage of *S. aureus* isolates from blood cultures reported as MRSA has recently stabilized, MRSA causes an estimated >5000 excess deaths and >250 000 excess hospital days annually in European countries [2], which shows that MRSA prevention and availability of effective therapeutic options are still public health priorities.

An investigation assessing the clonal structure of *S. aureus* from cases of invasive infections using *S. aureus* protein A (*spa*)

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typing demonstrated the presence of few predominant MRSA *spa* types and suggested selection and spread of a limited number of clones leading to regional clusters [3]. As studies found that such regional emergence of MRSA was fuelled by patient movements between different cooperating healthcare facilities [4], data on molecular characteristics of MRSA can add valuable information to conventional surveillance data (time, place, person) and can support the work of local infection control staff or public health authorities. This is also important, because in some countries new reservoirs for MRSA infections outside healthcare facilities (community-associated or livestock-associated cases) have recently gained greater impact [5].

Appropriate empirical antibiotic therapy of MRSA bacteraemia is essential for reduction of mortality [6]. Surveillance data on resistance phenotypes of MRSA can indicate effective therapeutic options and allow for early recognition of the emergence of MRSA harbouring new antibiotic resistance traits [7]. As the number of patients in hospitals with MRSA bacteraemia correlates with the number of all patients affected by an MRSA infection [8], mandatory notification of MRSA bacteraemia cases to regional health authorities is an important MRSA surveillance tool. Although a mandatory notification system for the detection of MRSA from blood cultures was established in Germany in 2009, there is currently no system for mandatory collection of MRSA isolates for the purpose of country-wide investigation of molecular types or clinically relevant antimicrobial resistance.

To address these issues, we performed this prospective characterization of MRSA isolates derived from blood cultures obtained from patients in the most populated German federal state during a 23-month period. All isolates were characterized using *spa* typing and antimicrobial resistance testing based on broth microdilution.

Methods

This study was carried out in the German federal state of North Rhine-Westphalia (NRW; 17.6 million inhabitants, i.e. 22% of the total German population; 370 hospitals, 120 247 hospital beds in 2013). Hospitals and microbiology laboratories were asked to voluntarily send every first MRSA isolate from blood cultures of each patient obtained between December 2011 and October 2013 to a central typing facility at the Institute of Hygiene, University Hospital Münster, together with anonymized information on the patient's age and sex. To yield geographical information, laboratories were asked to which local public health authority (on district level) the MRSA bacteraemia case was notified. Notification has to be made to the local health authority in the district where the patient officially resides or where the patient has his current location (e.g. hospital). All notifications are documented in a central database (http://www3.rki.de/SurvStat/). Geographical information was assessed using the Nomenclature of Units for Territorial Statistics defined by the European Commission.

Typing of isolates

All bacterial isolates were characterized by *spa* sequence-based typing as described previously [9] using primers spa_1113f (5′-TAA AGA CGA TCC TTC GGT GAG C-3′) and spa_1514r (5′-CAG CAG TAG TGC CGT TTG CTT-3′) or alternative primers spa_239f, spa_1717r [10]. For clustering of *spa* types and grouping in *spa*-clonal complexes (*spa*-CC) we used the Based Upon Repeat Pattern (BURP) algorithm of the StaphType™ software (Ridom GmbH, Münster, Germany) with default parameters as recommended previously [11]. Multilocus sequence typing (MLST) was performed for *spa* non-typeable isolates, isolates grouped as singletons and for those excluded from clustering in the BURP analysis [12]. For the other isolates, the MLST clonal complexes (CC) corresponding to the respective *spa*-CCs were assessed from the SpaServer website (www.spaserver.ridom.de), as it was demonstrated that there is a high concordance between BURP and MLST-based clustering results [13]. Statistical differences in the occurrence of *spa* types were calculated using chi-squared test or Fisher's exact test (IBM SPSS Statistics, version 22). *P*-values <0.05 were considered to be significant.

Antimicrobial susceptibility testing

All isolates were subjected to antimicrobial susceptibility testing using broth microdilution according to EUCAST guidelines and clinical breakpoints (version 4.0; www.eucast.org) at the Robert Koch-Institute, National Reference Centre for staphylococci and enterococci, Wernigerode branch, Germany. The MICs were determined for 17 substances from 15 antibiotic classes. If isolates were susceptible to oxacillin, they were excluded from the study. PCR for *cfr*, a mobile genetic element conferring linezolid resistance, was performed as described previously [14].

Results

Typing of isolates

Overall, 1952 MRSA isolates from 57 microbiological laboratories in NRW were *spa* typed. In 11 cases, two different isolates (with respect to susceptibility profiles and *spa* types) were collected from the same blood culture specimen (i.e. the 1952 isolates were obtained from 1941 different patients). The isolates were notified to authorities in 53 of the 55 districts of

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