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THE EVALUATION OF MRSA SURVEILLANCE CULTURES BY THE NUMBER AND COMBINATIONS OF ANATOMICAL SITES

VREDNOTENJE NADZORNIH KUŽNIN NA MRSA PO ŠTEVILU IN MESTU ODVZEMA

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ABSTRACT

Introduction. The identification of patients infected and/or colonised by methicillin resistant *Staphylococcus aureus* (MRSA) is necessary for the timely introduction of measures for infection control. We compared the diagnostic efficacy of combinations of MRSA surveillance swabs routinely taken by health institutions in the country.

Methods. All surveillance samples, which were sent for a microbiological analysis to detect MRSA with the culture method in 2014, in the three departments for medical microbiology of the National Laboratory for Health, Environment and Food, were included in this study.

Results. Among 65,251 surveillance cultures from 13,274 persons, 1,233 (2.1%) were positive (490 positive persons). Prevailing positive surveillance cultures were throat swabs (31.3%), followed by nose swab (31.2%), skin swab (18.9%), perineum (16.4%) and wound swabs (1.4%). The contribution of other samples, such as aspirate, urine and excreta, was under 1%. We found no statistically significant differences in the frequency of detection of a positive patient, if the combination of samples NTS (nose, throat, skin) or NTP (nose, throat, perineum) was analysed. However, statistically significant differences were confirmed when any of the anatomic sites would be omitted from the sets of NTP and NTS (chi square; $p<0.01$). Adding additional samples resulted in only 24 additional positive patients (4.9%).

Conclusions. The results indicate that increasing the number of surveillance cultures above three does not add much to the sensitivity of MRSA surveillance, the exception could be wound. The swabs from the perineum and from the skin are exchangeable.

Keywords:
surveillance sample, MRSA, MRSA control, meticillin-resistant *Staphylococcus aureus*

IZVLEČEK

***Izhodišča**. Odkrivanje bolnikov, okuženih in/ali koloniziranih s proti meticilinu odporno bakterijo S. aureus (MRSA), je nujno za pravočasni pričetek ukrepov obvladovanja okužb, povezanih z zdravstvom. Primerjali smo diagnostično učinkovitost kombinacije rutinsko poslanih nadzornih brisov na preiskavo za MRSA.*

***Metode**. V študijo smo vključili vse nadzorne kužnine, ki so bile poslane na mikrobiološko preiskavo za MRSA s kultivacijo v letu 2014 na treh oddelkih za medicinsko mikrobiologijo Nacionalnega laboratorija za zdravje, okolje in hrano.*

***Rezultati**. Med 65.251 nadzornimi kužninami od 13.274 oseb je bilo 1233 (2,1%) pozitivnih (490 posameznih pozitivnih oseb). Med pozitivnimi nadzornimi kužninami je prevladoval bris žrela (31,3%), sledili so bris nosu (31,2%), bris kože (18,9%), bris perineja (16,4%), rane (1,4%). Delež ostalih kužnin, kot so aspirat, urin, blato, je bil pod 1%. Ugotavljali smo, da ni statistično pomembnih razlik v deležu zaznanih nosilcev, če imamo kombinacijo nos, žrelo, koža ali nos, žrelo, perinej. Statistično pomembne razlike so, če iz seta nos, žrelo, perinej in nos, žrelo, koža, izločimo katerokoli mesto odvzema ($p<0,01$). Dodatni vzorci so doprinesli le 24 dodatnih pozitivnih bolnikov (4,9%).*

***Zaključki**. Brisa kože in perineja sta zamenljivi kužnini glede na zaznavanje pozitivnih bolnikov. Rezultati študije kažejo, da ni optimalno povečevati število nadzornih kužnin na več kot tri, ker ne prispeva veliko k občutljivosti, razen rane.*

Ključne besede:
nadzorne kužnine, MRSA, obvladovanje MRSA, proti meticilinu odporna bakterija Staphylococcus aureus

