# CAT
## Critically Appraised Topic

**Nasal Carriage of *S.aureus* in Ambulant Preoperative Patients : Performance Evaluation of Chromogenic ChromID™ MRSA/ ChromID™ *S.aureus* medium (bioMérieux), and Evaluation of MRSA Biodiversity in Ambulatory Patients.**

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### CLINICAL BOTTOM LINE

ChromID MRSA/ ChromID S.aureus biplate (BioMérieux) permits a reliable detection of both MRSA and MSSA in nasal screening samples after 48 h of incubation without an enrichment step. The sensitivities for both chromogenic sections were substantially higher (28-36%) after 48 h of incubation. Our study confirms that extension of the incubation time to 48 h enhances the sensitivity of MRSA/MSSA detection at the expense of a decreased specificity. However, ChromID MRSA remains to have an excellent specificity of 99.7% at 48 h.
Our study demonstrated a low MRSA proportion (1.2%) and genotypic heterogeneity of MRSA strains in an outpatient population. Our results also confirm that the majority of MRSA strains in the community belong to a restricted number of predominant clones.

### CLINICAL/DIAGNOSTIC SCENARIO

*Staphylococcus aureus* is a common inhabitant of the skin and mucosae. The ecological niches of *S. aureus* strains are the anterior nares of the nose, a principal endogenous reservoir for infection. The prevalence of continuous nasal carriage of methicillin-susceptible *S.aureus* (MSSA) is 20% (range 12-30%) and intermittent nasal carriage is estimated as 30% (range 16-70%).[26] Recently, a cross-sectional study in nine European countries in 32,206 healthy participants showed an overall crude prevalence of *S.aureus* nasal carriage of 21.6% and the one in Belgium was 19.4%.[9] The prevalence of methicillin-resistant *S.aureus* (MRSA) in the above-mentioned study was low and ranged from 0% in Sweden to 2.1% in Belgium.
The majority of MRSA carriers upon admission consist of patients with a history of MRSA colonization or infection, patients recently discharged from healthcare institutions or nursing homes residents (hospital-associated, HA-MRSA). MRSA strains with true community origin (community-associated, CA- MRSA) are genetically unrelated to HA-MRSA and frequently produce the Panton-Valentine leukocidin (PVL) toxin. They started circulating into hospitals and cause nosocomial infections.[29] The early detection of CA-MRSA strains in hospitals is of great importance because of their potential for easy transmission of the type IV SCC*mec* element and PVL toxin to nosocomial methicillin-susceptible *S. aureus* isolates.[30] A third MRSA reservoir is detected in livestock animals and persons in contact with them (livestock-associated, LA-MRSA).
*S.aureus* nasal carriage (MRSA as wel as MSSA) increases a patients risk for the development of a health care-associated infection, more specifically after cardiothoracic, orthopaedic and vascular surgery.[1,5,11,21,27]
*Donker et al.*reported the incidence of surgical site infections (SSI) in nasal carriers of *S.aureus* as 7.3%,

whereas in noncarriers the incidence was 3.8%.[5] For instance, *S.aureus* colonization is related to an increased risk of *S.aureus* mediastinitis after cardiothoracic surgery (odds ratio 4.6).[21] *Kalmijer et al*. reported a relative risk of 8.9 for SSI after orthopedic surgery in carriers of *S.aureus* compared with noncarriers.[11] Systematic review showed that MRSA colonization poses a 4-fold increased risk for infection compared with MSSA colonization and is associated with considerable morbidity and mortality.[20] Despite of this, methicillin-susceptible *S.aureus* remains responsible for approximately 50% of SSI's.[21] Thus, preoperative screening for nasal carriage of methicillin-susceptible *S.aureus* is at least equally important as screening for those of MRSA.

