Research article/Raziskovalni prispevek

DIVERSITY OF *spa* TYPES AMONG MRSA ISOLATES FROM MARIBOR UNIVERSITY HOSPITAL

RAZNOLIKOST MRSA *spa* TIPOV, IZOLIRANIH PRI BOLNIKIH IZ UNIVERZITETNEGA KLINIČNEGA CENTRA MARIBOR

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Abstract

Background *Typing of pathogens is an important part in control and prevention of health care-associated infections. For methilcillin-resistant S. aureus (MRSA) a new sequence-based and easily internationally comparable typing method, spa typing, was recently described. We have used this method to analyze the MRSA types present in our hospital and to compare them with types reported elsewhere.*

Methods *63 MRSA strains isolated from patients in Maribor University Hospital (MUH) during the year 2006 were spa typed. Typing data were compared to the patient hospitalization data to detect possible spatial and temporal clusters.*

Results *Sixty-three MRSA strains were distributed into 12 spa types. Seven spa types were represented only by a single isolate. The three most prevalent types (t001, t288, t003) included 80 % of all strains. The most prevalent type, t001, was present in many different wards and during entire year. This type is also one of the mostly isolated types worldwide. High prevalence of other two types seems to be associated with small scale transmission events.*

Conclusions *Most of the spa types present in MUH are well known and widespread also elsewhere in Slovenia, in other EU countries and worldwide. Typing has helped us to follow the introduction of different MRSA types to the hospital environment and to detect occasional transmissions.*

Key words *MRSA; typing; spa types; health care-associated infections*

Izvleček

Izhodišča *Tipizacija patogenih mikroorganizmov lahko doprinese k nadzoru in preprečevanju bolnišničnih okužb. Za tipizacijo sevov S. aureus je bila opisana nova metoda, ki temelji na določanju nukleotidnega zaporedja gena spa, ki kodira protein A. Rezultati so zato za razliko od do sedaj uporabljanih metod zelo lahko primerljivi med laboratoriji. Z novo metodo smo zato želeli preiskati seve, izolirane v naši bolnišnici, ugotoviti raznolikost prisotnih tipov ter jih primerjati z drugimi bolnišnicami.*

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Metode *Vsem sevom MRSA, izoliranim pri bolnikih Univerzitetnega kliničnega centra Maribor v letu 2006, smo določili spa tip. Rezultate tipizacije smo nato primerjali s podatki hospitalizacije posameznega bolnika ter tako sledili morebitne časovne in prostorske povezave.*

Rezultati *Triinšestdeset MRSA sevov smo uvrstili v 12 spa tipov. Sedem spa tipov je imelo le po enega predstavnika. Trije najpogostejši spa tipi (t001, t288, t003) so predstavljali 80 % vseh preiskanih sevov. Najpogostejši tip t001 je bil prisoten na številnih oddelkih v celem letu. Visok delež drugih dveh tipov pa je najverjetneje povezan s prenosi manjšega obsega.*

Zaključki *Večina MRSA tipov spa, ki so bili prisotni v UKC Maribor v letu 2006, je pogostih tudi drugje v Sloveniji, v Evropi in v svetu. S pomočjo tipizacije smo lahko natančneje sledili vnos MRSA v našo bolnišnico ter prenose znotraj bolnišnice.*

Ključne besede *sevi MRSA; tipizacija; spa tipi; bolnišnične okužbe*

Introduction

Staphylococcus aureus is among the leading bacterial pathogens causing a spectrum of diseases ranging from superficial skin infections to toxin mediated diseases and serious infections like pneumonia, endocarditis and sepsis. Methicillin – resistant *Staphylococcus aureus* (MRSA) is defined by a presence of a specific *mecA* gene which renders those strains resistant to all beta-lactam antibiotics. In addition, MRSA strains often possess other resistance genes (e. g. for macrolides, tetracyclines and aminoglycosides) and hence infections are difficult to treat.