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1 INTRODUCTION

The identification of patients infected and/or colonised by MRSA is necessary for the timely introduction of infection control measures. If MRSA infection/colonisation is determined only with clinical samples, some 20–30% of patients carrying MRSA are not detected (1). The hidden reservoir of carriers is actively searched by surveillance cultures. We try to identify MRSA carriers in hospitals to prevent its spreading to other patients. The effectiveness of the identification of MRSA carriers depends on the optimal selection of the anatomic region of sampling for the surveillance cultures, the number and type of surveillance cultures, transport conditions, the selection of a medium for primary inoculation (preliminary enrichment, non-selective or selective mediums), the methods of pathogen identification and the proper method for the detection of antibiotic sensitivity. Several studies analysed the sensitivity of different anatomic sites of the MRSA surveillance sampling, as well as the effect of the combination of different anatomical sites (2, 3). The studies of the effectiveness of different methods of pathogen detection are difficult to compare, as they are of different designs. Almost each state and institution is using its own variant of carrier detection, adapted to the characteristics of the endemic strain.

The microbiological diagnostics has to be rapid and reliable, but also rational. The rationality may be increased by the optimisation of taking the appropriate number of surveillance swabs from the most appropriate anatomic locations. In our study, we compared the diagnostic efficacy of combinations of MRSA surveillance swabs routinely taken by health institutions in the country.

2 METHODS

Slovenia has an area of 20,273 km2 and a population of 2 million. Seven departments of Medical Microbiology of the National Laboratory for Health, Environment and Food (NLZOH) cover more than 13 hospitals, primary care and long-term care facilities, in Slovenia. In our analysis, three departments of the NLZOH, located in Maribor, Celje and Kranj, participated, which serve 6 hospitals, primary care and long-term care facilities, and represent one third of Slovenian population.

All the surveillance cultures included in this study were sent for microbiological detection of methicillin-resistant bacteria *Staphylococcus aureus* (MRSA) from 1 January 2014 to 31 December 2014. The microbiological diagnostics and the computer analysis were performed according to the standard procedures. Data were extracted from the MBL Programme (Infonet version 22.0, Kranj, Slovenia) for surveillance cultures. The programme Kocka 21 (Infonet, version 4.26.0) was used for the analysis of positive results.

2.1 Samples

For each patient who was screened for the MRSA carriage, we got the surveillance swabs from one or more anatomic sites. If two or more swabs from the same patient were sent, the term “Surveillance sample set (SSS)” was used. The labellings for surveillance swabs from similar anatomic sites were grouped into one category (Table 1). We might get the surveillance swabs of the same patient on more than one occasion, so the same patient could be repeatedly represented. For the analysis of sensitivity, only SSSs with more than 3,000 patients were considered. The types of SSSs analysed were nose, throat and skin (NTS) at the Department of medical microbiology in Celje (DMM Celje); nose, throat, perineum (NTP) at the DMM in Kranj; and both at DMM in Maribor.

Table 1. NOSE (the original questionnaire).

Category	Anatomic sites				
Nose	Nose	Naso-pharynx			
Perineum	Perineum	Anus	Perianal	Rectum	
Skin	Skin	Groin	Axilla	Ear	Swabs of stoma (tracheostoma, gastrostoma, ileostoma and colostoma), cannula and tubus
Wound	Wound	Pressure sore	Lesion		
Tracheal aspirate	Trachea aspirate	Trachea swabs			
Urine	Urine				
Faeces	Excreta				

2.2 Laboratory Methods

The swabs for the MRSA detection were inoculated on to the chromogenic medium (MRSA smart (bioMerieux, Paris, France) orCHROMagar MRSA (CHROMagar, Paris, France)) and in the liquid medium (THBS) (Oxoid, Basingstoke, United Kingdom). After 24-hour incubation, the liquid medium was subcultured on a hard chromogenic medium (MRSA Smart, CHROMagar MRSA). All S. aureus isolates were confirmed by standard diagnostic procedures. The identification was performed with MALDI-TOF technology (Bruker Daltonik GmbH, Bremen, Germany) or by DNase activity test, the latex agglutination test and the catalase test.

