Journal of Hospital Infection 88 (2014) 72–77

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

spa typing alone is not sufficient to demonstrate endemic establishment of meticillin-resistant *Staphylococcus aureus* in a low-prevalence country

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ARTICLE INFO

Article history:
Received 16 August 2013
Accepted 17 June 2014
Available online 10 July 2014

Keywords:
Meticillin-resistant
Staphylococcus aureus
Norway
Nursing home
Outbreak
PFGE

SUMMARY

Background: The prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) in Norway is low but increasing. Over the last decade, numerous nursing homes have experienced MRSA outbreaks. One genetic lineage, *spa* type t304, has been identified at multiple nursing homes and has caused large outbreaks lasting for several years.
Aim: To evaluate whether *spa* typing is sufficient for the detection of MRSA spread and endemic establishment in a low-prevalence area, using *spa* type t304 as the test organism.
Methods: All *spa* type t304 isolates detected in 1991–2010 in the most densely populated area of Norway were included. Time and place of bacterial sampling were recorded. The isolates were analysed using multi-locus sequence typing, staphylococcal cassette chromosome *mec* typing, detection of *lukS/F-PV* and pulsed-field gel electrophoresis (PFGE).
Findings: In total, 181 *spa* type t304 isolates were identified in three of 23 municipalities. Most (91%) of the isolates could be linked to 13 nursing homes, eight of which experienced outbreaks. PFGE analysis revealed three PFGE types, consisting of 19 PFGE patterns; 95% of the isolates were PFGE type 2. In total, PFGE types 2 and 3 accounted for 99% of all nursing home isolates, and included isolates from different nursing homes, different outbreaks and different time periods. Additional genetic analyses did not further differentiate between the *spa* type t304 isolates.
Conclusion: MRSA *spa* type t304 appears to have established itself as an endemic genetic lineage in the study area. *spa* typing does not provide sufficient resolution when investigating the spread of an endemic-like genetic lineage in a low-prevalence area, and should be supplemented by additional typing techniques.

Introduction

The incidence of meticillin-resistant *Staphylococcus aureus* (MRSA) in Norway is relatively low. However, the Norwegian Institute of Public Health has reported an increase in incidence

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http://dx.doi.org/10.1016/j.jhin.2014.06.008

over recent years, both in the community and in healthcare institutions.[1] Nearly 1% of the Norwegian population live in nursing homes.[2] In 2006, 18% of identified cases of MRSA in Norway were in nursing home residents, and nursing homes have experienced both large and small outbreaks over the last decade.[3,4] Nursing homes are seen as reservoirs of MRSA, and provide ideal conditions for colonization and spread as the residents often have chronic illnesses and are exposed to multiple antimicrobial agents.[5] Once MRSA is introduced, spread between patients and healthcare workers could result in an outbreak, which could lead to further spread to local hospitals when affected residents require hospital treatment.[6] Affected staff members may facilitate microbial spread to other nursing homes or to patients receiving home care due to dual conditions of employment.

A recent report from the Health Agency in Oslo revealed MRSA in 22 out of 50 nursing homes between 2005 and 2011.[4] Staphylococcal protein A (*spa*) typing displayed a heterogeneous genetic composition. The majority of *spa* types were represented in less than 2% of the isolates, but one *spa* type, t304, represented 52% of MRSA isolates, affected 11 nursing homes and was the sole *spa* type appearing every year over the seven-year study period. *spa* type t304 was the causative agent of the largest MRSA outbreaks in nursing homes in Oslo, and has also caused several small outbreaks.[7,8] *spa* type t304 is detected globally but with low frequency (http://spa.ridom.de). One exception is in Latin America, where the prevalence of *spa* type t304 is higher.[9,10] Previous studies of MRSA in Norway have revealed a diverse composition of genetic lineages, and fluctuations in the incidence of these genetic lineages over time.[8,11] Thus, the appearance of *spa* type t304 in several nursing homes in a limited geographic area over a relatively short period of time raises the question of clonal spread and endemic settlement.

