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PHENOTYPIC AND GENOTYPIC PROPERTIES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED IN HUNGARY, 1997–2000*

HEDDA MILCH, JUDIT PÁSZTI, TÍMEA ERDŐSI AND MAGDOLNA HETZMANN

"B. Johan" National Center for Epidemiology,
Gyáli út 2–6, P.O. Box 64, H-1966 Budapest, Hungary

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An account is given on the activity of the National Center for Phage Typing of Staphylococci in Hungary in the period between 1997 and 2000 related to methicillin resistant *Staphylococcus aureus* (MRSA) strains originating mainly from hospital infections and sporadic cases. The rate of multiresistant MRSA strains has decreased gradually from 98.1% in 1997 to 74.6% in 2000, accordingly the typability by phages showed a considerable improvement by the international basic phages. Resistance pattern of MRSA strains became narrower in the period of the examinations. With the exception of erythromycin the rate of resistance decreased probably as a consequence of the increased use of erythomycin. The typing method was completed with the phenotypic and genotypic characterization of macrolide resistance. Among 73 MRSA strains type A was the most frequent macrolide resistance group, while type B, C1 and C2 occurred rarely. Type A was frequent also among the few MSSA and CNS strains. Out of the 168 examined *S. aureus* strains *ermA* genes occurred in 81.5%; in MSSA and CNS strains *ermC1* genes were frequent, both genes are responsible for the target modification. The *msrA* gene, encoding the increased efflux, occurred only in CNS strains. Comparing the results obtained by phenotyping (phage typing) and genotyping (AP-PCR) methods it is of note that MRSA strains which proved non-typable by phage typing gave suitable results by the AP-PCR.

Keywords: MRSA strains, phage typing, genotyping, antibiotic resistance

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were first described in 1961 as "Celbenin" resistant Staphylococci [1]. Their clinical and epidemiological significance has increased gradually since 1980 in the European countries [2–5], in the USA [6] and in Australia [7]. In the 1990s MRSA has become worldwide the most significant nosocomial pathogen [8-12]. Examinations on the virulence of MRSA and methicillin sensitive *S. aureus* (MSSA) led to various conclusions. While in Hong Kong it was found that MRSA and MSSA strains had similar virulence [13], comparison of mortality rate in nosocomial bacteriaemia with MRSA and with MSSA showed that it was three times higher in patients of MRSA group [14]. It can be stated that MRSA infections usually cause serious infections, leading to difficulties in their treatment because the strains are not only resistant to all the β-lactam antibiotics, their resistance rates to other antibiotics are also high [15, 16], and in the recent years low-level resistance has emerged even to glycopeptides, vancomycin and teicoplanin [17, 18]. With regard to the serious infections and dangerous nosocomial outbreaks it is important from epidemiological point of view to determine the type of the MRSA strains with a suitable typing method.

In this paper an account is given of investigations on the phenotypic and genotypic properties of MRSA strains isolated from nosocomial infections and from sporadic cases in Hungary between 1997 and 2000.

Materials and methods

Staphylococcal strains

In the period between 1997 and 2000 altogether 3407 *S. aureus* (2693 MSSA, 698 MRSA and 16 coagulase negative Staphylococci /CNS/) were isolated from different nosocomial or sporadic infections in the County Institutes of National Public Health and Medical Officer Services (NPHMOS). For the macrolide resistance studies the following strains were selected: 145 MRSA, 18 MSSA and 16 CNS.

Phenotyping

Staphylococcal phages used for typing

International basic set for phage typing *S. aureus:* 29, 52, 52A, 79, 80(I), 3A, 3C, 55, 71 (II), 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85(III); 94, 96(V), 81, 95 (Misc) (originating from Public Health Laboratory Service (PHLS), Colindale, London [19,

20]) and the MRSA type phages: 616, 617, 618, 620, 622, 623, 625, 626, 629, 630 [21] were used. Phage typing was carried out by the method of Blair and Williams [22].

Antibiotic susceptibility testing methods

Oxacillin sensitivity was determined by disc diffusion [23] using Mueller-Hinton medium (Oxoid). The disc contained 10μg oxacillin (Human, Budapest). In addition to oxacillin the following antibiotics were tested: ampicillin (AM), streptomycin (SM), tetracycline (TE), gentamicin (GM), vancomycin (VA), erythromycin (EM), oleandomycin (OL), lincomycin (LI), spiramycin (SP).

