

*Epidemiological typing of methicillin-resistant *Staphylococcus aureus* isolates from surgical wounds and other sites of patients in the Medical Centre Ljubljana*

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ABSTRACT

Background. Methicillin-resistant *Staphylococcus aureus* (MRSA) frequently causes colonisation and infection of wounds and other sites in hospitalised patients. It evolved by the acquisition of the chromosomal gene *mecA* in a previously susceptible *Staphylococcus aureus* (MSSA). This gene encodes a novel penicillin-binding protein PBP 2a, with a low affinity for methicillin and all other β -lactam antibiotics.

Materials and methods. Fifty-two MRSA isolates: 36 from surgical wounds and 16 from other sites (2 from blood, 9 from drains and 5 from intravascular catheter tips) of patients at Departments of Surgery of the Medical Centre Ljubljana, Slovenia, were typed by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA to determine the number and distribution of clones.

Results. MRSA isolates belonged to 6 genotypes or clones (A–F); 46 to clone A, which was epidemic and 6 to clones B–F. In surgical wounds 31 of 36 MRSA belonged to clone A and 5 to other clones (clones B, C, D, E and F, one isolate each). In other sites 15 of 16 isolates belonged to clone A and one to clone C.

Discussion. Epidemic MRSA clone A, which is genetically related to the South German clone, was prevalent. Other MRSA clones were found sporadically in wounds, but almost not in other sites. Some of them could belong to the other internationally spread clones, but in others, horizontal spread of genes from MRSA to MSSA could have occurred.

KEY WORDS

Staphylococcus aureus, methicillin-resistant, surgical wounds, epidemiological typing, distribution of clones, Slovenia

Introduction

Since methicillin-resistant *Staphylococcus aureus* (MRSA) was first described in 1961, most countries have reported varying proportions of MRSA among *Staphylococcus aureus* isolates in hospitals and long-term care facilities (1, 2). Recently, the appearance of community-acquired MRSA strains has been described (3,4). In the USA these are becoming a rather frequent cause of

skin infections even in the young immunocompetent persons without MRSA risk factors. Methicillin resistance in previously methicillin-susceptible staphylococci is due to the acquisition of the chromosomal gene *mecA* which encodes a novel penicillin-binding protein PBP 2a, with a low affinity for methicillin and all other β -lactam antibiotics (5,6). Hospital acquired MRSA are

usually multi-resistant, and therapy with β -lactam antibiotics and antibiotics of most other groups is not successful, therefore glycopeptide antibiotics should be used (6). An exception represents the community-acquired MRSA which is resistant only to β -lactam antibiotics and is susceptible to the majority of non- β -lactam preparations.

Like methicillin-susceptible *Staphylococcus aureus* (MSSA), MRSA colonises nasopharynx, skin and perineal region. Up to 1% of patients admitted to acute-care facilities where MRSA is endemic, may become colonised, with dissemination occurring essentially via the hands of healthcare workers. Once colonised with MRSA, 20-60% of patients in acute care and 3-15% in chronic care facilities will subsequently develop infection (7). Risk factors for infection after colonisation are: recent antibiotic therapy, an intensive care unit (ICU) stay, intravenous catheterization, presence of wounds and prior colonisation with MRSA. Most frequent infections caused by MRSA are: skin and soft tissue infection, bone infection, pneumonia, bacteremia, etc (8).

The proportion of MRSA in European countries ranges from <1% in Scandinavia to >30% in Spain, France and Italy (2). In Slovenia, the prevalence of MRSA from blood in the year 2001 was 20%, but it decreased to 14% in the year 2002 (9). The prevalence of MRSA from wounds in the Medical Centre in Ljubljana in the year 2001 was 17.3% (10). In institutions where MRSA has been endemic for an extended period, eradication is unlikely to occur due to the large occult reservoir of colonised patients circulating among acute and chronic care facilities and the community. However, the investigators have found that active surveillance, extensive patient screening and intensive control measures (improved hand hygiene practices, early implementation of contact isolation, decolonization in some patients) decrease the MRSA reservoirs, with a subsequent reduction in the rate of infection (6,7,11).

