

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Poland: further evidence for the changing epidemiology of MRSA

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SUMMARY

This study reports the isolation of CA-MRSA strain which was found to colonize the nasal mucosa of a patient undergoing haemodialysis treatment. The MRSA was subjected to molecular analysis by Pulsed Field Gel Electrophoresis (PFGE), multiplex PCR assay for staphylococcal cassette chromosome *mec* (*SCCmec*) typing, and PCR detection of the *pvl* gene encoding for Panton-Valentine leukocidin. The analyzed MRSA harbored the *SCCmec* type IV and the *pvl* gene—two unique genetic markers of CA-MRSA. The PFGE pattern of the strain corresponded to the common European CA-MRSA (MLST Type ST80). Moreover, the strain was only resistant to β -lactam agents and tetracycline. This study adds further evidence for the changing epidemiology of MRSA and indicates the ability of CA-MRSA to affect persons with established risk factors in addition to previously healthy individuals.

KEY WORDS: Community-acquired methicillin-resistant *Staphylococcus aureus*, Haemodialysis

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INTRODUCTION

Carriage of *Staphylococcus aureus* in the anterior nares appears to play a key role in the epidemiology and pathogenesis of infections, particularly among patients with predisposing risk factors such as those receiving haemodialysis (Kluytmans *et al.*, 1997; von Eiff *et al.*, 2001). This patient population has been considered to be

prone to a higher colonization rate and subsequent invasion by *S. aureus* largely due to immune deficiencies and repeated violation of the skin and mucosa anatomical barriers (Nouwen *et al.*, 2001; Peacock *et al.*, 2002). Moreover, the striking ability of this bacterium to acquire resistance against a wide spectrum of antibiotics exemplified by the emergence and worldwide spread of methicillin resistance renders *S. aureus* a subject of great concern in hospitals and other health care settings (Hiramatsu *et al.*, 2002; de Mattos *et al.*, 2003; Crisóstomo *et al.*, 2001). Recent evidence suggests that the epidemiology of methicillin-resistant *S. aureus* (MRSA) has changed, since this microorganism, besides having established itself as a major nosocomial pathogen, has begun to appear outside the hos-

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pital environment as well. This phenomenon has led to a substantial alteration of the conventional view of MRSA, which can no longer be regarded as confined to nosocomial settings. The MRSA associated with community infections are referred to as *community-acquired* or *associated MRSA* (CA-MRSA) and pose a serious threat due to their significant pathogenic potential which, according to the recent literature data, even exceeds the virulence of hospital-acquired MRSA (Voyich *et al.*, 2005), and the ability to affect otherwise healthy people (Anonymous 1999; Faria *et al.*, 2005; Vandenesch *et al.*, 2003; Anonymous 2005). This study reports the isolation of the CA-MRSA from the nasal mucosa of a patient undergoing haemodialysis treatment.

MATERIALS AND METHODS

The nasal swab was collected in February 2004 during screening of 43 haemodialysed patients treated at the Nephrology Department of the University Hospital in Lublin, Poland for staphylococcal nasal colonization. The carrier of the CA-MRSA strain (which was also the only MRSA cultured from the above mentioned patients) was a 56-year-old male. At the time of swab collection the patient had been on haemodialysis for 11 months. Before admission to the haemodialysis ward, he had received continuous peritoneal dialysis (CAPD) from January 2001 until March 2003. The patient developed peritonitis caused by *Candida albicans* during the CAPD therapy and underwent 2-month hospitalization from January to March 2003. In addition to chronic renal failure, the patient suffered from other severe co-existing conditions including malignancy (true polycythaemia) and hepatitis C. The patient had an endogenous arteriovenous fistula as a vascular access site.

The processing of the nasal specimen as well as the preliminary identification of the staphylococcal isolate were conducted according to conventional microbiological procedures. In addition, the isolate was characterized to the species level using the API STAPH system according to the manufacturer's instructions (BioMérieux, France). Antibiotic susceptibility testing was performed using the Vitek automated system (BioMérieux, France) and the agar disc diffusion

method according to recommendations given by the National Reference Centre for Antimicrobial Susceptibility Testing in Poland (NRCAST) (Hryniewicz *et al.*, 2004).