The resistance phenotype of macrolide-lincosamide-streptogramin B resistance (MLSR) Macrolide type

The placing of the EM, OL, SP and LI antibiotic discs were carried out according to Jánosi' s method [24]. Evaluation of and conclusion to the constitutive or inductive character of the resistance was made according to Weisblum [25–26]. On the basis of these characteristics there are 5 distinguishable phenotype classes: A, B, C1, C2 and other.

The macrolide phenotypes group A: constitutive MLSR strains; group B: inducible (partial) PMSR strains; group C1: EM inducible MLSR strains; group C2: EM, OM inducible MLSR strains [27–29].

Genotyping

Arbitrarily primed-PCR (AP-PCR)

AP-PCR typing was carried out according to Welsh and Mc Clelland [30] with slight modifications. DNA preparation and purification was made by Promega Wizard genomic DNA purification kit, according to the manufacturer's protocol.

The amplification was made in Techne Progene PCR apparatus during 42 cycles through the following temperature profiles: 96°C 5 min, after two cycles: 96°C 1 min, 40°C 1 min and 72°C 1 min continued in 40 "standard" cycles: 96°C 1 min, 60°C 1 min and 72°C 1 min. The amplified DNAs were separated by agarose gel electrophoresis and ethidium bromide staining was made during 15 min. The photography was made under UV transilluminator and the evaluation by Gel-Doc 2000 documentation apparatus (Bio-Rad) by the aid of Quantity One evaluation program.

As subtype of the same type of an isolate was regarded when there was one band difference among the patterns [31].

Detection and identification of macrolide resistance genes in MRSA, MSSA and CNS strains

Demonstration of erm genes encoding target modification

Oligonucleotide primer pairs: *ermA-F* 21 nucleotides, *ermA*-R 24 nucleotides [32], *ermB-F* 21 nucleotides, *ermB-R* 24 nucleotides [33], *ermC-F* 21 nucleotides, *ermC-R* 24 nucleotides [34].

Demonstration of genes encoding increased efflux (from S. epidermidis *strains)*

Oligonucleotide primer pairs: *msrSA-343F* 20 nucleotides, *msrSA-1237* – 21 nucleotides [35].

Primers were selected as described by Sutcliffe [36] and Nakajima [37]. Specificity of different primers were tested using DNA extracts of the reference strains containing: *ermA* gene (chromosome): *S. aureus* ISP 447; *ermB* gene (plasmid): *S. aureus* 8325 (pI258); *ermC* gene (plasmid): *S. aureus* MS 13837; *msrSA* gene (plasmid): *S. aureus* 8325 (pMC 38).

Preparation of template DNA

A single colony of each isolate was picked up from an agar plate and resuspended in 100µl of distilled H_2O, and boiled for 10 min. They were placed to -70°C from 2 to 24 hours. The supernatant was collected after spinning for 2 min. in a microcentrifuge.

The DNA concentration of boiled extracts was determined at 260 nm (Genesis UV Spectrophotometer) [38]. Phenol-chloroform extracted DNAs were prepared as described by Kado and Liu [39] and Katsurashima [40]. Purification of DNA was made by Wizard Kit (Promega), as described in the "Protocol".

Amplification protocol

PCR was carried out in 50µl volumes that contained 20µg bacterial genomic DNA or boiled extracts for each resistant determinant.

The "master" contained: PCR Buffer (Promega), 200µM dNTP, 1.5mM $MgCl_2$ 0.5µM primer, 1.25 E Taq.

A Progene (Techne) thermal cycler was used for 1 cycle 94°C 2 min 15 s and 30 cycles of amplification (94°C, 45 s, 55°C, 30 s, 72°C, 1 min 45 s and then 1 cycle 72°C, 10 min). The amplified DNA was separated in 15 µl aliquots and electrophoresed in 1.6% agarose gels containing 0.5 µg/ml ethidium bromide and photographed under UV light.

Fig. 1. Typability of MSSA strains by international basic phages

Results

Phage pattern by basic and MRSA phages

Typability of 3391 *S. aureus* strains (2693 MSSA and 698 MRSA) by basic and MRSA phages is shown in Fig. 1 and Fig. 2. MSSA strains were typable by basic phages in 91.8%, MRSA strains in 57.6%, while by MRSA phages in 73.5%.