Some strains of *S. aureus* are more likely than others to cause epidemics of staphylococcal infection and

the same may be true for some strains of MRSA, such as the epidemic MRSA strains described in Australia and England (5). Monitoring and limiting the intra and interhospital spread of MRSA strains require the use of efficient and accurate epidemiological typing systems (12). There are numerous reports of recent MRSA outbreaks from various European countries (13,14,15). Authors report the limited number of MRSA clones that circulate in Europe (13,14). Murchan described three clusters of genetically related MRSA clones, which have spread internationally and one of them contains strains from Finland, Belgium, United Kingdom, Southern Germany and also from Slovenia (16).

The macrorestriction analysis of chromosomal DNA using pulsed-field gel electrophoresis (PFGE) is a reference method for *Staphylococcus aureus* typing and can be combined with other methods (17). Our aim was to use PFGE to determine the number and distribution of clones in MRSA isolates from wounds and other sites at Departments of General Surgery, Neurosurgery, Cardiac, Abdominal, Thoracic and ICU-Surgery in a short time period in the Medical Centre Ljubljana, Slovenia.

Materials and methods

Fifty-two MRSA isolates collected from 52 patients at Departments of General Surgery, Neurosurgery, Cardiac, Abdominal, Thoracic and ICU-Surgery of the Medical Centre Ljubljana, Slovenia, were typed by PFGE. 36 isolates were from surgical wounds and 16 from other sites (2 from blood, 9 from drains and 5 from intravenous catheter tips). Isolates of *Staphylococcus aureus* were identified by coagulase and DNA-se tests. Methicillin resistance was determined by oxacillin 1 μ g disk and confirmed by oxacillin plate (Mueller Hinton agar containing 4% Na Cl and 6 μ g/ml oxacillin) and susceptibility to penicillin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, vancomycin, rifampin, gentamicin, ciprofloxacin and teicoplanin was determined by the disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (18). MRSA were typed by PFGE. The restriction enzyme (which is normally used for staphylococci) was *Sma* I (Roche) (19). Electrophoresis was carried out in 1.2% (wt/vol) agarose gel (Bio Rad) and a running buffer, containing 0.5xTBE, with a CHEF DR III drive module (Bio Rad, Hercules, California). Running conditions were initial and final pulse times of 1 and 80 s and a run time of 37 h at 14°C. After electrophoresis DNA of each isolate yielded 15-20 fragments from 10 to 700 kbp, representing a PFGE pattern. Isolates with identical PFGE patterns belonged to the same clone and were epidemiologically related, isolates with one to three band differences were its subclones and were related to the main clone, while isolates with more differences were different clones (17,19,20).

Table 1. Distribution of clones of 52 methicillin-resistant *Staphylococcus aureus* isolates from wounds and other sites at the Departments of General Surgery, Neurosurgery, Cardiac, Abdominal, Thoracic and ICU-Surgery.

Clone	No isolates	Subclones	Wounds	Other sites
A	46	4	31	15
B	1	0	1	0
C	2	1	1	1
D	1	0	1	0
E	1	0	1	0
F	1	0	1	0
Total	52	5	36	16

Results

Fifty-two MRSA isolates belonged, according to their PFGE patterns, to 6 genotypes or clones (A–F); 46 isolates belonged to clone A, which was epidemic and 6 to 5 other clones (B–F), which were sporadic. Assignment of isolates to different clones was based on more than three bands difference in their PFGE pattern. In surgical wounds all of the clones were found; 31 of 36 MRSA belonged to clone A, which had four subclones (A1–A4) and 5 to other clones (clones B, C, D, E and F, with