The acquisition pathways for MSSA and MRSA are mainly different. *San Juan et al.*demonstrated in a case-control study endogenous pathogenesis in most cases of postoperative MSSA mediastinitis, whereas exogenous acquisition pathway was typical for MRSA mediastinitis.[21] Defining the acquisition routes of SSI is crucial for designing specific preventive measures. Hospital infection control measures are the major factors for preventing MRSA SSI. Nevertheless, decolonization of nasal carriers became a common component of infection control strategies. Multiple studies performed in orthopaedic and general surgery have demonstrated that preoperative screening for nasal carriage of *S.aureus* and thereafter decolonization with mupirocin and chlorhexidine reduce the risk for the development of nosocomial SSI's.[3,12,13,19]

The early identification of MRSA/MSSA carriers is essential for the implementation of decontamination and infection control measures. Considerable efforts have been made to develop reliable media for the rapid and accurate detection of those micro-organisms in screening samples. Recently, a selective chromogenic biplate, ChromID MRSA/ ChromID S.aureus (bioMérieux), has been developed for the isolation and rapid identification of *S.aureus* (MRSA en MSSA), but there are no reports of the efficacy of this medium with screening samples.

It is common practice in our hospital to screen all preoperative patients for nasal carriage of MRSA during preoperative consultation, nasal carriers of methicillin-susceptible *S. aureus* are not detected however. We believe that the chromogenic medium for the simultaneous identification of methicillin-susceptible and methicillin-resistant *S.aureus* can be very useful for the detection of those two micro-organisms in preoperative patients. Therefore, we evaluated the chromogenic biplate on routine preoperative samples.
Also, we were interested in the biodiversity of the MRSA strains isolated from this outpatient population screened for MRSA in the preoperative setting.

## QUESTION(S)

1. What is the performance of chromogenic ChromID™ MRSA/ ChromID™ S.aureus medium (bioMérieux, Marcy l' Etoile, France) for the simultaneous identification of methicillin-susceptible and methicillin-resistant *S.aureus* in screening nasal samples?

2. What is the biodiversity of MRSA in the screening samples of an outpatient population?

## SEARCH TERMS

*MeSH Database (PubMed): MeSH term: "* Staphylococcus aureus, methicillin resistant, nasal screening swabs, chromogenic media, performance, MRSA, molecular typering "

1) *PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)*
2) *Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), National Guideline Clearinghouse (http://www.ngc.org/), Institute for Clinical Systems Improvement (http://www.icsi.org), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane, Health Technology Assessment Database (http://www.york.ac.uk/inst/crd/htahp.htm)*
3) *National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/)*
4) *UpToDate Online version 12.2 (2004)*

## RELEVANT EVIDENCE/REFERENCES


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METHODS

*Patients*

Ambulatory patients from the preoperative consultation were screened between March and June 2013. Nasal screening samples ($n$ = 1,200) were collected with the liquid Amies E-swab® (Copan, Brescia, Italy). Each sample was assigned an Excel-generated randomization code.

*Media and inoculation*

E-swab tubes were vortexed, and swabs were used to inoculate both ChromID *S.aureus* (SAID) and ChromID MRSA sections of the chromogenic biplate. The order in which samples were inoculated on the two sections was random. Mannitol salt agar (MSA, homemade), a selective growth medium for *Staphylococcus species*, was used as the gold standard medium. A broth enrichment method was not used in this study. All plates were read after 16-24 h (referred to as 24 h) of incubation at 37°C in ambient air and again after a further overnight incubation equivalent to 42-48 h (referred to as 48 h of incubation) according to the manufacturer's instructions.

*Detection of S. aureus*

On the SAID section the colony appearances considered suspicious were white with a green shine, pale blue-green, blue-green to green after 18-24 hours of incubation and blue-green or green after 42-48 hours of incubation. On the MRSA section the colonies considered suspicious were pale-green or green after 18-24 hours of incubation and green or dark-green after 42-48 hours.Yellow-pigmented colonies on MSA medium were regarded as presumptive *S. aureus* strains.