Antibiotic resistance of MSSA and MRSA strains

Antibiotic resistance of the MSSA and MRSA strains examined in 1997–2000 are demonstrated in Fig. 3 and Fig. 4. Among the 3391 *S. aureus* strains 20.6% were oxacillin resistant. Oxacillin resistant MSSA strains occurred annually between 8.8% and 36.0%. Tetracycline resistance of MSSA strains varied between 21.0% and 26.1%, gentamicin resistance between 0.5% and 1.9%; streptomycin resistance between 0.5% and 2.9%, erythromycin resistance between 3.8% and 6.5%. Antibiotic resistance of MRSA strains showed significantly higher values: tetracycline, gentamicin and streptomycin resistance was the highest in 1997 (92.3%, 90.3%, 90.3%, respectively)

and decreased after 1998, but increased again in 2000. On the contrary the erythromycin resistance showed the highest value in 2000 (93.4%).

Multiresistant MSSA strains occurred in 1.3%, while multiresistant MRSA strains in 83.5% (Fig. 5).

Fig. 2. Typability of MRSA strains by international MRSA phages

Antibiotic resistance according to phage type pattern

Occurrence of antibiotic resistance proved to be different according to phage type pattern and the year of examinations. In case of 83A complex tetracycline resistance e.g. occurred in 1997, 1998, 1999 and 2000 in 28.5%, 10.0%, 55.9% and 49.6%, respectively. Non-typable strains showed the highest antibiotic resistance in every year with all the examined antibiotics (Fig. 6).

Macrolide resistance types

For identification of macrolide type 107 erythromycin resistant MRSA, MSSA and CNS strains were selected. Among 73 MRSA strains type A was found in 91.8%, type B in 2.7%, C1 in 4.1% and C2 in 1.4%. Out of 18 MSSA erythromycin resistant strains 9 strains were of type A, 8 strains were of macrolide type C2 and one of type C1. Out of the 16 CNS strains 11 were of macrolide type A, two strains of type C2 and three strains were non-typable (Table I).

Fig. 3. Antibiotic resistance of MSSA strains
TE=tetracycline, GM=gentamicin, SM=streptomycin, EM=erythromycin

Fig. 4. Antibiotic resistance of MRSA strains. For abbreviations see Fig. 3

Fig. 5. Multiresistance of MRSA and MSSA strains

Macrolide resistant genes of MRSA, MSSA and CNS strains

Macrolide resistance genes were determined in 145 MRSA, 18 MSSA and 16 CNS strains. Table II shows the occurrence of macrolide resistance genes according to macrolide types. The most frequent *ermA* gene occurred in every macrolide type in MRSA and MSSA strains. Macrolide resistance genes of MRSA strains were: *ermA*, *ermA+C*; macrolide resistant genes of MSSA strains were *ermC*, *ermA* and unknown. Macrolide resistance genes of CNS strains were *ermA*, *ermC*, *msrA*, unknown and one CNS strain carried presumably a macrolide gene of *new type* (resistance pattern: EM^s, OL^r, LI^r, Sp^r). Table III shows the occurrence of macrolide resistance genes of 168 examined *S. aureus* strains. The most frequent were the *ermA* genes (81.5%) in MRSA strains, *ermC* genes in MSSA, as well as in CNS strains. The *ermA+C* gene occurred only in 2.4%, while the *ermC* in 13.1%.

Fig. 6. Antibiotic resistance of frequent *S. aureus* phage groups or type patterns. For abbreviation see Fig. 3

Fig. 6. (continued)

Table I

Macrolide type and antibiotic resistance pattern of MRSA, MSSA and CNS strains in Hungary, 1997–1999

Antibiotic resistance	No. of examined Strains	No. of macrolide types				
		A	B	C1	C2	Nt
OX PC TE GM SM EM	67	65	–	1	1	–
OX PC TE GM EM	4	1	2	1	–	–
OX PC EM	2	1	–	1	–	–
No. of MRSA strains	73	67	2	3	1	–
PC TC EM	2	1	–	–	1	–
PC SM EM	1	–	–	–	1	–
PC EM	15	8	–	1	6	–
No. of MSSA strains	18	9	–	1	8	–
OX PC TE GM SM EM	3	2	–	–	1	–
OX PC TE SM EM	4	2	–	–	–	2
OX PC GM SM EM	1	1	–	–	–	–
OX PC TE GM EM	1	1	–	–	–	–
OX PC GM EM	1	1	–	–	–	–
OX PC SM EM	3	2	–	–	1	–
OX PC EM	3	2	–		–	1
No. of CNS strains	16	11	–	–	2	3
Total No of strains	107	87	2	4	11	3
Macrolide types in %		81.3	1.9	3,7	10.3	2,8