The sensitivity of S. aureus isolates was tested with the standardised disc diffusion method, according to the CLSI (1.1. to 31.3. 2014) and EUCAST (1.4. to 31.12.2014) (4, 5). Minimal inhibitory concentration (MIC) determination of oxacillin and vancomycin was performed using the E-test (bioMerieux, France).

2.3 Statistical Methods

The results are presented descriptively: the number of received samples, the number of positive samples, the number of positive surveillance sample sets (SSSs) and the number of positive patients in each DMM of the NLZOH. All SSSs with at least one positive swab were included in the next analysis phase. For positive SSSs, the number of positive surveillance cultures per patient were counted and grouped into categories according to combinations of anatomical locations. For SSSs with positive surveillance cultures on more regions, the diagnostic sensitivity decrease was calculated if the results of the swabs from certain anatomic points were not considered. The sensitivity of different sample combinations was analysed only in the group of patients where the same samples (SSSs) were taken: respectively, in the group of patients with swabs from the NTP and in the group with swabs from the NTS. The statistical significance of the differences between the proportions of positive patients regarding the SSS used for specimen sampling was checked with the Chi-squared test.

3 RESULTS

In the year 2014, a total of 65,251 surveillance cultures (SC) were received (Table 2). The proportion of positive surveillance cultures was 1.89% (the range among laboratories was from 1.3 to 2.7%). Of 13,274 persons, 490 (3.69%) were positive for MRSA.

Table 2. The type and number of SC for MRSA analysis.

	Maribor	Celje	Kranj	Total
The number of received SC	32,389	21,500	11,362	**65,251**
The number of positive SC	430	492	311	**1,233**
The % of positive SC	1.3%	2.3%	2.7%	**1.9%**
The number of SSS	no data***	7,105	3,565	**/**
The number of positive SSS	231	260	170	**661**
The % of positive SSS	no data***	3.7%	4.8%	**/**
The number of examined persons*	6,141	5,050	2,083	**13,274**
The number of positive persons**	152	203	135	**490**
The % of positive persons	2.5%	4.0%	6.5%	**3.7%**
Positive SC in one anatomic site	101	106	83	**290**
Positive SC in two anatomic sites	65	80	45	**190**
Positive SC in three anatomic sites	58	72	34	**164**
Positive SC in four or more anatomic sites	7	2	8	**17**

*- if repeated surveillance swabs were received from a single patient, such patient was counted once only
**- each MRSA positive patient was counted once only
***- the data management in Maribor did not allow to group the swabs of MRSA negative patients into “Surveillance sample set (SSS)”.
SC: surveillance cultures

The rates of positive samples are presented in Table 3. Throat swabs were the most prevalent surveillance swabs taken (31.3%), followed by nose (31.2%), skin (18.9%) and perineum swabs (16.4%).The most frequent among all MRSA positive surveillance cultures were nasal swabs (33.9%) followed by throat (29.4%), skin (18.5%), perineum (13.1%) and wound swabs (4.22%). Other samples, such as the aspirate, urine and faeces, represented less than 0.8% of all surveillance samples and less than 1% of positive cultures.

Table 3. The type and number of surveillance cultures (SC) sent for the MRSA detection, and the proportion of positive results.

SAMPLE	Total (%)	Positive (%)	% of positive swabs taken form this location
Nasal swab	20,378 (31.2)	418 (33.9)	2.1
Throat swab	20,426 (31.3)	363 (29.4)	1.8
Skin swab	12,319 (18.9) %	228 (18.5)	1.9
Perineum swab	10,707 (16.4)	161 (13.1)	1.5
Wound swab	894 (1.4)	52 (4.2)	5.8
Tracheal aspirate/sputum	200 (0.3)	3 (0.2)	1.5
Vaginal swab	13 (0.0)	0 (0.0)	0.0
Urine	289 (0.4)	4 (0.3)	1.4
Stool	25 (0.0)	4 (0.3)	16.0
TOTAL	65,251 (100)	1233 (100)	1.9