Because of MRSA importance in healthcare-associated infections (HAIs), several typing methods were established and were used to confirm the identity of the strains in particular outbreak or for monitoring the spread of certain MRSA types in different geographic regions.[1] For local level surveillance the pulsed-field gel electrophoresis (PFGE) was mostly used while multi-locus sequence typing (MLST) was the method of choice for larger pylogenetic studies.[2] PFGE gives a good results and correlates well with epidemiological data, however, the results are extremely difficult to compare between two or more laboratories. Recently, a new sequence based typing method, a *spa* typing, was introduced.[3] It is based on determination of a sequence for protein A gene *(spa)* specific for *S. aureus.* Method can be applied for methicillin-sensitive (MSSA) and MRSA strains. Parts of *spa* gene are highly variable and currently more than 4000 versions of *spa* gene are known. Each unique *spa* gene represents a *spa* type designated as t001, t002 ... The typing results for different hospitals or laboratories are therefore easily compared at national and international level. Additionally, *spa* typing is discriminative enough to enable the detection of epidemiologically related strains and their differentiation from unrelated strains.[4]

The prevalence of patients colonized or infected with MRSA is one of the indicators for quality of healthcare-associated infection control worldwide and is since 2006 included also in monitoring of quality of Slovenian hospitals.[5–8] At Maribor University Hospital MRSA cases were found only sporadically until 1999. In this year first MRSA outbreak was detected and the number of patients has substantially increased.[8] As a result a register of MRSA positive patients was introduced, standard infection control measures were increased and some additional measures were introduced (immediate reporting of new MRSA isolations to the patient's ward and to the Infection Control Team (ICT), daily visits of ICT members to all MRSA positive patients, decolonization of MRSA patient, tagging of MRSA positive patients in hospital database, regular screening of high risk patients at admission).[8] Currently, the MRSA rates at MUH are stable. The number of new MRSA positive patients is constantly decreasing since the year 2004 and was for the studied period (year 2006) 0.14 positive patients/100 admittances (Table 1). Similarly is the proportion of MRSA strains among all *S. aureus* strains low and was 6.9 for the year 2006[8] (Table 1).

Table 1. *New MRSA positive patients at Maribor University Hospital.*

Tab. 1. *Novoodkriti pacienti z MRSA v Univerzitetnem kliničnem centru Maribor.*

Year	Admitted patients	New MRSA patients	MRSA/100 admittances	Percentage (%) MRSA/all *S. aureus*
Leto	Sprejemi	Novi pacienti z MRSA	MRSA/100 sprejemov	Delež (%) MRSA/vsi *S. aureus*
1998	50237	71	0.14	7.0
1999	50520	114	0.23	10.0
2000	52111	73	0.14	6,4
2001	53971	53	0.10	4.5
2002	55245	66	0.12	5.4
2003	55137	85	0.15	6.7
2004	56822	109	0.19	8.9
2005	56205	82	0.15	7.4
2006	54470	75	0.14	6.9
2007	55029	68	0.12	6.1

To further improve the surveillance and control of MRSA in MUH we have *spa* typed MRSA isolated during one year interval in our laboratory (only 63, the others were isolated in other laboratories) with the aim to determine the diversity of MRSA types present in our hospital environment and to compare them to *spa* types described worldwide.[9] This is a first report on *spa* typing of consecutive MRSA isolates from a Slovenian hospital.

Methods

MRSA screening

Patients with at least one of the following risk factors are included in the screening: transfer from another hospital or from long-term care facility, previous hospitalization within last 12 months, previous known colonization with MRSA, patients with chronic wounds, all patients at critical care units at admission and at dismission, patients undergoing clean elective and implant surgery, patients on peritoneal dialysis, patients with more than 24-hour room contact with a confirmed MRSA carrier, health care workers at the time of epidemia.[10]

Isolation and characterization of MRSA

For isolation, conventional culture media were used. For surveillance swabs (nose, throat, skin) MRSA-screening plate (MRSA ID, bioMerieux) and Trypticase soy broth with added NaCl were used. The susceptibility testing to oxacillin and cefoxitin was performed with disk diffusion method according to NCLLS Performance Standards for Antimicrobial Susceptibility Testing. MRSA strains were confirmed by amplification of *mecA* gene. All strains were frozen at –70 °C until further characterization. Only the first isolate per patient was stored.

spa typing

MRSA strains were thawed and cultured on blood agar. A single colony was transferred to a fresh plate and after 24 hours of incubation the culture was used for DNA isolation with QIAamp DNA Kit (QIAgen). *spa* region was amplified using primers and conditions as described by Harmsen et al.[3] and sequenced by commercial service (MWG, Martinsried, Germany). *spa* types were assigned using the Ridom StaphType software (Ridom GmbH, Wurzburg, Germany).[3]

Results

In the year 2006 *S. aureus* was isolated in 1088 patients hospitalized in MUH. Seventy-five of them (6.9 %) were colonized with MRSA. Altogether 63 MRSA strains from 20 different wards were finally available for *spa* typing.

