

## METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE AMONG HOSPITALIZED PATIENTS AND HEALTHCARE WORKERS IN THE CLINICAL CENTER OF SERBIA

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**Abstract** - The aim of the present study was to provide the first comprehensive analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage among patients and healthcare workers (HCWs) in the largest healthcare facility in Serbia. Specimens from anterior nares obtained from 195 hospitalized patients and 105 HCWs were inoculated after broth enrichment onto chromogenic MRSA-ID medium. In total, 21 of 300 specimens yielded MRSA. Among hospitalized patients, 7.7% were colonized with MRSA, and 5.7% HCWs were colonized with MRSA. Five out of 21 (23.8%) tested MRSA strains were classified as community-associated MRSA (CA-MRSA), and four of them were isolated from HCWs. The remaining 16 MRSA strains had characteristics of healthcare-associated MRSA (HA-MRSA), and two of them were isolated from HCWs. The HA-MRSA strains isolated from HCWs were indistinguishable from HA-MRSA of the same cluster isolated from patients. This finding reveals the circulation of HA-MRSA strains between patients and HCWs in the Clinical Center of Serbia.

**Key words:** Methicillin-resistant *Staphylococcus aureus*, nasal carriage, patients, healthcare workers

### INTRODUCTION

*Staphylococcus aureus* is an important pathogen of serious infections in humans. It is always a challenge to treat infections caused by *S. aureus*, particularly isolates resistant to methicillin and related beta-lactams. Methicillin-resistant *S. aureus* (MRSA) was first reported in the early 1960s, and rapidly increased and spread in the 1980s. Nowadays, MRSA is endemic in most hospitals in the world (Chen et al., 2010). Colonized patients are the chief source of MRSA in hospitals and colonized healthcare workers (HCWs) may be involved in the transmission of MRSA between patients (Albrich and Harbarth, 2008). Although multiple body sites can be colo-

nized in humans, the anterior nares are the most frequent carriage site for MRSA. In the acute care hospital setting, MRSA colonization is associated with a higher risk of nosocomial infection and increased hospital costs (Patel et al., 2008). Reduction of MRSA colonization is the most effective measure for preventing dissemination of MRSA. Therefore, a rapid and sensitive detection method to identify MRSA carriers is crucial in MRSA infection control. Active surveillance cultures for MRSA are now part of clinical practice recommendations in both Europe and the United States. This includes both patients and HCWs (Verkade et al., 2011). Studies of MRSA carriage have been performed in various geographic regions, but to our knowledge, no data

concerning MRSA carriage in Serbia have been published so far.

The aim of the present study was to provide the first analysis of MRSA nasal carriage in hospitalized patients and HCWs in the largest healthcare facility in Serbia and to determine the genetic lineages of the circulating MRSA.

## MATERIALS AND METHODS

### *Screening samples*

The Clinical Center of Serbia (CCS) in Belgrade is the largest healthcare facility in Serbia, with 3 300 beds. For the present investigation, nasal swabs from both nares were taken from 195 hospitalized patients and 105 HCWs.

### *Culture methods*

After the collection, all samples were processed within 2 h. Each swab was dipped in 3 mL of Mueller-Hinton broth (MHB; bioMérieux, France) supplemented with 6.5% NaCl, vortexed for 30 s, and incubated for 24 h at 35°C, in air. 50 µL of the suspension was inoculated onto chromogenic medium MRSA-ID (bioMérieux, France). All inoculated solid media were incubated at 35°C, in air, and read after 48 h of incubation.

### *Identification of isolates*

In accordance with the manufacturers' instructions, a colony suggestive of MRSA was subcultured onto Columbia blood agar, and later confirmed by PCR for *nuc* (Brakstad et al., 1992) and *mecA* (Bignardi et al., 1996) genes.

### *Susceptibility testing*

Susceptibility to antibiotics (cephoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, penicillin, pristinamycin, rifampin, teicoplanin, tetracycline, tobramycin, trimethoprim/

sulfamethoxazole and vancomycin) was performed by disk diffusion method in accordance to the CLSI recommendations (CLSI, 2010), or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendation (EUCAST, 2011).

### *Panton-Valentine leukocidin (PVL) determination*

Presence of PVL was determined by previously described PCR protocol (Lina et al., 1999). *S. aureus* ATCC 49775 was used as the positive control.

### *DNA Typing*

Determination of SCC $mec$  types was performed by PCR using primers and protocol as described by Boye et al. (2007). *S. aureus* strains HT20020290, HT20020285, HT20030826, HT20040068, HT20060580 and HT20020274 served as the control.

