

Surveillance report

EMERGENCE AND DISSEMINATION OF THE METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* USA300 CLONE IN DENMARK (2000-2005)

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The problem of methicillin resistant *Staphylococcus aureus* (MRSA) is increasing worldwide, and the spread of MRSA in the community challenges infection control since it is no longer restricted to hospital settings but involves private homes, places of work and kindergartens [1]. Furthermore, community acquired (CA)-MRSA may circumvent existing hospital infection control, since patients are rarely screened at admission. In the United States, the predominant CA-MRSA is defined by the Center for Disease Control (CDC) as the USA300 (ST8) clone. USA300 primarily causes skin and soft tissue infections (SSTI) in the community [2], but healthcare acquired infections with USA300 are rapidly emerging in the United States [3,4]. Comparison of the Danish collection of MRSA from 1997-2005 with the USA300 reference strain showed that USA300 has been introduced into Denmark on several occasions. Between 2000 and 2005, we identified 44 isolates which in addition to identical pulsed-field gel electrophoresis (PFGE) pattern shared other molecular characteristics with USA300: *spa* type t008 or closely related variants, Pantone-Valentine leukocidin (PVL) positive and Staphylococcal Cassette Chromosome *mec* (*SCCmec*) type IVa. The isolates primarily caused SSTI, but cases of invasive infections were also encountered. The number of USA300 has increased several-folds in Denmark from 2003 to 2005 (2, 11 and 28 new cases, respectively) and with the experience from the US in mind, this is of great concern, especially as it is observed in a country with a long reputation for controlling MRSA.

Background

During the past decade, community acquired infections with methicillin resistant *Staphylococcus aureus* (CA-MRSA) have been observed with increasing frequency. Distinct genetic lineages associated with CA-MRSA infections have been determined through typing and their geographic dissemination evaluated [5]. In the United States (US), the predominant CA-MRSA clone is the USA300, characterised by a particular pulsed field gel electrophoresis (PFGE) pattern, staphylococcal protein A (*spa*) type t008, multi locus sequence type 8 (ST8), Staphylococcal Cassette Chromosome *mec* (*SCCmec*) type IVa and encoding Pantone-Valentine leukocidin (PVL) [2]. USA300 is the single most prevalent MRSA clone obtained from skin and soft tissue infections (SSTI) infections in several metropolitan areas across the US [6], and has for instance been transmitted in relation to contact sports [7], and prison inmates [8]. However, an increasing number of reports describe USA300 as a rapidly emerging cause of hospital infections causing severe infections such as septicaemia, and neonatal death [4].

Transatlantic spread of USA300 has recently been documented in a case report, but the extent to which it is disseminated in Europe is still unknown [9].

Denmark has been a low prevalence country, with MRSA comprising <1% of all blood isolates for three decades [10]. Recently, the number of new MRSA cases (infected persons and/or carriers) in Denmark has increased rapidly, from 100 in 2002 to 243, 549 and 864 cases in 2003, 2004 and 2005, respectively [11] with a large proportion of infections being community acquired.

In Denmark, the European CA-MRSA clone (ST80) has been the predominating cause of CA-MRSA for a decade (1995-2004). However, a remarkable increase in MRSA isolates belonging to clonal complex 8 (CC8) has been recognised in different parts of the country and especially in the Copenhagen area since 2003.

This article describes the presence of USA300 in Denmark between 2000 and 2005, and gives epidemiological characteristics of its dissemination.

Materials and methods

As a part of the national surveillance system, all MRSA isolated from infections or from healthy carriers in Denmark since 1988 has prospectively been referred to and stored at Statens Serum Institut (SSI) on a voluntary basis. Since 1997 the first isolate from each MRSA case has been subjected to PFGE typing (n=1986) and isolates assigned to clonal complex based on *spa* and MLST typing of representative isolates in each PFGE cluster. The results have prospectively been registered in a database with each patient reported only once. In the present report, all MRSA isolates belonging to CC8 in the period 1997-2005 were investigated.

