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In the centre of an epidemic: Fifteen years of LA-MRSA CC398 at the University Hospital Münster

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ABSTRACT

Ten years after initial publications on livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in 2005, we report on the course of the LA-MRSA CC398 epidemic among patients of the University Hospital Münster. This tertiary care facility is located in the Dutch-German border region (EUREGIO), which is characterized by a high density of livestock production and is hence a hotspot for the occurrence of LA-MRSA CC398. Taking advantage of the unique opportunity to track the emergence and spread of MRSA CC398 among humans from the very beginning of the epidemic until today, a total of 6555 non-duplicate MRSA isolates from all screenings and clinical specimens cultivated within the period from 2000 to 2014 were included in the analysis. Retrospectively, the first MRSA CC398 isolate (*spa* type t034) was obtained from a screening specimen of a patient in 2000, which represents one of the first human-associated LA-MRSA CC398 isolates reported in Europe. After sporadic detections between 2000 and 2004, this clonal lineage accounted for 9.6% of all local MRSA in 2005; a proportion which increased to 35% in 2013 and became stable since then. Considering the period from 2000 to 2014, the group of MRSA CC398 isolates comprised a total of 45 different *spa* types among which t011 (48.3%), t034 (39.3%) and t108 (3.5%) were predominant and so far unreported types were found. Overall, LA-MRSA CC398 emerged rapidly during the past decade, developed enormous sublineage diversity and contributed substantially to the total burden of MRSA colonization and infection at the hospital.

1. Introduction

During the past decade, MRSA emerged increasingly in livestock most notably in pigs (Voss et al., 2005). In Germany and other European countries, the majority of livestock-associated (LA) MRSA are associated with clonal complex 398 (CC398) and *S. aureus* protein A (*spa*) types t011, t034, t108 and t1451 (Köck et al., 2009). Epidemiological investigations observed that LA-MRSA CC398 was breaking the species barrier followed by colonization and infection of humans in particular of those with close contact to livestock animals (Köck et al., 2009). Direct livestock contact is considered to be the major risk factor for zoonotic transmission of MRSA CC398 (van Loo et al., 2007; Graveland et al., 2010). Nevertheless, several studies indicated that indirect livestock exposure, such as household contacts or farm visits, are also responsible for acquiring colonization (Cuny et al., 2009; Graveland et al., 2011; van Cleef et al., 2011a,b). The first cases of acute human infections with MRSA CC398 were reported in France and the Netherlands (Voss et al., 2005; Wulf et al., 2008) followed by several reports from other parts of Europe (Köck et al., 2009; van Loo et al., 2007; Wulf et al., 2012). The North-Western border-region (EUREGIO) between the Netherlands and Germany is characterized by a high density of livestock farming (http://www.atlas-agrarstatistik.nrw.de/) with 1.38 million head of cattle, 6.67 million pigs and 9.82 million chickens or turkeys (Landwirtschaftskammer NRW, 2012). Hence, there is a considerable zoonotic source for LA-MRSA CC398 in this region (Köck et al., 2013; Schaumburg et al., 2012). While the prevalence of MRSA CC398 among humans was found to be rather low in other parts of Germany (Schaumburg et al., 2012; Layer et al., 2012), the EUREGIO was shown to represent a hotspot for human MRSA CC398 carriage and infection (Köck et al., 2013). In this report, we summarized

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data about the occurrence, spread and clonal types of LA-MRSA CC398 at the University Hospital Münster (UKM) located in the German part of the EUREGIO in order to reflect the local epidemic of this MRSA clonal lineage since the occurrence of first isolates in screening and clinical specimens.

2. Methods

The UKM is a 1450-bed University Hospital located in North-Western Germany. From 2006 on, a general admission screening of all patients (i.e. taking a nasal swab of every inpatient admitted in all departments of the hospital except psychiatry) was implemented at the UKM; before 2006 screening was performed inconsistently and only for those patients considered to be at high risk for MRSA carriage. At the UKM, strict isolation precautions according to the national recommendations are implemented for all patients known to be MRSA positive (Kommission für Krankenhaushygiene und Infektionsprävention am RKI, 1999; Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) beim Robert Koch-Institut, 2014). MRSA isolates were identified and confirmed as previously described (Becker et al., 2006) and every first isolate of each patient (irrespectively whether derived from a screening swab or a clinical specimen) was characterized by *spa* typing (Harmsen et al., 2003; Mellmann et al., 2006). MLST of t899 isolates were performed as described elsewhere (Enright et al., 2000) and analyzed using SeqSphere+ (Ridom GmbH, Münster, Germany). All typing results (2000–2014) were entered in a central database (StaphType™, Ridom GmbH, Münster, Germany). For this report, duplicate isolates of the same patient were excluded. The Based Upon Repeat Pattern (BURP) algorithm of the RidomStaphType software was used for clustering all MRSA isolates in the database into *spa*-clonal complexes (*spa*-CCs) with preset parameters as described elsewhere (Mellmann et al., 2008). The percentage of MRSA CC398 cases on all annual MRSA cases was assessed by Cochran Armitage test of linear trend using XLSTAT software ($p < 0.05$ was considered significant).