The presence of *agr* type was assessed by multiplex PCR (Lina et al., 2003). *S. aureus* strains RN6390, RN6607, RN8465, and RN4850 served as positive controls.

Multiple-locus variable-number tandem-repeat assay (MLVA) was performed as described (Sabat et al., 2003).

## RESULTS

### *Isolation of MRSA from nasal swab specimens*

In total, 300 swabs were taken from hospitalized patients and HCWs. A total of 21 of the 300 participants (7%) in the study carried MRSA. Among hospitalized patients, 7.7% were colonized with MRSA, and of the tested HCWs, 5.7% were colonized with MRSA (Table 1).

### *Characteristics of MRSA isolates*

Twenty-one MRSA isolates were analyzed in this study and summarized results are presented in Table 2. In all, 19 isolates (90.5%) were resistant to one

**Table 1.** Presence of MRSA in nasal swab specimens obtained from hospitalized patients and HCWs in the CCS, Belgrade.

Result for:	Number (%) of individuals with result	
	positive	negative
Patients	15 (7.7)	180 (92.3)
HCWs	6 (5.7)	99 (94.3)
Total	21 (7.0)	279 (93.0)

**Table 2.** Characteristics of 21 MRSA strains isolated from nasal swab specimens obtained from hospitalized patients and HCWs in CCS, Belgrade.

MLVA type (number of strains)	SCC <sub>mec</sub> type	<i>agr</i> type	PVL positive strains	Number of patients / HCWs in which the MLVA type was isolated	Resistance profile (>50% of isolates)*
A (9)	I	2	0	8 / 1	GEN, KAN, TOB, ERY, CLI, CIP
B (4)	I	2	0	3 / 1	GEN, KAN, TOB, CIP
C (2)	III	1	0	2 / 0	GEN, KAN, TOB, ERY, CLI, CIP, RIF, TET
D (2)	V	1	1 (patient)	1 / 1	GEN, KAN, TOB, TET, CHL & other strain was susceptible to all tested antibiotics except beta- lactams
E (1)	V	2	0	0 / 1	GEN, KAN, TOB, CHL
F (1)	V	1	0	0 / 1	susceptible to all tested antibiotics except beta- lactams
G (1)	IV	3	1 (HCW)	0 / 1	KAN
H (1)	I	2	0	1 / 0	GEN, KAN, TOB, ERY, CLI, CIP

\* GEN, gentamicin; KAN, kanamycin; TOB, tobramycin; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; RIF, rifampin; TET, tetracycline; CHL, chloramphenicol.

or more antibiotics beside beta-lactam antibiotics, while only 2 (9.5%; both isolated from HCWs) were susceptible to all other tested antibiotics except beta-lactams. Multidrug resistance, i.e. resistance to three or more antibiotics with different mechanism of action, was observed in 17 (80.9%) isolates; it should be noted that all strains were already resistant to one, beta-lactam class of antibiotics. All tested MRSA strains were susceptible to fusidic acid, trimethoprim/sulfamethoxazole, vancomycin, linezolid, pristinamycin and mupirocin, while 19 (90.5%) isolates were resistant to gentamicin, 20 (95.2%) to kanamycin, 19 (90.5%) to tobramycin, 11 (52.4%) to erythromycin, 11 (52.4%) to clindamycin (only two strains showed inducible type of resistance), 17 (80.9%) to ciprofloxacin, 2 (9.5%) to rifampin, 3 (14.3%) to tetracycline and 4 (19.0%) to chloramphenicol.

Based on MLVA typing, the MRSA could be divided into eight different MLVA types (A-H) (Table

2). The largest group (42.9%) consisted of isolates belonging to MLVA type A. This MLVA type carried SCC<sub>mec</sub> type I, belonged to *agr* type 2 and showed resistance to gentamicin, kanamycin, tobramycin, erythromycin, clindamycin and ciprofloxacin. The MLVA type A MRSA isolates was predominantly found in patients (88.9%), whereas a larger diversity of isolates was found among HCWs (Table 2). MRSA isolates obtained from HCWs primarily contained the smaller SCC<sub>mec</sub> elements type IV and V.

Correlation between resistance patterns and MLVA was good, except for MLVA type D, where one strain isolated from HCWs was susceptible to all tested antibiotics except beta-lactams, and the other, isolated from patient, was multiresistant (Table 2).