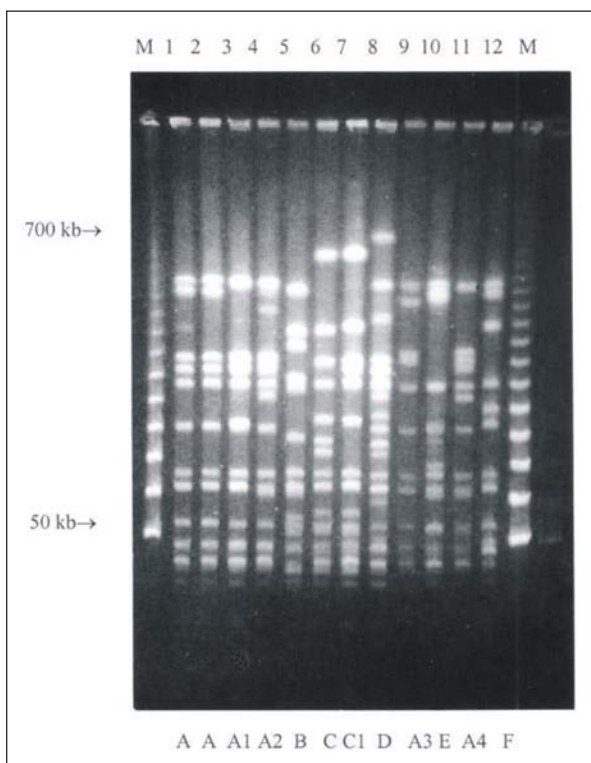


Figure 1. Pulsed-field electrophoresis (PFGE) patterns of clones A–F and their subclones (A1–A4, C1) present in 52 MRSA isolates collected from wounds and other sites at the departments of General Surgery, Neurosurgery, Cardiac, Abdominal, Thoracic and ICU-Surgery of the Medical Centre Ljubljana, Slovenia: M=marker; 1–12= PFGE patterns of MRSA isolates.

one isolate each). In other sites 15 of 16 isolates belonged to clone A and one only to clone C, subclone C1 (Table 1, Figure 1). The isolates of clone A were resistant to oxacillin, penicillin, erythromycin, clindamycin, gentamicin and ciprofloxacin, while they remained susceptible to trimethoprim/ sulfamethoxazole, vancomycin, rifampin and teicoplanin. Isolate of clone B differed from clone A in susceptibility to erythromycin and clindamycin and resistance to rifampin, isolates of clone C in resistance to rifampin, and the isolate of clone E in susceptibility to gentamicin. Isolates of clones D and F didn't differ from clone A in antibiotic susceptibility.

Discussion

The epidemic MRSA clone A was widespread in surgical units of our hospital and 86% of MRSA isolates belonged to this clone. The clone A was even more frequent than the clone described in the study of Wichelhaus who detected by phage typing and PFGE one dominating (epidemic) clone to which 50% or more isolates in five German hospitals belonged (21). From wounds 83% of MRSA isolates belonged to clone A and its subclones and 17% to clones B–F, whereas from other sites 94% MRSA belonged to clone A. Different clones may differ also in antibiotic susceptibility, but it is not obvious (6).

All clones were multi-resistant, which is the characteristic of hospital-acquired MRSA. Our clones B, C and E differed from the clone A in antibiotic susceptibility, but clones D and F didn't. Murchan has proved, by PFGE and by computer comparison of PFGE patterns internationally, that our clone A is very similar to the South German clone which is widespread in Europe (16). It is probable that the epidemic clone in our hospital spread so widely because of the combination of its great propensity to spread and of insufficient infection control practices. In wounds, but almost not in other sites, clones B–F appeared sporadically. These clones should be compared to other internationally spread clones, because some could belong to them (for example clone C was related to the Iberian clone).

For an analysis of community-acquired MRSA, isolates from outpatients should be collected, their antibiotic susceptibility determined and the typing performed.

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