Clinical and epidemiological information

Since 1999, epidemiological and clinical data have been registered for patients and healthy carriers by their primary MRSA isolate. The data has been obtained from hospital discharge summaries and general practitioner (GP) records. The following clinical data were recorded: reason for specimen collection (infection or screening), infection onset, risk factor for acquisition of MRSA, and infected body site (skin and soft tissue, blood, respiratory tract, bone/joint, urinary tract or postoperative wound).

For classification of the infection onset, we used a recent definition for MRSA infections [12] including five possible types

of MRSA infections: (i) hospital acquired (HA); (ii) imported (IMP); (iii) community onset (CO-MRSA) infections with no identified risk factors (CO-NR); (iv) CO-MRSA infections with an identifiable community risk factor (CO-CR), for example infected persons with other family members as known MRSA carriers/patients and (v) CO-MRSA infections with identified healthcare risk factors (CO-HCA), for example persons living in residential homes for elderly people or with a history of hospitalisation in the previous 12 months. In the present report the CO-CR and CO-NR will be grouped together as CA-MRSA.

Molecular characterisation

PFGE. Small macrorestriction profiles were performed according to the HARMONY protocol [13] and analysed using Bionumerics 4.6 (Applied Maths, Sint-Martens-Latem, Belgium). The Danish isolates were compared to the US reference strain, kindly provided by Fred C. Tenover, CDC, Atlanta, USA. Spa typing and MLST were performed as previously described [14,15]. The spa type (t) and MLST sequence types (ST) were assigned through the Ridom (<http://www.ridom.de>) and MLST databases (<http://www.mlst.net>), respectively.

SCCmec types I-V and mecA confirmation were determined by two multiplex PCR strategies [16,17] and PVL was detected as previously described [18].

Results

Through the period 1999-2005, 516 out of 1986 MRSA cases belonged to CC8. Based on PFGE and detection of PVL genes, 44 (8.5%) of these isolates were found to be USA300.

By SCCmec and spa typing, all these isolates harboured SCCmec type IVa, and spa type t008 or variants thereof (t068, t211, t304 and t622).

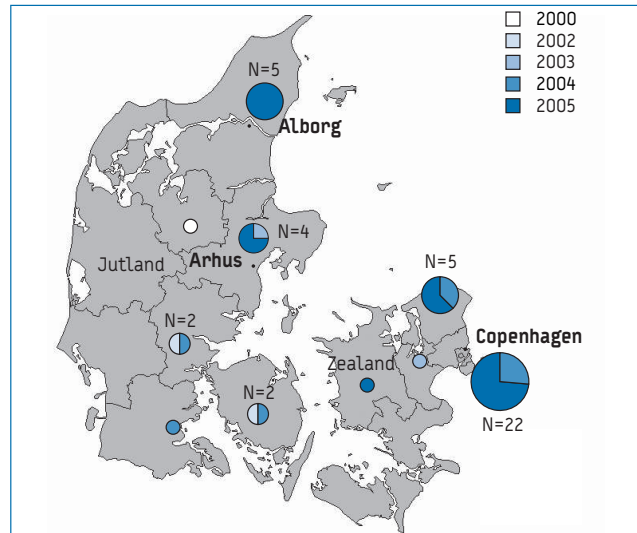
Discharge summaries were obtained for 42 of the patients, but there was insufficient patient information for two of the cases. In 41 cases these isolates caused infections, and one isolate was found as result of screening a family member to a hospitalised person infected with USA300.

Discharge summaries suggested that 28/41 (68%) of the USA300 infections had community onset (CO-MRSA), and import (IMP) and hospital acquired (HA) infections were re-reported in two and 11 cases, respectively. Healthcare (CO-HCA) or community (CO-CR) risk factors were recognised in four cases each. In the remaining 20 cases no risk factors (CO-NR) were identified. Thus, 24/41 (59%) were regarded as true CA-MRSA infections.