The proportions of positive anatomical locations and sensitivity of cultures regardingthe anatomical location and combinations of swabs from different locations are shown in Table 4. The largest proportion of positive patients in the NTP set was detected by the nasal swab (67.7%). The same applies also for the NTS sets (66.7%). This means that the nasal swab was the most sensitive specimen in these two sets. The perineum swab was the most frequent sample being single positive in the NTP. With the exclusion of the perineum from the standard set of swabs, 15.3% of MRSA positive patients would have gone undetected. The skin swab was the most frequent sample being single positive in the NTS. With the exclusion of the skin swab from the standard set of swabs, 15.6% of MRSA positive patients would have been lost. We compared the statistical significance of the added sensitivity of the perineum swab in the NTP (29 single perineum positive patients out of 189) with the added sensitivity of the skin swab (43 single skin positive patients out of 276) in the NTS. No statistically significant difference was found between the sensitivity of the skin swab and the perineum swab (chi square 0.353; $p>0.05$), which means the skin and perineum were exchangeable cultures in respect of the MRSA carriage detection.

In the case that we decrease the number of surveillance swabs from three to two per patient, the smaller proportion of MRSA positive patients would be lost in the NTP set, if the throat swab would have been excluded (12.7), and in the NTS set, if the nose (12.7%) or throat (13.0%) swab would have been excluded (Table 4 B and D). However, statistically significant differences were confirmed; when any of the anatomic sites would have been omitted from the sets of the NTP and NTS (chi square; $p<0.01$).

Considering swabs from additional anatomic locations, we identified only 24 additional positive patients (4.9%). Those locations were the wound (19), stool (3), tracheal aspirate (1) and urine (1). Among them, the most frequently positive sample was the wound swab.

Table 4. The sensitivity of surveillance cultures regarding the anatomical location and combination of swabs.

A MRSA positive locations in the nose, throat, perineum (NTP) set.

A combination of locations	Total (%)
The number of MRSA positive patients with the nose, throat, perineum set	189 (100)
The number of patients with the MRSA in all three swabs	59 (31.2)
The number of patients with the MRSA in the nose and perineum	23 (12.2)
The number of patients with the MRSA in the nose and throat	18 (9.5)
The number of patients with the MRSA in the throat and perineum	8 (4.2)
The number of patients with the MRSA in the nose only	28 (14.8)
The number of patients with the MRSA in the perineum only	29 (15.3)
The number of patients with the MRSA in the throat only	24 (12.7)

NTP: nose-throat-perineum; SSS: Surveillance sample set

We took the NTP as a kind of "gold standard" (obviously, that would mean 100% sensitivity, because we had no other method which would show us how many colonized patients were not detected with the NTP) to somehow put the sensitivities of other SSS into perspective.

B The sensitivity of different combinations of surveillance swabs included in the NTP SSS.

SSS	Sensitivity
NTP	100
NT	86.7
NP	87.3
TP	85.2
N	67.7
T	57.7
P	62.9

N: nose; T: throat; P: perineum

C MRSA positive locations in the SSS NTS.

A combination of locations	Total (%)
The number of MRSA positive patients with the NTS set	276 (100)
The number of patients with the MRSA in all three swabs	74 (26.8)
The number of patients with the MRSA in the nose and skin	35 (12.7)
The number of patients with the MRSA in the nose and throat	40 (14.5)
The number of patients with the MRSA in the throat and skin	13 (4.7)
The number of patients with the MRSA in the nose only	35 (12.7)
The number of patients with the MRSA in the skin only	43 (15.6)
The number of patients with the MRSA in the throat only	36 (13.0)

NTS: nose-throat-skin; SSS: Surveillance sample set

D The sensitivity of different combinations of surveillance swabs included in the NTP SSS.

SSS	Sensitivity
NTS	100
NT	75.0
NS	87.0
TP	75.0
N	66.7
T	59.1
S	34.0

N: nose; T: throat; S: skin

4 DISCUSSION

We aimed to establish the appropriate number of surveillance cultures and appropriate combination of surveillance cultures to detect patients colonized with the MRSA. We found that the combination of three samples of the NTS (nose, throat, skin) or NTP (nose, throat, perineum) was significantly more sensitive in detecting the MRSA colonisation over single or double anatomic site swabs with the skin and perineum being exchangeable. Adding additional samples resulted in 4.9% of additional positive patients, with the majority of additionally identified patients being MRSA positive in the wound swab.