Sixty-three MRSA strains were grouped into 12 different *spa* types (Table 2). Seven *spa* types had only a single representative strain. One of them (t2986) was a new *spa* type. The three most prevalent MRSA types in year 2006 (t001, t288 and t003) represented 80 % of all MRSA patients and five most prevalent MRSA types represented 89 % of all MRSA strains.

More than half of all isolates (58.7 %) belonged to the type t001. This type was present in ten different wards and over entire year. The second most numerous type, t288, represented 12.7 % of all typed MRSA strains and was probably associated with a short outbreak as six out of eight strains were found in a single ward within a 2 months time. The third most prevalent type, t003, was associated with elderly care facility from where it was introduced twice to our hospital and was then on both occasions found in at least one additional patient in the same ward.

Table 2. *Distribution of spa types of MRSA at Maribor University Hospital in year 2006.*

Tab. 2. *Spa tipi MRSA in njihov delež UKC Maribor v letu 2006.*

spa type *spa* tip	Number of strains Število sevov	Percentage of the type Delež med vsemi tipi v %
t001	37	58.73
t288	8	12.69
t003	5	7.93
t041	3	4.76
t030	3	4.76
t355	1	1.59
t458	1	1.59
t020	1	1.59
t102	1	1.59
t201	1	1.59
t005	1	1.59
t2986	1	1.59

Patients with non-prevalent *spa* types were all colonized already at admittance and have came to MUH from other hospitals and health care facilities from Slovenia (Ljubljana, Murska Sobota, Hrastovec) and from abroad (Great Britain, Kosovo).

Discussion

We have typed the MRSA strains isolated in patients hospitalized during a one year period in MUH. The typing results combined with patient hospitalization data have helped us to clarify part of the MRSA epidemiology at MUH. A single type, t001 (which belongs by MLST to ST-5,ST-222, and ST-228) seems to be endemic in our hospital and was present during entire year and at many different wards. The same type was detected also in patients admitted from home or from a long-term care facility, suggesting that this type is prevalent in entire Maribor region. The endemic spread of such highly successful types finally leads to a lack of discrimination in local hospital epidemiology. To overcome this situation, it is necessary to use the combination of different typing methods.[4]

Although several studies on molecular characterization of MRSA isolates from different Slovenian hospitals are published,[11, 12] to date none of them reported the *spa* types, therefore the comparison to situation in other Slovenian hospitals is not possible at the time. However, Slovenian MRSA strains isolated between 1981 and 1998 were included in a large comparative study[13] and twelve Slovenian strains were distributed into 6 *spa* types. Interestingly, already at that time type t001 was the most prevalent and was found in six of twelve strains from our country. Two other types (t030 and t201) were the same as reported in present study. International database[9] provides the list of all currently known *spa* types with information of their relative frequency among all strains reported to the database and with information on countries where the given type is present. All MRSA types detected in MUH except one (t2986) were already included in *spa* database. Two of the three *spa* types that repre-

sented 80 % of all MRSA strains in MUH are also among the most prevalent in the Ridom database. Type t003 is actually the mostly reported type worldwide and is present in Europe, USA and among our neighbouring countries in Austria and Croatia. Type t001 is the eighth most common in the database and is reported in almost all European countries (including Slovenia), of the neighbouring countries in Austria, Croatia and Italy. Our *spa* types also have substantial overlap (t003, t001, t041, t030) with ten most prevalent types in German national reference centre for 6 months period in the year 2006.[4] On the other hand there is type t288, the second most common type in MUH, rarely reported in the international database. This additionally support the observation that high number of MRSA t288 due to the short outbreak situation in a single ward.

Conclusions

MRSA strains were typed into 12 *spa* types. Most of the *spa* types predominant in our hospital are also very often isolated in other countries and/or were reported for previous Slovenian MRSA isolates. Using the *spa* typing for characterization of MRSA isolates for a one year time interval we were able to follow the introductions to and transmissions within our hospital.

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