## DISCUSSION

Laboratory-based screening for MRSA colonization

of patients and HCWs is a key element in enabling control measures and early therapeutic decisions. Rapid diagnostic testing safely reduces the number of unnecessary isolation days, but only chromogenic screening, and not PCR-based screening can be considered as cost-saving (Wassenberg et al., 2010). Mannitol salt agar is still the most frequently used medium for isolation of *S. aureus*, but recently several chromogenic media for the rapid detection of MRSA have become available (Verkade et al., 2011). Beside this, several studies in the past few years confirm the importance of broth enrichment for accurate detection of MRSA in clinical sample (Verkade et al., 2011) and therefore we decided to use both methods (broth enrichment and chromogenic media) for the isolation of MRSA in our study.

The anterior nares are considered the primary colonization site of *S. aureus*, and therefore most screening programs require the acquisition of a swab specimen from the anterior nares only. The prevalence of *S. aureus* nasal carriage among healthy adults ranges from approximately 20% to 30%, with higher prevalences in overcrowded populations (Albrich and Harbarth, 2008; Chen et al., 2010). The results of examination of MRSA carriage may significantly vary between different studies and the reason may reflect true differences in MRSA carriage, usually as the consequence of a prevalence of MRSA in the investigated hospital. However, differences in study design, such as duration of the study, investigation of a particular population (e.g. patients on dialysis), investigation during an epidemic, investigation in a particular unit (e.g. ICU), different media used for isolation of MRSA, time of incubation of media, etc., may have significant impact on obtained results. MRSA carriage among patients was reported to be 5.9% to 15.6% in nares (Nahimana et al., 2006; Wang et al., 2009). In our study, 7.7% of hospitalized patients were colonized with MRSA. In the largest review of studies evaluating the MRSA carriage rate among HCWs, the mean nasal MRSA carriage in HCWs was 4.1% in 104 studies, range 0-59% (Albrich and Harbarth, 2008). In our study, among 105 tested HCWs, 5.7% were colonized with MRSA.

As far as we know, the present study is the first from Serbia to evaluate the susceptibility of MRSA strains from patients and HCWs to linezolid, fusidic acid and mupirocin. All the MRSA isolates recovered from nasal carriers were susceptible to linezolid, fusidic acid, mupirocin and pristinamycin. A possible explanation is that the use of these antibiotics in Serbia is limited. To date, mupirocin has not been used for the eradication of MRSA in nasal carriers at our hospital. Local therapy with mupirocin has been shown to eliminate MRSA nasal colonization in both patients and HCWs.

There was a relationship between methicillin resistance and resistance to other antibiotics, as noted in previous investigations (Nahimana et al., 2006; Chen et al., 2011). Therefore, this is a major problem in the treatment of MRSA infections. Our study also supports the observation of a relationship between methicillin and aminoglycoside resistance in MRSA. More than 90% of MRSA were resistant to gentamicin, tobramycin and kanamycin.

Hospital-associated MRSA (HA-MRSA) are typically multiresistant, *agr* type 1 or 2, and share a type I, II or III *SCCmec*, while community-associated MRSA (CA-MRSA) strains are multiply susceptible, share a type IV or V *SCCmec* and frequently the PVL locus (Vandenesch et al., 2003; Chen et al., 2012). Among MRSA strains isolated in this study, five strains could be classified as CA-MRSA because they carried *SCCmec* type IV or V, two of them were susceptible to all tested antibiotics except beta-lactams, while one was resistant only to kanamycin beside beta-lactams. Predominantly CA-MRSA isolates were isolated from HCWs. It is interesting that one MLVA type D CA-MRSA was isolated from HCWs and the other MLVA type D CA-MRSA from a hospitalized patient (Table 2). However, the strain isolated from the HCW was susceptible to all tested antibiotics except beta-lactams, and the other, isolated from a patient, was multiresistant. It is possible that the strain isolated from the patient, as well as other multiply-resistant CA-MRSA strains, acquired resistance to non-beta-lactam antibiotics in order to be able to survive in

the hospital environment, where the antibiotic selective pressure is high.

The remaining 16 MRSA strains had characteristics of HA-MRSA, they carried SCCmec I or III, *agr* 1 or 2, and all were resistant to different antibiotics beside beta-lactams. Among these 16 strains, two isolates (MLVA type A and B) were recovered from HCWs. These HA-MRSA strains isolated from HCWs were indistinguishable from HA-MRSA isolated from patients, indicating a circulation of HA-MRSA strains between patients and HCWs in the CCS.