AP-PCR types of MRSA strains

Typing results of 12 MRSA strains are demonstrated in Table IV and Fig. 7 by comparison the phenotyping (phage typing with two phage-sets) and genotyping (AP-PCR typing) results. The strains were isolated from different nosocomial infections in 1997, 1998 and 1999. Strains 1/97 and 2/97 originated from the burn ward of the same hospital, one from a hygienic sample, the other from a patient, but the phage type and antibiotic resistance pattern were different supported also by their different (type 1 and 2) AP-PCR types. In the traumatology ward of hospital "E", three MRSA strains were isolated from three different patients (6/97, 7/97, 8/97).

Table II

Macrolide resistance genes of MRSA and CNS strains according to macrolide types

	MRSA (145)								MSSA (18)				
Macrolide type (MT)	A			B2	C1	C2	ND		A		C1	C2	
No. of MT	67			2	3	1	72		9		1	8	
Macrolide resistance gene (MRG)	ermA	erm A+C	ND	erm A	Erm A	erm A	erm A	erm A+C	erm C	ND	erm A	erm C	UK
No. of MRG	59	2	6	2	3	1	70	2	8	1	1	7	1

	CNS (16)							
Macrolide type (MT)	A			C2		NT		
No. of MT	11			2		3		
Macrolide resistance gene (MRG)	erm A	erm C	ND	msr A	UK	msr A	new type	ND
No. of MRG	1	7	3	1	1	1	1	1

Two strains isolated from two different patients were identical with each other according to phage type, antibiotic resistance pattern and AP-PCR type (type 1) alike. The phage type (by MRSA phages) of the third strain originating from the third patient (8/97) proved to be not definitely different, but the AP-PCR typing gave different result (type 2). In the surgery of Hospital "F" one strain was isolated from wound infection of a patient (9/98), and three strains from wound, throat and nose swab samples of another patient (10/98, 11/98, 12/98). These latter strains were not typable by basic phages, but proved to be identical by MRSA phages and were identical or nearly identical by AP-PCR typing (type 1 and 1/1). A strain from the wound infection of the other patient (9/98) proved to be different from the former strains by AP-PCR typing (type 5). Strains 3/99, 4/99 and 5/99 originated from sporadic cases in different hospitals and proved to be different from each other based on phage typing and AP-PCR typing alike (types 1, 3 and 4).

Table III

Multiresistance of MSSA and MRSA strains*

Year	Staphylococci	No. Of strains	Multiresistant strains	
			No	%
1997	MSSA	553	2	0.4
	MRSA	208	204	98.1
1998	MSSA	849	22	2.6
	MRSA	82	74	90.2
1999	MSSA	695	3	0.4
	MRSA	73	55	75.3
2000	MSSA	596	9	1.5
	MRSA	335	250	74.6
1997 –2000	MSSA	2693	36	7.3
	MRSA	698	583	83.5

*resistant to 3 or more antibiotics

Discussion

For many years phage typing was the method of choice for investigation of MRSA epidemiology since its discriminatory capability had been demonstrably higher than that of other phenotypic tests [41]. Since 1974 there have been valuable data on the occurrence of MRSA strains in Hungary [42], and since 1990 on their multiresistance and typability by phages [43]. The epidemiological usefulness of phage typing was indisputable also in Hungary [44]. Nevertheless the method requires a large number of typing phages, so it is labour consuming and difficult to standardize. Antibiograms are inadequate for differentiation purposes. Among genotypic methods the plasmid analysis was the first method applied widespread. Many reports indicated the usefulness of plasmid analysis, but the method had its limitations, e.g. similar, but not identical patterns and isolates without plasmid could not reliably be distinguished from similar isolates. Complex typing (phage typing and plasmid profile) of MRSA was applied by Kozarsky et al. [45] and Witte et al. [46].

Following the first molecular technique (plasmid analysis), various molecular methods have been developed and used for typing of MRSA strains. Comparative investigations of traditional and molecular methods for typing isolates of *S. aureus*,

especially of phage typing and pulsed-field gel electrophoresis (PFGE) showed the superiority of PFGE typing [47].