3. Results

Overall, 6555 MRSA isolates (one isolate per patient) cultivated within the period from 2000 to 2014 were included in the analysis. MRSA isolates were associated with both colonization (76.4%; $n = 5008$ isolates from classical screening specimens including nasal, pharyngeal, superficial skin swabs) as well as potentially with infection derived from clinical specimens (19.9%; $n = 1307$). For 240 isolates (3.7%), information about the specimen of origin was missing. All MRSA isolates exhibited 451 different *spa* types of which t003 (23%), t032 (21.1%), t011 (11.7%), t034 (9.6%), t008 (2.8%), t004 (2.1%), t001 (1.5%), t002 (1.2%), t014 (1.1%) and t020 (1.1%) were the ten most frequently detected. The BURP algorithm clustered the *spa* types into 16 *spa*-CCs and 20 singletons. 36 *spa* types with less than five repeats were excluded. MLST typing of the *spa* t899 isolates revealed that all isolates ($n = 6$) belonged to ST9.

Among the 6555 MRSA isolates from human patients, 1591 isolates (24.3%) belonged to *spa* types related to MLST CC398 according to previous publications (Schaumburg et al., 2012; Köck et al., 2013). Based on the BURP algorithm, *spa* types related to MLST CC398 were grouped into *spa*-CC011 including 45 different *spa* types; t011 (48.3%; $n = 768$), t034 (39.3%; $n = 626$), t108 (3.5%; $n = 55$), t1451 (1.8%; $n = 29$), t2011 (1%; $n = 16$), t571 (0.6%; $n = 11$), t2582 (0.6%; $n = 9$), t2576 (0.5%; $n = 8$), t898 (0.4%; $n = 6$) and t1255 (0.4%; $n = 6$) being predominant (Fig. 1).

During the analyzed period of 15 years, altogether, 1436 (90.3%) MRSA CC398 isolates were obtained from screening specimens and 124 (7.8%) MRSA CC398 isolates were derived from clinical specimens (Table 1). Retrospectively, the first MRSA CC398 isolate at the UKM appeared in 2000, was derived from a nasal screening sample and was associated with *spa* type t034. Thereafter, the number of patients colonized with MRSA CC398 in screening specimens increased from five in 2002 to 244 in 2013 and 186 in 2014. The number of patients from whom MRSA CC398 was first obtained from clinical specimens ranged between one in 2002 and 22 in 2010 (Fig. 2).

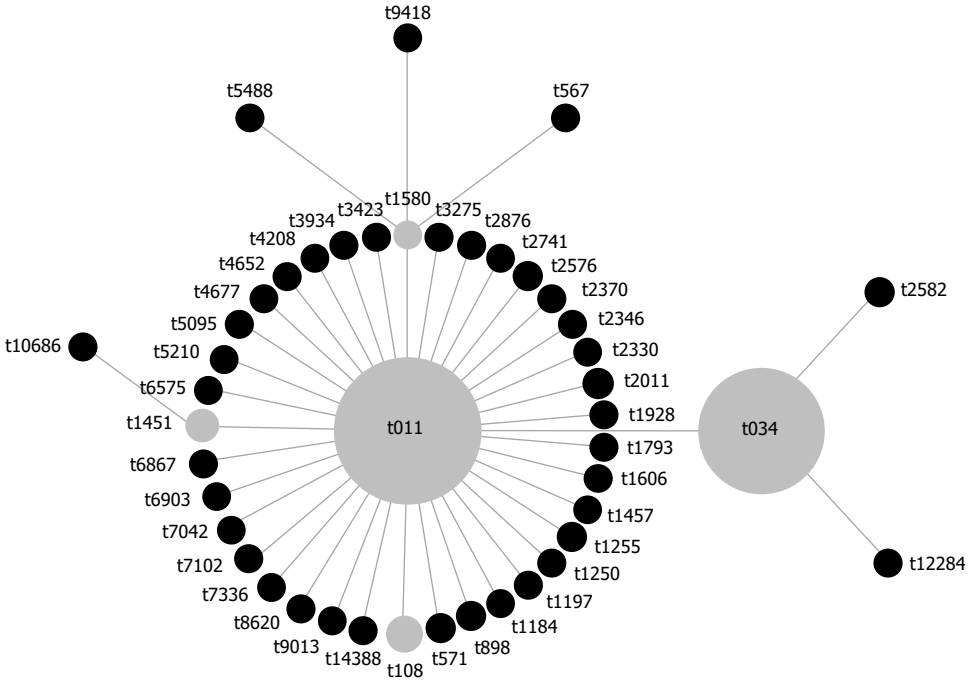

Fig. 1. Population snapshot for MRSA CC398 by BURP analysis.
BURP clustering of all local MRSA isolates resulted in the spa-clonal complex (CC) 011, which comprises CC398 isolates. Each dot represents a unique *spa* type and the *spa* type t011 represents the group founder with the highest founder score. Dot diameters are proportional to the quantity of corresponding *spa* types in the database.