*Confirmatory identification and antimicrobial susceptibility testing*

Confirmatory identification of *S. aureus* from the evaluated media and/or MSA was performed by Matrix Assisted Laser DesorptionIonization – Time Of Flight Mass Spectrometer (MALDI-TOF MS) (Bruker Daltonics). Susceptibility testing to confirm or to exclude the oxacillin resistance was performed on *S. aureus* strains grown on the biplate or MSA agar. At first, disk diffusion test was performed on Mueller-Hinton agar (MHA) with a 30µg disk of cefoxitin (Neo- Sensitabs™, Rosco). A bacterial suspension equivalent to a 0.5 McFarland standard was prepared and thoroughly streaked onto MHA with a sterile cotton swab. A cefoxitin disk was placed on the inoculated MHA after which, the plate was incubated at 35°C in ambient air for 18-24 h. The breakpoints for MRSA were interpreted as defined by the Clinical and Laboratory Standards Institute (CLSI): $\geq$ 22 mm -oxacillin sensitive; $\leq$ 21 mm- oxacillin resistant.

Secondly, the presence of penicillin-binding protein 2α was assessed with a latex agglutination test (Alere™, PBP2α Culture Colony Test, USA). Testing was performed according to the manufacturer's instructions on the colonies with a cefoxitin susceptibility diameter of $\leq$ 21 mm. If both requirements were met, namely, positive agglutination and resistance to cefoxitin, the species was considered MRSA.

All confirmed MRSA isolates were additionally tested for the presence of the *mec*A gene by means of real-time PCR (GeneXpert®, Cepheid) directly on the sample (Amies medium of E-swab).

*Interpretative criteria*

The performance of the ChromID S.aureus and ChomID MRSA compartments of the biplate was evaluated against gold standards. For *S.aureus* compartment, the gold standard was defined as MALDI-TOF identification of *S.aureus* from the MSA and/or ChromID S.aureus compartment after 48 h of incubation. For MRSA, the gold standard was defined as MALDI-TOF identification of *S.aureus* on MSA and/or ChromID MRSA compartment with a positive PBP2α Culture Colony Test and oxacillin resistance after 48 h of incubation.

*Molecular typing*

Molecular typing of all MSRA stains was performed at the National Reference Laboratory for *S.aureus* by *spa*-typing as previously described.[32] The *spa* types were clustered into Clonal Complexes (*spa*-CC) with Based Upon repeat (BURP) algorithm using default parameters. Multilocus sequence types (MLST) were determined using the "permutuation nomenclature", based on *spa* typing results, as previously described.[32] SCC*mec* typing was performed using multiplex PCR for *ccr* and *mec* gene complexes.[32]

*Statistical analysis*

Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) were calculated using Microsoft Excel 2010.

RESULTS

A total of 1,200 nasal screening samples from 1,200 ambulatory preoperative patients were collected in our hospital between March and June 2013.

From the 1,200 surveillance specimens, 311 (25.9%) *S.aureus* isolates were recovered; 14 (1.2%) were MRSA. For both sections of ChromID MRSA/ ChromID S.aureus medium sensitivity, specificity, PPV, and NPV was calculated at 24 h and 48 h. (Table 1)

**Table 1. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ChromID S.aureus and ChromID MRSA sections.**

| Medium | Incubation time | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| ChromID MRSA | 24 h | 57.1 | 100 | 100 | 99.5 |
| | 48 h | 92.9 | 99.7 | 81.3 | 99.9 |
| SAID | 24 h | 66.2 | 99.0 | 95.8 | 89.3 |
| | 48 h | 94.2 | 93.6 | 83.7 | 97.9 |

Following confirmation of the organism's identification as *S. aureus* and its susceptibility to oxacillin, the sensitivities of ChromID MRSA section at 24 h and 48 h of incubation were 57.1% and 92.9%, respectively. Specificities of ChromID MRSA at 24 and 48 h were very high: 100% and 99.7%, respectively.