Among 65,251 surveillance samples, 1.9% were positive for the MRSA, confirming that Slovenia is among the countries with low prevalence of MRSA carriers (6). However, we detected marked differences in regions of Slovenia, with the eastern part detecting three times less MRSA colonised patients compared to the western part. Prevailing surveillance cultures sent to laboratories were throat swabs (31.3%), followed by nasal swabs (31.2%). Around two thirds of MRSA colonised patients were identified by nasal swabs. However, although rarely taken, the highest positive rate was found for stool cultures. The most frequent indication for the stool sample is detection of the MRSA in the intestine, in known MRSA carriers before decolonisation, therefore those patients are a selected population, and results should be discussed concerning these facts.

Similarly, Ide et al. showed that the nasal swabs provide both the largest number of overall MRSA isolates as well as the largest number of MRSA isolates found at a single site (7). This suggests that the nose is the most important MRSA screening site. In contrast some other studies, we found the largest proportion of positive throat samples, confirming that the throat swab is also an important specimen (8, 9). The sensitivity of the MRSA carrier detection increased when throat, nose and perineum swabs were taken simultaneously (7, 9).

A longitudinal follow up study of MRSA colonised patients showed that nasal swabs had the sensitivity and the negative predictive value of 93% and 95%, respectively, compared to the axilla, groin and perineum, with the sensitivity and the negative predictive value of less then 39% and 69%, respectively (10). When the number of screening samples from the same patient was increased with simultaneous sampling from the nose, throat and perineum, the sensitivity increased to 98.7%, with the negative predictive value of 99.8%.

The nose was the most sensitive anatomic site with the best prediction value for the detection of the MRSA and also MSSA carriage (11). Persons colonized in the nose are often colonized at more anatomic areas simultaneously. The study of Cursino et al. showed that the use of only nasal swabs is insufficient, with sensitivity of 67%, compared to four anatomical site sampling (the nose, anus, perineum, and oropharynx) (3). Even in the combination of two anatomic sites, the detection of the carriers was too low, being 80% with the nose and pharynx sampled simultaneously.

In our study, the sensitivity of different sample combinations was analyzed only in the groups of patients in which the same surveillance sample sets were taken, that is, in the group of patients with cultures from the nose, throat, perineum, and the group from the nose, throat and skin cultures. Those two groups of patients

were also the largest, as those SSS are recommended by the National Committee for Healthcare-associated Infection Control and Prevention in Slovenia (12). Our analyses demonstrated that the nose was the most prevalent positive culture in the NTP and NTS SSS, with over two thirds of MRSA colonised patients positive at that location. By the exclusion of the perineum sample from the NTP SSS, a large proportion of MRSA positive patients (15.3%) would have been lost; the same would happen in the NTS set, if the skin would be excluded (15.6%). A smaller proportion of MRSA positive patients would be lost, if the throat or nose would be excluded (12.7-13.0%). The results of our study thus indicate that the use of three surveillance cultures is reasonable in the majority of subjects. The exception might be the adding of the wound swab in patients who present with the wound, as also proposed by Ide and Dutch Workingparty on Infection Prevention (7, 13, 14).

The highest sensitivity is obtained by the combination of sampling sites. Datta et al. sampled 6 sites in ICU (intensive care unit) patients. Combining the nose and throat culture, they were detecting 95% of colonised patients (9).

In dermatological patients, the best sensitivity was obtained by combining nose, wound and skin lesion swabs (15). German dermatologists recommend the combination of nose, skin and skin-lesion smears as surveillance samples.

In newborns, the best surveillance samples for the MRSA are the navel swab (68% sensitivity) or the combination of the nose and navel swab (91% sensitivity). In pregnant women, the sensitivity of the nasal swab was 67% and the sensitivity of the nose and throat combination 80% (3).

In a meta-analysis, McKinnell and colleagues showed that, by adding additional locations to nasal swabs, approximately one-third more colonised subjects are identified, regardless of the severity of patients and prevalence of the MRSA (16). Adding pharyngeal swabs identifies 21% more patients compared to the nasal swab alone; rectal swabs 20%; wound swabs 17%; and axillar swabs 7%.