The PFGE typing of *Sma*I digested DNA was performed by a modification of a previously described method (Bannerman *et al.*, 1995). Briefly, each tube containing 250 µl of NET Buffer [10 mM Tris, 1 mM EDTA, 10 mM NaCl] was inoculated with the relevant bacterial culture. The suspension was then mixed with 200 units of achromopeptidase (Leonard *et al.*, 1995) [Sigma, UK] and an equal volume of 2% SeaPlaque Agarose [Flowgen, UK] cooled to 50°C. Agarose/cell mixture was immediately loaded into block molds [Bio-Rad Laboratories Ltd, UK] and allowed to solidify at 4°C. Solidified blocks were subsequently dispensed into appropriate test tubes containing lysis buffer (6mM Trizma base, 100 mM EDTA, 1 M NaCl, 0.5% Brij 58, 0.2% sodium deoxycholate, 0.5% lauroyl sarcosine) and incubated in a waterbath at 50°C for 60 min. After incubation, the lysis buffer was removed from the tubes and the blocks were washed three times for 10 min at 50°C each in TE buffer (10 mM Trizma base, 1 mM EDTA). One quarter of each agarose block was then digested with *Sma*I (10 units/µl) at 30°C for 3 hours and loaded into the wells of 1% PFGE certified agarose gel [Bio-Rad Laboratories Ltd, UK]. Electrophoresis was performed in 0.5 x TBE buffer (44.5 mM Trizma base, 44.5 mM boric acid, 1 mM EDTA) [Biowhittaker, UK] by the contour-clamped homogenous electric field method with a CHEF system [Bio-Rad Laboratories Ltd, UK]. Fragments were separated with a linear ramped pulse time of 6.8-63.8 s for 23 hours at 14°C. Gels were subsequently stained with ethidium bromide solution (1 µg/ml) for 30 min, visualized under UV and photographed. The PFGE pattern was matched with the MRSA PFGE pattern database of worldwide MRSA clones in the Scottish MRSA Reference Laboratory.

The PCR method for *SCCmec* typing was based on the work of Oliveira & de Lencastre (2002). Primers for Locus E (243-bp) were not included in the assay used in this study. The DNA template was obtained by the suspension of several staphylococcal colonies in NET buffer containing 100 U achromopeptidase (Kobayashi *et al.*, 1994; Leonard *et al.*, 1995) and incubation of the sus-

pension at 50°C for 10-15 minutes. Two microlitres of the DNA template were then added to 12.5 µl of Reddy Load PCR mix (0.2 mM of each dNTPs, 3 mM MgCl₂, 0.625 units of Taq polymerase, Taq buffer (20 mM Trizma base, 50 mM KCl), and dye [Abgene, UK]) containing *SCCmec* primers. Tissue culture grade water [Sigma, UK] was added to give a final volume of 25 µl. Cycling conditions for the *SCCmec* typing were as follows: 1 cycle of 94°C (4 min), 30 cycles of 94°C (30s), 52°C (30s), 72°C (1 min), followed by a final cycle of 72°C for 4 min. Six microliters of the PCR products were loaded into 1.5% Phorecus agarose [Biogene, UK] and electrophoresis was performed in 0.5xTBE buffer at 180 volts for 2.5 h. The gels were subsequently stained with 1 µg/ml ethidium bromide [Sigma, UK] for 30 min, visualized under UV, and photographed.

The PCR assays for the detection of the *nuc* and *pvl* genes were based on published primers and methods (Brakstad *et al.*, 1992; Lina *et al.*, 1999). The bacterial DNA extraction was conducted as for the *SCCmec* typing. Two microliters of the DNA template were subsequently added to 12.5 µl of Reddy Load PCR mix (0.2 mM of each dNTPs, 3 mM MgCl₂, 0.625 units of Taq polymerase and Taq buffer and dye [Abgene, UK]) containing 25 pmol of each primers. Tissue culture grade water [Sigma, UK] was added to give a final volume of 25 µl. Cycling conditions for the *nuc/pvl* detection were as follows: 1 cycle of 95°C (5 min), 30 cycles of 95°C (30s), 55°C (30s), 72°C (1 min), and 1 final cycle of 72°C for 5 min. Six microliters of the PCR product were loaded into 1.5% Phorecus agarose [Biogene, UK] and electrophoresis was performed in 0.5 x TBE buffer at 180 volts for 3 h. The gels were subsequently stained with 1 µg/ml ethidium bromide [Sigma, UK] for 30 min, visualized under UV, and photographed.