For the ChromID S.aureus section, the sensitivities at 24 h and 48 h of incubation were 66.2% and 94.2%, respectively. The specificity of this section was high at 24 h of incubation (99.0%) with a slight decrease to 93.6% at 48 h of incubation. (Table 1)

Micro-organisms producing false positive reactions on the evaluated media are shown in Table 2.

**Table 2. Micro-organisms producing false positive reactions on SAID and Chrom ID MRSA after 48 h of incubation.**

| Organism | No of strains isolated | |
|---|---|---|
| | SAID | Chrom ID MRSA |
| *Micrococcus luteus* | 23 | |
| Coagulase-negative staphylococci | 20 | |
| *Rothia mucilaginosa* | 4 | |
| *Bacillus sp.* | 2 | 2 |
| *Aerococcus viridans* | 1 | |
| *Listeria monocytogenes* | 1 | |
| *Enterococcus faecalis* | | 1 |
| **Total** | 51 | 3 |

For all confirmed MRSA strains (14) there were no discrepancies between the two susceptibility testing methods (cefoxitin, latex agglutination test) and real-time PCR.

In the national reference laboratory for *S. aureus* the 14 MRSA strains were confirmed with multiplex PCR for the detection of 16SrRNA, *mec*A and *nuc* genes. (Table 3)

**Table 3. Confirmation of MRSA: susceptibility testing, PBP2α latex agglutination test, and PCR of MRSA strains.**

| MRSA strain | Cefoxitin diameter (mm) | Oxacillin susceptibility | PBP2α latex agglutination test | Real-time PCR *mec*A (GeneXpert) | Multiplex PCR | | |
|---|---|---|---|---|---|---|---|
| | | | | | *mec*A | *nuc* | 16SrRNA |
| 0504-100 | 14 | R[a] | pos[b] | pos | pos | pos | pos |
| 1004-75 | 14 | R | pos | pos | pos | pos | pos |
| 1504-189 | 15 | R | pos | pos | pos | pos | pos |
| 1704-80 | 14 | R | pos | pos | pos | pos | pos |
| 2304-164 | 14 | R | pos | pos | pos | pos | pos |
| 2304-193 | 11 | R | pos | pos | pos | pos | pos |
| 2404-51 | 16 | R | pos | pos | pos | pos | pos |
| 1405-135 | 14 | R | pos | pos | pos | pos | pos |
| 2105-211 | 12 | R | pos | pos | pos | pos | pos |
| 2705-160 | 14 | R | pos | pos | pos | pos | pos |
| 2905-165 | 14 | R | pos | pos | pos | pos | pos |
| 0306-168 | 13 | R | pos | pos | pos | pos | pos |
| 0506-120 | 14 | R | pos | pos | pos | pos | pos |
| 0306-187 | 12 | R | pos | pos | pos | pos | pos |

a resistant

b positive

Ten *spa* types were detected for 14 MRSA strains, clustering into 2 *spa* Clonal Complexes (CC) and 3 singletons (Table 4). Thirteen MRSA strains had a type IV (2B) SCC*mec* element, the remaining strain was assigned to SCC*mec* type V (5C2). Overall, MRSA were assigned to 6 different MRSA clones as defined by MLST typing (Table 4), the majority of which corresponded to ST45-IV (43%) and ST5-IV (29%). Two MRSA belonged to the livestock-associated MRSA lineage ST398.