The carriage in the alimentary tract is detected by the surveillance culture from the throat, the perineum and the rectum (17). Lautenbach et al. confirmed that nostrils are the prime MRSA colonized site with the throat as the second, and that more anatomic points have to be sampled to reach 90% sensitivity with previously confirmed colonised patients (18). If the throat smear had not been taken, 5-7% of colonized patients would have been lost. The anatomic areas of colonization were different with the CA-MRSA and HA-MRSA. The groin and the perineum were the sites most often colonized in CA-MRSA positive patients.

The Dutch Guideline on the Laboratory Detection of Methicillin-resistant *Staphylococcus aureus* proposes that the surveillance samples from patients and medical staff include the nasal swab (both front nostrils), the throat and perineum or rectal swab (14). The perineal carriage is frequent and in some carriers it is a unique point of colonization. Perineal and rectal swabs are of comparable sensitivity for the MRSA carriage detection. Some previous studies also showed that the groin swab could be used as an alternative to the perineum swab, but for the moment, there is not enough evidence for such a recommendation. It is also important to consider that MRSA density in the axila and perineum is lower than in the nose. Therefore, the preliminary enrichment in a liquid enrichment medium is even more important with these samples (19). Lauerdale et al. found that 66.2% of MRSA colonised patients would be lost, if only direct cultures from nostrils were used without additional samples from the nose, axilla and perineum, and without the use of a liquid enrichment medium (20). In our study, the contribution of the enrichment medium was estimated to be 26.8% (21).

The Dutch experts propose additional sampling sites according to the clinical status and age of the patients: the sputum, if the patient has a productive cough, or aspirate in intubated patients; the skin swab in case of skin-lesions, including eczema; the urine in the case of urine catheter; and the navel swab with newborns. The results of the large study demonstrate that the cultivation of catheter or drainage entering point swabs has no additional value in the MRSA colonization detection (14).
To optimize the costs of MRSA surveillance programs, it should be borne in mind that the MRSA carriage without decolonization lasts for months, so in most cases, it is not reasonable to repeat the surveillance sampling with a MRSA positive patient (22).

The pooling of multiple anatomical site swabs of a patient into a single culture seems an attractive way to decrease costs of the MRSA surveillance; however, we advise against such an approach (21). The first reason is that the location of individual colonization affects the clinical decision with regard to the treatment strategy. The second reason is that pooling of clinical samples disturbs the performance of the classical diagnostic procedure and decreases the sensitivity to 86%, compared to the diagnostics of individual samples.

There are limitations to our study. It relied on the retrospective analysis of routine cultures. The surveillance standards differed in different health care institutions, so many patients could not be included in the analysis of the prevalent SSS due to missing swabs from certain locations. We were not aware of the characteristics of the patients, that is, whether they were hospitalised in medical wards or ICU. We were also not aware whether

positive patients were only colonized, or whether some also had a clinically important MRSA infection. There were also no data of prior decolonization. It was also not clear whether wound swabs, tracheal aspirates and faeces were sent as surveillance specimens, or whether those were clinical specimens. On the other hand, we were able to analyze a very large database of routinely obtained data, thus reflecting everyday clinical practice.

The effective MRSA surveillance depends largely on the adequate laboratory detection of MRSA. The MRSA carrier detection can be enhanced with the use of liquid enrichment media and the inclusion of additional anatomic sites alongside the nasal swab. The results of our study verify that the selection of the surveillance proceduress is very important in the optimisation of surveillance samples. It seems prudent to combine swabs from the nose, skin and gastrointestinal locations. It is rarely benefitial to increase the number of the surveillance samples over three, the exception could be the wound or other skin lesion swab. There is no need to combine smears from the perineum and from the skin, as they are exchangeable.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

All the data analyzed in this study were collected at the National Laboratory for Health, Environment and Food, without information about the identity of individuals diagnozed with MRSA colonisations, according to the Contagious Diseases Act, Health Care Databases Act and Communicable Diseases Reporting Regulation. The study was conducted in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki).

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