The first USA300 was isolated in Denmark in 2000 by a GP in a rural area (Viborg), followed by two cases from different parts of the country in 2002, and another two cases in 2003. One of the cases in 2003 had been working in Canada when he visited a GP in Denmark with abscesses on his chest. In 2004, 11 new cases were encountered, six of them in the Copenhagen area. In 2005, the number increased to 28 cases, of which 16 were found in the Copenhagen area. Travel to the US was reported for two patients in 2004 and five patients in 2005. In addition, two had traveled in the east Asia and another two cases reported unspecified travel, which makes import a suspected source for 11/42 (26%) of all cases. The annual distribution of USA300 cases in Denmark is summarised in the figure.

FIGURE

Annual distribution of USA300 MRSA cases for each county in Denmark, 2000-2005*



* Where no numbers are written, a dot represents a single case

SSTIs accounted for 90% (38/42) of the infections: two patients suffered from respiratory tract infections and one had a deep seated wound infection.

Discussion

The finding of USA300 in Denmark illustrates the ease of MRSA spread between countries and continents. Import of USA300 was very likely on several occasions, and travel to the US was highly overrepresented among patients where travel destination was noted by the physicians. However, domestic spread seems to be the most prominent way of dissemination in Denmark, especially in 2004 and 2005. A single transatlantic event of transmission has previously been reported [9]. In Belgium, three cases of PVL positive isolates with spa type t008 were reported in 2005 and in the Netherlands an increase in PVL positive ST8 isolates from 2002-2003 have been detected [19,20]. Some of these isolates may likely be identical to USA300, but confirmation by PFGE has only been reported once in a tertiary care center in the Netherlands [21]. The USA300 clone may therefore already be disseminated in several European countries.

In the US, the USA300 clone has proven successful in causing CA-MRSA as well as healthcare acquired infections [3,4]. It is therefore of concern that it now seems to have been established in the Danish community, causing 2%-5% of the annual MRSA infections in the period 2002-2005.

The general increase in MRSA cases in Denmark during the study period could be due to increased surveillance activity. However, only one USA300 isolate was found by screening, while all the other isolates reported caused infections, so the increase of reported USA300 isolates does not seem to be a study artifact. Since 1995, ST80 has been the predominant CA-MRSA in Denmark, but USA300 is now competing for the same niche, which may cause either a general increase in CA-MRSA or a shift in the clonal distribution. So far, the dissemination of USA300 has primarily occurred in the Copenhagen area, which is also the

most densely populated area, thereby supporting the hypothesis of community spread. In Denmark, USA300 has also been found in a few hospitalised patients, indicating that PVL positive MRSA could also become a healthcare-associated problem in Denmark as observed in the US [6]. In contrast, spread of ST80 isolates into hospitals has been reported only occasionally [12]. At present, the national recommendations for infection control of MRSA in Denmark primarily concern precautions and interventions in hospital settings, while interventions against CA-MRSA infections are not included.

All persons who have been in contact with foreign hospitals outside the Nordic countries and the Netherlands are screened for MRSA at admission to Danish hospitals. However, domestic patients admitted to hospitals are only screened when they have known risk factors for carrying MRSA, resulting in an unhindered access to hospital settings by patients carrying CA-MRSA with no established risk factors for MRSA.

It is therefore of concern if USA300 turns out to be a successful nosocomial pathogen as indicated by the experience from the US [6]. In order to diminish entry and transmission of CA-MRSA (including USA300) into hospitals, it is important to increase awareness of MRSA as the cause of SSTI or other typical staphylococcal infections. Increased use of diagnostic sampling from SSTI should be considered, both in primary healthcare and in hospitals. Furthermore, the use of proper hand hygiene routines is known to be the most effective way to prevent transmission of MRSA.

In conclusion, the number of USA300 isolates has increased several-fold in Denmark since 2003. With the US experience in mind, this is of great concern, especially since this is observed in a country with a long reputation for controlling MRSA.