Several studies have questioned whether *spa* typing is sufficiently discriminatory to follow the dissemination of an endemic clone, or if a combination of genotyping techniques would provide more accurate results.[12–14] *spa* typing, together with identification of the genes *lukS/F-PV*, is the standard typing method for MRSA in Norway.[15]

The aim of this study was to evaluate whether *spa* typing and *lukS/F-PV* identification are sufficient for the detection of MRSA clonal spread and endemic establishment in a low-prevalence country. *spa* type t304 was used as the model organism, and the following typing techniques were applied for the evaluation process: multi-locus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing and pulsed-field gel electrophoresis (PFGE).

Materials and methods

The study area consisted of Oslo and Akershus counties, which include 23 municipalities, the capital city Oslo, and a multitude of large and small cities and rural areas. Approximately 24% (1.2 million) of the Norwegian population live in the study area. All individuals, with few exceptions, from whom MRSA was isolated between 1991 and 2010 were included in the study. The MRSA isolates had been genotyped routinely[15,16] at Akershus University Hospital, and the results were registered along with epidemiological data in a MRSA database. The *spa* types were assigned using Ridom StaphType Version 2.0.3 (Ridom GmbH, Münster, Germany). The MRSA database was accessed and a search for *spa* type t304 was performed. Only the first *spa* type t304 isolate for each individual patient was included. The following data were recorded: place and date of bacterial sampling, and whether or not the isolate was regarded as a sporadic case or part of an outbreak. An outbreak was defined as two or more cases with identical *spa* types that were epidemiologically linked by individual and time or place.

All *spa* type t304 isolates were subjected to MLST and SCC*mec* typing, except in outbreak settings where a representative selection of the outbreak isolates were typed. The laboratory work was performed at Akershus University Hospital as described previously.[16,17] The MLS types (STs) were assigned using the MLST database (http://www.mlst.net). All isolates were subjected to PFGE typing at Oslo University Hospital, Ullevål using the HARMONY protocol described by Murchan *et al.*[18] with modifications. A 1.2% agarose gel was prepared and the switch time was 5–60 s for 23 h using Gene Navigator (Pharmacia, NY, USA). Lambda Ladder PFGE Marker and NCTC 8325 were used as reference standards. PFGE profiles were analysed and compared using Molecular Analyst Software Fingerprinting Version 1.6 (Applied Maths, Sint-Martens-Latem, Belgium), based on Dice coefficients, and represented by unweighted pair grouping by mathematical averaging with 1.25% band position tolerance and optimization of 0.5%. A similarity coefficient of 80% was selected to define PFGE types. The gels were also analysed by visual interpretation of the banding patterns, by three trained specialists, according to the criteria of Tenover *et al.*[19]

Ethics

The Norwegian Regional Ethics Committee South-East considered that ethical approval was not required for this study (Ref. No. REK sør-øst 2013/669). The study was approved by the representative of privacy protection at Akershus University Hospital (Ref. No. 13-060).

Results

spa type t304 first appeared in the study area in 1998 and was detected each year until 2010, with the exception of 2001–2003 (Table I). In total, 181 individuals, representing 18% of all individuals in the MRSA database, were infected or colonized with *spa* type t304 over this 13-year period. Of the 181 individuals, 165 (91%) could be linked to nursing homes, 10 (6%) could be linked to hospitals, and four (2%) had their bacterial samples collected by a general practitioner (GP). For two individuals (1%), the location for bacterial sampling was unknown.

spa type t304 was identified in three neighbouring municipalities. In 2013, 56 nursing homes existed in these three municipalities. The number of nursing homes changed over the study period, and it was not possible to identify the exact number of nursing homes for each year. *spa* type t304 was detected in 13 nursing homes (N1–N13), eight of which experienced outbreaks (N1–N8); and in three hospitals (H1–H3), one of which experienced an outbreak (H2). Six of the outbreaks were small, involving two to eight individuals, and ended shortly after infection control measures had been activated (Table I). Three outbreaks (N2, N3 and N4) were large in size, including 20, 82 and 34 individuals, respectively, and lasted for up to five years (Table I).