**Table 4. Molecular typing of MRSA strains.**

| strain ID | age | sex | *spa* type | *Spa* CC [1] | SCC*mec* type | MRSA clone [2] |
|---|---|---|---|---|---|---|
| 2404-51 | 73 | W | t655 | CC740 | IV(2B) | ST45-IV |
| 0506-120 | 73 | W | t739 | CC740 | IV(2B) | ST45-IV |
| 1704-80 | 89 | W | t740 | CC740 | IV(2B) | ST45-IV |
| 0306-187 | 84 | W | t740 | CC740 | IV(2B) | ST45-IV |
| 1004-75 | 87 | W | t3836 | CC740 | IV(2B) | ST45-IV |
| 2105-211 | 51 | W | t10634 | CC740 | IV(2B) | ST45-IV |
| 2705-160 | 61 | M | t002 | NF1 | IV(2B) | ST5-IV |
| 0306-168 | 70 | M | t002 | NF1 | IV(2B) | ST5-IV |
| 2304-193 | 70 | M | t002 | NF1 | IV(2B) | ST5-IV |
| 1504-189 | 67 | W | t447 | NF1 | IV(2B) | ST5-IV |
| 0504-100 | 54 | M | t011 | Singleton 1 | IV(2B) | ST398-IV |
| 2905-165 | 35 | W | t011 | Singleton 1 | V(5C2) | ST398-V |
| 1405-135 | 55 | M | t021 | Singleton 2 | IV(2B) | ST30-IV |
| 2304-164 | 35 | M | t267 | Singleton 3 | IV(2B) | ST97-IV [3] |

1 *spa* CC, *spa* clonal complex; NF1, no founder 1.
2 Defined as combination of MLST clonal complex (CC) and SCC*mec* type. derived by using the permutuation nomenclature
ST97 by the Ridom Spa Server (http://www.spaserver.ridom.de/)

## DISCUSSION

*ChromID S.aureus (SAID) section*

In our study, the SAID section for the detection of MSSA performs moderate at 24 h of incubation with a sensitivity of 66.2%. The study of *Perry et al*. conducted on wound swabs reported, however, a better sensitivity of SAID plate after 24 h of incubation, namely 96.8%.[18] Of all *S.aureus* strains that were not recovered on SAID within 24 h of incubation, 60% didn't grow on MSA within this incubation period. The majority of these strains were isolated in small numbers after additional incubation (there were no more than 3 colonies) of in the mixture of other commensal flora, which probably accounted for the failure to detect *S.aureus* after 24 h of incubation. As we observed in this study, some experience is needed for the recognition of suspected colonies after 16-24 h of incubation. Some colonies were tiny white with a discrete green shine better observed against a white background. To note that the sensitivity after 24 h of incubation might be better, if those colonies were taken into account. The sensitivity of the SAID section, however, significantly improved at 48 h of incubation and reached 94.2%.

The specificity of SAID was very high at 24 h of incubation (99.0%) and decreased to 93.6% after 48 h of incubation. A high specificity of SAID was also reported in previous studies.[4,18] In our study, species responsible for false-positive results were mainly *Micrococcus luteus* or coagulase-negative staphylococci.

Other genera were also observed, namely *Rothia mucilaginosa, Bacillus sp., Aerococcus viridans, Listeria* and *Enterococcus faecalis*. The colonies of other genera distinctly differed from those of *S.aureus* by size or color with the exception of *S. intermeduis.* A few colonies of *S. intermeduis* were morphologically similar to those of *S.aureus*, but with MALDI-TOF, as used routinely in our laboratory, avoids misclassification of this species as true positive.

*ChromID MRSA section*

In our study, the ChromID MRSA section for MRSA detection in screening nasal samples of ambulatory population demonstrated a low sensitivity (57.1%) after 24 h of incubation.

Previous authors showed better sensitivity of ChromID MRSA after 24 h of incubation by using MRSA strains for the evaluation.[14,24] However, these evaluations do not reflect real-life conditions (high inocula yield, no potential inhibitory effect of the commensal flora). A higher sensitivity may be achieved by the inclusion of an enrichment step, although this method delays the reporting of results for an additional 24 h. [6,16,25] The inclusion of an

enrichment step for the detection of MRSA in the nose and throat specimens *only* is also recommended by the manufacturers.

A broth enrichment method was not included as part of our evaluation, which might have increased the yield of MRSA. It must be pointed out that studies performed on clinical screening samples without enrichment broth showed a sensitivity similar to ours after 24 h of incubation ranging from 51% to 67.9%.[6,16,17,22,25] Different mechanisms can influence the detection of MRSA, including low inocula in screening samples, overgrowth of commensal flora or insufficient homogenisation by vortexing.