RESULTS AND DISCUSSION

The CA-MRSA isolate reported in the present study was found to colonize the nasal mucosa of a patient undergoing haemodialysis therapy. Although this isolate was not associated with the development of invasive infection in its carrier this is a significant finding taking into account the well-documented virulence and clinical im-

portance of CA-MRSA (Anonymous 1999; Holmes *et al.*, 2005; Etienne, 2005) as well as high susceptibility of haemodialysed patients to infections of staphylococcal etiology following asymptomatic mucosal colonization (Kluytmans *et al.*, 1997).

The MRSA isolate met laboratory criteria characteristic of CA-MRSA (Vandenesch *et al.*, 2003, Anonymous, 2005). It demonstrated the presence of two unique and stable molecular markers of CA-MRSA including the *SCCmec* type IV (Figure 1) and the *pvl* locus (Figure 2) encoding for the Panton-Valentine leukocidin (PVL). In addition, the cultured MRSA isolate possessed a distinct PFGE profile characteristic of one of the recognized CA-MRSA clones (Figure 3) (Vandenesch *et al.*, 2003). This PFGE pattern of the isolate corresponded to the multilocus sequence type (MLST) - ST80, a clone which has been reported in several European countries (Hanssen *et al.*, 2005; Faria *et al.*, 2005; Vandenesch *et al.*, 2003; Holmes *et al.*, 2005). It should also be mentioned that the PFGE pattern of the CA-MRSA isolate differed from the healthcare-associated multiresistant MRSA clone identified in seven wards (Neurology, Surgery, Cardiology, Cardiosurgery, Laryngology, Intensive Care Unit, and Ortho-

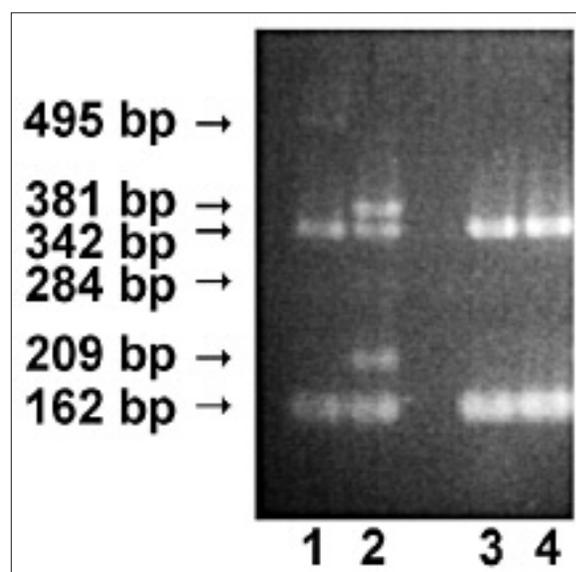


FIGURE 1 - The *SCCmec* typing of the MRSA isolate colonizing anterior nares of the haemodialysed patient. Lane 1 - NCTC 10442 Reference Strain (Type I); Lane 2 - Mu50 strain (Type II); Lane 3 - EMRSA-15 (Type IV); Lane 4 - the CA-MRSA isolate (Type IV).

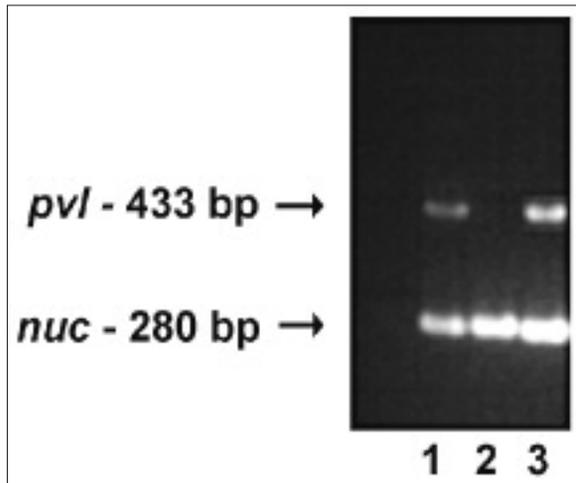


FIGURE 2 - The PCR detection of the *nuc* and *pvl* genes in the MRSA isolate colonizing anterior nares of the haemodialysed patient. Lane 1 - Positive Control strain (*nuc* and *pvl* positive); Lane 2 - Negative Control strain (*nuc* positive, *pvl* negative); Lane 3 - the CA-MRSA isolate (*nuc* and *pvl* positive).