References

1. Urth T, Juul G, Skov R, Schonheyder HC. Spread of a methicillin-resistant *Staphylococcus aureus* ST80-IV clone in a Danish community. *Infect Con-trol Hosp Epidemiol.* 2005;26(2):144-9.
2. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB et al. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. *J Clin Microbiol.* 2006;44(1):108-18.
3. Kourbatova EV, Halvosa JS, King MD, Ray SM, White N, Blumberg HM. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 clone as a cause of health care-associated infections among patients with prosthetic joint infections. *Am J Infect Control.* 2005;33(7):385-91.
4. Seybold U, Blumberg HM, Halvosa SJ, Kourbatova E, King MD. Emer-gence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype in health care-associated bloodstream infec-tions. *J Investig Med.* 2005;53:S306.
5. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carry-ing Panton-Valentine leukocidin genes: worldwide emergence. *Emerg In-fect Dis.* 2003;9(8):978-84.
6. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB et al. Methicillin-resistant *S.aureus* infections among patients in the emergency department. *N Engl J Med.* 2006;355(7):666-74.
7. Centers for Disease Control and Prevention. Methicillin-Resistant *Staphylococcus aureus* Infections Among Competitive Sports Participants --- Colorado, Indiana, Pennsylvania, and Los Angeles County, 2000--2003 Vol 52, No MM33;793 . *MMWR Morb Mortal Wkly Rep.* 2003;52(33):793-5.
8. Centers for Disease Control and Prevention. Public health dispatch: out-breaks of community associated methicillin- resistant *Staphylococcus aureus* skin infections- Los Angeles County, California, 2002-2003. *JAMA.* 2003;289(11):1377.
9. Tietz A, Frei R, Widmer AF. Transatlantic spread of the USA300 clone of MRSA. *N Engl J Med.* 2005;353(5):532-3
10. Members of the Danish *Staphylococcus aureus* bacteremia group. Annual report on *Staphylococcus aureus* bacteraemia cases. 2004. Statens Se-rum Institut. Available from: www.ssi.dk/sw3425.asp
11. Agersø Y, Emborg HD, Heuer OE, Frøkjær Jensen V, Seyfarth AM, Hammerum AM, et al. DANMAP 2005- Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. 2005. Available from: http://www.danmap.org/pdfFiles/Danmap_2005.pdf
12. Faria NA, Oliveira DC, Westh H, Monnet DL, Larsen AR, Skov R et al. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. *J Clin Microbiol.* 2005;43(4):1836-42.
13. Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE et al. Harmonization of pulsed-field gel electrophoresis protocols for epi-demiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European labo-ratories and its application for tracing the spread of related strains. *J Clin Microbiol.* 2003;41(4):1574-85.
14. Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hos-pital setting by using novel software for spa repeat determination and da-tabase management. *J.Clin.Microbiol.* 2003;41(12):5442-8.
15. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus se-quence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin.Microbiol.* 2000;38(3):1008-15.
16. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylo-coccal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J.Clin.Microbiol.* 2005;43(10):5026-33.
17. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identifica-tion of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2002;46(7):2155-61.
18. Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D et al. Commu-nity-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leu-kocidin. *Clin.Infect.Dis.* 2002;35(7):819-24.
19. Denis O, Deplano A, De Beenhouwer H, Hallin M, Huysmans G, Garrino MG et al. Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-Valentine leucocidin genes in Belgium. *J Antimicrob Chemother.* 2005;56(6):1103.
20. Wannet WJ, Heck ME, Pluister GN, Spalburg E, van Santen MG, Huijsdens XW et al. Panton-Valentine leukocidin positive MRSA in 2003: the Dutch situation. *Euro Surveill.* 2004;9(11):28-9. Available from: <http://www.eurosurveillance.org/em/v09n11/0911-222.asp?langue=02&>
21. Gonzalez BE, Rueda AM, Shelburne SA 3rd, Musher DM, Hamill RJ, Hulten KG. Community-associated strains of methicillin-resistant *Staphylococcus aureus* as the cause of healthcare-associated infection. *Infect Control Hosp Epidemiol.* 2006 Oct;27(10):1051-6.

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