In summary, we demonstrated that broth enrichment and chromogenic MRSA-ID medium are the optimal choice for the isolation of nasal MRSA carriage. The results of this study showed that patients and HCWs of the CCS are colonized with MRSA, and that the prevalence of nasal carriage is higher in the patient group. CA-MRSA more frequently colonized HCWs, while hospitalized patients are more frequently colonized by HA-MRSA. Our study also showed the exchange of MRSA between patients and HCWs.

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## REFERENCES

- Albrich W.C. and S. Harbarth (2008) Health-care workers: source, vector, or victim of MRSA? *Lancet Infect. Dis.* **8**:289-301.
- Bignardi G.E., Woodford N., Chapman A., Johnson A.P., and D.C. Speller (1996) Detection of the *mec-A* gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. *J. Antimicrob. Chemother.* **37**, 53-63.
- Boye K., Bartels M.D., Andersen I.S., Møller J.A., and H. Westh (2007) A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I-V. *Clin. Microbiol. Infect.* **13**, 725-727.
- Brakstad O.G., Aasbakk K., and J.A. Maeland (1992) Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J. Clin. Microbiol.* **30**, 1654-1660.
- Chen C.B., Chang H.C., and Y.C. Huang (2010) Nasal methicillin-resistant *Staphylococcus aureus* carriage among intensive care unit hospitalised adult patients in a Taiwanese medical centre: one time-point prevalence, molecular characteristics and risk factors for carriage. *J. Hosp. Infect.* **74**, 238-244.
- Chen F.J., Siu L.K., Lin J.C., Wang C.H., and P.L. Lu (2012) Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university. *BMC Infect. Dis.* **12**, 343.
- CLSI (2010) Performance standards for antimicrobial susceptibility testing; Eighteenth Informational Supplement. CLSI document M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute.
- EUCAST (2011) European Committee on Antimicrobial Susceptibility Testing (EUCAST), Breakpoint tables for interpretation of MICs and zone diameters, Version 1.3, January 2011.
- Lina G., Piemont Y., Godail-Gamot F., Bes M., Peter M.O., Gauduchon V., Vandenesch F., and J. Etienne (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis.* **29**, 1128-1132.
- Lina G., Boutite F., Tristan A., Bes M., Etienne J., and F. Vandenesch (2003) Bacterial competition for human nasal cavity colonization: role of *Staphylococcal agr* alleles. *Appl. Environ. Microbiol.* **69**, 18-23.
- Nahimana I., Francioli P., and D.S. Blanc (2006) Evaluation of three chromogenic media (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **12**, 1168-1174.
- Patel M., Weinheimer J.D., Waites K.B., and J.W. Baddley (2008) Active surveillance to determine the impact of methicillin-resistant *Staphylococcus aureus* colonization on patients in intensive care units of a Veterans Affairs Medical Center. *Infect. Control Hosp. Epidemiol.* **29**, 503-509.
- Sabat A., Krzyszton-Russjan J., Strzalka W., Filipek R., Kosowska K., Hryniewicz W., Travis J., and J. Potempa (2003) New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *J. Clin. Microbiol.* **41**, 1801-1804.
- Vandenesch F., Naimi T., Enright M.C., Lina G., Nimmo G.R., Hefernan H., Liassine N., Bes M., Greenland T., Reverdy M.E., and J. Etienne (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis.* **9**, 978-984.
- Verkade E., Ferket M., and J. Kluytmans (2011) Clinical evaluation of Oxoid Brilliance MRSA Agar in comparison with bioMerieux MRSA ID medium for detection of livestock-

associated methicillin-resistant *Staphylococcus aureus*. *J. Med. Microbiol.* **60**, 905-908.

Wang C.Y., Wu V.C., Chen Y.M., Su C.T., Wu K.D., and P.R. Hsueh (2009) Nasal carriage of methicillin-resistant *Staphylococcus aureus* among patients with end-stage renal disease. *Infect. Control Hosp. Epidemiol.* **30**, 93-94.

Wassenberg M.W., Kluytmans J.A., Box A.T., Bosboom R.W., Buiting A.G., van Elzakker E.P., Melchers W.J., van Rijen M.M., Thijsen S.F., Troelstra A., Vandembroucke-Grauls C.M., Visser C.E., Voss A., Wolffs P.F., Wulf M.W., van Zwet A.A., de Wit G.A., and M.J. Bonten (2010) Rapid screening of methicillin-resistant *Staphylococcus aureus* using PCR and chromogenic agar: a prospective study to evaluate costs and effects. *Clin. Microbiol. Infect.* **16**, 1754-1761.