Table IV

Phage types and AP-PCR type of MRSA strains

Strain No./ year	Origin	Test sample	Phage pattern by		AP-PCR type	Antibiotic resistance pattern
			Basic phage-set	MRSA phage set		
1 /97	Hospital "A" Burn ward	nose	NT	630	1	OX PC SM TE GM EM OL LI SP
2 /97	Hospital "A" Burn ward	hygenicsa mple	75 (III)	618/623/63 0	2	OX PC TE GM EM OL LI SP
3 /99	Hospital "B" Accident surgery	wound	NT	NT	1	OX PC SM TE GM EM OL LI SP
4 /99	Hospital "C" Vascular surgery	wound	3C (II)	NT	3	OX PC TE
5 /99	Hospital "D" Otolaryngology	throat	116 (II)	NT	4	OX PC EM OL LI SP
6 /97	Hospital "E" Traumatology	nose	NT	622/630	1	OX PC SM TE GM EM OL LI SP
7 /97	Hospital "E" Traumatology	blood culture	NT	622/630	1	OX PC SM TE GM EM OL LI SP
8 /97	Hospital "E" Traumatology	pharynx	47/54 (III)	622	2	OX PC SM GM EM OL LI SP
9 /98	Hospital "F" Surgery	wound	NT	NT	5	OX PC TE GM EM OL LI SP
10 /98	Hospital "F" Surgery	wound*	NT	618/620/62 6/630	1	OX PC SM TE GM EM OL LI SP
11 /98	Hospital "F" Surgery	throat*	NT	618/620/62 6/630	1/1	OX PC SM TE GM EM OL LI SP
12 /98	Hospital "F" Surgery	nose*	NT	618/620/62 6/630	1	OX PC SM TE GM EM OL LI SP

Which should be the international standard typing method for the MRSA isolates? That was the question of Weller [41] who listed the advantages of different phenotypings such as antibiogram, phage typing, serotyping, whole cell protein analysis, immunoblotting, multilocus enzyme electrophoresis (MLEE), zimotyping and genotypings including: plasmid analysis, restriction enzyme analysis (REA), ribotyping, insertion sequences (IS), *mecA*: Tn554 probetyping, binary typing, pulsed-field gel electrophoresis (PFGE), PCR typing: coagulase gene typing, protein A gene typing, random amplified polymorphic DNA (RAPD), arbitrarily primed-PCR (AP-PCR), repetitive element sequence based PCR (rep PCR).

Fig. 7. AP-PCR patterns for 12 MRSA strains used in this study
1: markers, 2: negative control, 3–14: From left to right strains are numbered as in Table IV from 1/97 to 12/98

The conclusion was that only the phage method has been standardized for international use, but owing to the high proportion of non-typable isolates and poor reproducibility it should be completed by the "gold standard" PFGE method, which is highly discriminatory, but time consuming, and the international standardization is missing. According to Weller only the binary typing (based on Southern hybridisation) can be used as a single method.

In the present work 3391 *S. aureus* strains isolated between 1997 and 2000 were examined for phagetype-pattern and antibiotic sensitivity. 27.3% and 8.8% of strains originating from 1997 and 1998 proved to be MRSA, respectively. In 2000 the rate of

MRSA strains increased to 36.0%. The reason was that in 2000 special attention was given to the nosocomial infections.

When non-typable MRSA strains are isolated from a hospital outbreak, it cannot be stated which of the strains are responsible for the outbreak. In a number of cases high proportion of MRSA isolates originating from hospital outbreaks remain non-typable [48]. In England and Wales e.g. in the framework of a survey of MRSA infections carried out in 1990 only 3.8% of isolates were typable [49]. In a former examination 46.3% of our non-typable *S. aureus* strains became typable using heat treatment [50]. In the present work the non-typable MRSA strains became typable by basic phages in 56.7% using heat treatment.

Comparing the antibiotic resistance rates of MRSA strains obtained in the present study to those found by Witte [51] in the same period in Germany it turns out that the rates were significantly higher in Hungary than in Germany (e.g. in 1999 the MRSA strains showed tetracyclin resistance in 16.0% in Germany, and in 65.8% in Hungary). The reason for this significant difference can rather be the abuse of antibiotic therapy than the spreading of different clones.