The manufactures recommend an incubation period of 18-24 h and a reincubation for a further 24 h of all negatives. In the real-life laboratory setting, the incubation period of culture plates can be shorter than 22-24 h, even < 18 h. The shorter incubation time (<22 h), can decrease the recovery rate and the sensitivity, as shown in some previous studies. [6,15] In other study, however, no differences in MRSA recovery were observed after incubation for 22 h compared with the reading after 16-18 h.[16]

ChromID MRSA performed better after 48 hours of incubation with a higher sensitivity: 92.9% versus 57.1% at 24 hours. The sensitivity of the ChromID MRSA at 48 hours in our study is in agreement with those of previous studies using screening swabs without enrichment broth. [6,16,22,25]

The specificity of ChromID MRSA at 24-h read time was excellent (100%). That allows reporting green colonies from nose specimens as MRSA after a 24-h read time without further confirmation as recommended by the manufacturers. The specificity at 48-h read time is slightly decreased (99.7%) due to an occurrence of false positive results, namely from *Bacillus sp*. and *Enterococcus faecalis*. This observation is in agreement with that of other investigators who compared different chromogenic media, including MRSA ID, for MRSA detection. [6,14,16,17,22,24,25]

*Molecular typing*

The defining feature of MRSA is the staphylococcal cassette chromosome *mec* (SCC*mec*), a mobile genetic element that carries the central determinant for broad-spectrum beta-lactam resistance encoded by the *mec*A gene. It is now common practice to define MRSA clones by the combination of SCC*mec* type and the chromosomal background in which SCC*mec* resides. (http://www.staphylococcus.net; http://www.SCCmec.org)

Our study showed a genotypic heterogeneity of MRSA strains in an ambulatory population: 10 unique *spa* types were detected in 14 MRSA strains. Genotypic heterogeneity of MRSA strains in an outpatient population of nine European countries was recently shown by *den Heijer et al* [9], suggesting low spread of MRSA in the community. Strains carrying SCC*mec* type IV, previously thought to be a marker of CA-MRSA, represent the largest group in our study (92.9%). According to data of the national reference laboratory SCC*mec* type IV is predominant in Belgium, namely 52.1% of all MRSA stains in 2011. (http//www.mrsa.be)

The main MRSA clones detected in our study were ST45-IV (42.9%) and ST5-IV (28.6%). Two Belgian national surveys showed that more than 90% MRSA were classified into five clones.[32] The majority of MRSA clones found in our study belong to the predominant clones as shown by previous national surveys.

Two clones (ST398-IV, ST398-V) are typically associated with livestock animals and the persons in contact with them (no data available on their potential contact with livestock).

The proportion of LA-MRSA in ambulatory patients found in our study (14%) is much higher than the one found in hospitalized patients (0.6%) in the last national survey.[32]

CONCLUSION

ChromID MRSA/ ChromID S.aureus biplate (BioMérieux) permits a reliable detection of both MRSA and MSSA in nasal screening samples after 48 h of incubation without an enrichment step. The sensitivities for both chromogenic sections were substantially higher (28-36%) after 48 h of incubation. Our study confirms that extension of the incubation time to 48 h enhances the sensitivity of MRSA/MSSA detection at the expense of a decreased specificity. However, ChromID MRSA remains to have an excellent specificity of 99.7% at 48 h.
Our study demonstrated a low MRSA proportion (1.2%) and genotypic heterogeneity of MRSA strains in an outpatient population. Our results also confirm that the majority of MRSA strains in the community belong to a restricted number of predominant clones.

## TO DO/ACTIONS

1) To think about the possibility of the implementation the Chrom ID MRSA/ Chrom ID S.aureus biplate (BioMérieux) for simultaneous MRSA/MSSA detection in nasal screening samples of patients undergoing breast surgery.

2) Alternatively: " treat all strategy" with decontamination of preoperative patients.