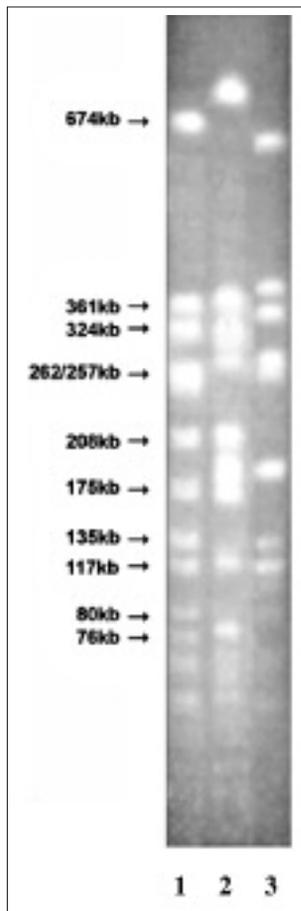


FIGURE 3 - The PFGE analysis of the CA-MRSA isolate cultured from the haemodialysed patient. Lane 1 - PFGE Reference Standard (*S. aureus* strain NCTC 8325); Lane 2 - haemodialysed patient CA-MRSA isolate with PFGE pattern characteristic of the "European" CA-MRSA clone; Lane 3 - the multiresistant healthcare-associated MRSA clone.

paedics) of the University Hospital in the same period (Szczepanik *et al.*, 2007) (Figure 3).

The relative susceptibility of this isolate to antibiotics also met the criteria of CA-MRSA which is described as non-multiresistant in nature (Anonymous, 2005; Aires de Sousa, 2003). The MRSA isolate was found resistant only to the beta-lactams (oxacillin MIC \geq 8; ampicillin MIC \geq 16; ampicillin/sulbactam MIC \geq 32; ceftazolin MIC \geq 32) and tetracycline (MIC \geq 16). The resistance of ST80 CA-MRSA against tetracycline has also been observed in other reports. In contrast, the isolate from this study was not resistant to fusidic acid as reported in other studies (Holmes *et al.*, 2005; Vandenesch *et al.*, 2003). The reports of this clone (ST80) in other European countries (including Norway, Denmark, France, Switzerland, and the United Kingdom) have in most cases noted an association with the community (Hanssen *et al.*, 2005; Faria *et al.*, 2005; Vandenesch *et al.*, 2003; Holmes *et al.*, 2005). In contrast, the first ST80 reported in 1998 was isolated from the hospital environment in Greece from the catheter of a premature baby one week after birth (Aires de Sousa *et al.*, 2003). These authors also reported the isolation of ST80 from hospitalized renal failure patients again indicating that these MRSA were acquired in the hospital. The results of our study were similar as the CA-MRSA strain was cultured from a hospitalized patient with several risk factors predisposing for MRSA acquisition including the dialysis therapy, a history of hospitalization, and severe conditions accompanying the chronic renal failure such as malignancy and hepatitis C. Nevertheless, since the carrier was not screened for MRSA at the time of hospital admission and had no known history of acquiring the microorganism during his hospital stay, the possibility of colonization in the community before or after hospitalization cannot be excluded. Moreover, the attempts to elucidate the origin of the strain were further complicated by the fact that, in addition to the history of hospitalization, the patient had regular and direct contacts with the health care system due to the dialysis treatment he had received for three years before the date of CA-MRSA isolation. Our assumptions regarding the healthcare associated origin of the strain can be supported by the previous reports on still low prevalence of CA-MRSA among persons without

traditional risk factors predisposing for MRSA acquisition (Salgado *et al.*, 2003).

It must be mentioned that the ST80 MRSA type, in addition to the present study, has already been observed in Poland in a single healthcare center. Molecular analysis revealed that the clone carried the type IV *SCCmec* and demonstrated both heterogeneous resistance to methicillin and a wide susceptibility to other classes of antimicrobials (Krzysztoń-Russjan *et al.*, 2005).

To conclude, this study reports the isolation of another CA-MRSA isolate in Poland and adds further evidence for the changing epidemiology of MRSA and the international spreading of this group of staphylococci represented here by a common European clone. Although the source of the CA-MRSA strain remains unclear, the results of the study support previous investigations (Salgado *et al.*, 2003) indicating the ability of CA-MRSA to colonise patients with established MRSA risk factors in addition to previously healthy and immunocompetent individuals.

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