Multiresistance of MSSA and MRSA strains suited to the expectations: in the whole period of examination MSSA strains were multiresistant in 1.3% while MRSA strains in 83.5%. The difference in the occurrence of multiresistance is reflected in the effectiveness of phage-typing.

Witte gave an account about a narrower resistance pattern of MRSA strains in Germany after 1995 [51]. MRSA strains showed similar changes in Hungary: multiresistance decreased gradually from 1997 to 2000. The decrease was connected with lower tetracycline, streptomycin and gentamicin resistance rates, presumably due to the altered therapy with new antibiotics.

The significance of resistance plasmids was emphasized convincingly by Lacey [52]: "The rapid diversification in the properties of the staphylococcus over the last 20 years has resulted chiefly from alterations in plasmid carriage, by transfer of plasmids and phages between cells." In Hungary Jánosi [25] published data about different *S. aureus* phage patterns and plasmid profiles. Plasmids (3.7 kb, 2.9 kb and 1.25 kb) coding chloramphenicol, tetracyline, streptomycin and gentamicin resistances occurred in the 83A phage type complex. Rosdahl [53] investigated *S. aureus* strains isolated from hospitalized patients in Denmark for antibiotic resistance and phage-type in 1996, 1997, 1998 and 1999 and found methicillin resistant strains in 4.4%, 4.6%, 4.5% and 4.1%, respectively; gentamicin resistance in less than 0.7%, erythromycin resistance in less than 3.9%. The reason for the low rates of the antibiotic resistances was the applied antibiotic policy.

According to phagetype pattern there was a significant difference in the occurrence of antibiotic resistance. Strains of phage group III and those non typable by international basic phages showed the highest resistance to tetracycline, gentamicin, streptomycin and erythromycin in every year of observation.

An increased use of erythromycin in the recent years has led to an increasing resistance to erythromycin, especially in MRSA strains [51]. In the present study the highest frequency (93.4%) of erythromycin resistance occurred among MRSA strains which originated from nosocomial infections in the year 2000.

According to the large-scale examinations of Jánosi [25] performed from 1978 to 1989 with more than 10 thousand *S. aureus* strains, 15% were resistant to MLS antibiotics. In the present work from 1997 to 2000 MSSA strains were resistant to MLS antibiotics between 3.8% and 6.5%, while the MRSA strains between 75.6% and 93.4%.

In the former examinations in Hungary [28] the constitutive MLSR strains group A were the most frequent phenotypes. The PMSR (partial macrolide-streptogramin B resistant) phenotype (group B) strains were frequent in 1980–81 and diminished thereafter, group C1 and C2 diminished after a short ascending period. In our examinations in 1997 to 1999 with comparatively a fewer number of MRSA strains the macrolide type A occurred in 91.8%, the groups B, C1 and C2 were rare.

Resistance to macrolides in bacteria evolves through three mechanisms: (1) modification of the antibiotic target mediated by the *erm* gene; (2) enhanced efflux, mediated by the *msr, erp. mef* and *mre* genes; (3) inactivation of antibiotics by *ere* or *ere*-like and other inactivating enzymes. Sutcliffe et al. [36] and Schmitz et al. [54] examined the macrolide resistance genes of *S. aureus*. On the basis of their results we searched for *ermA, ermB*, and *msrA* genes of MRSA strains. We found *ermA* and, *ermA+C* genes in MRSA strains; *ermA* and *ermC* genes in MSSA strains and *ermA, ermC* and *msrA* genes in CNS strains. Unknown (not identified) genes were detected in MSSA and CNS in 1.8%.

In MSSA and MRSA strains originating from German hospitals [54] *ermA* genes were found in 21% and 40.3%, *ermC* genes in 46.5% and 35.8%, and unknown genes in 12.0% and 10.4%, respectively.

Since in Hungary there was an increase of infections caused by MRSA strains, and the phage typing methods – effective to MSSA strains – gave unsatisfactory results for the typing of MRSA strains we completed the phage typing method with the AP-PCR typing based on the observations of van Belkum [55] who made a comparison between phage typing and AP-PCR typing with satisfactory results. In a former cooperation we supplemented MRSA phage typing with PFGE typing [56] with

acceptable results. Our examinations concerning AP-PCR typing of nosocomial MRSA strains gave suitable typing results of the strains non typable by phages.

In this publication we give a review on our present possibilities concerning MRSA typing. It is hoped that international efforts will result in a generally accepted DNA based typing method, the need for which was emphasized recently also by van Belkum [57].

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