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Chapter 4.2

Comparison of two PCR-based methods and conventional culture for the detection of nasal carriage of *Staphylococcus aureus* in pre-operative patients

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Abstract

Nasal carriage is an important risk factor for the development of post-operative infections with *Staphylococcus aureus* and pre-operative treatment with mupirocin in carriers reduces the post-operative infection rate. Therefore, it is important to identify nasal carriage rapidly. Two polymerase chain reaction (PCR) techniques were compared to conventional culture in surgical patients. In 404 consecutive patients, nasal swabs were taken for pre-operative screening for the nasal carriage of *S. aureus*. The performance of the Roche Staphylococcus Kit on Lightcycler (Roche; RSA) and the Becton Dickinson (San Diego, CA) GeneOhm StaphSR assay on Smartcycler (Cepheid; BDSA) were compared with semi-quantitative culture. The sensitivity for culture, RSA and BDSA compared to the gold standard was 98.2, 82.0 and 85.6%, respectively, and the specificity was 100, 98.3 and 99.3%, respectively. The lower sensitivity of both PCR techniques was associated with samples with low bacterial loads. The RSA and BDSA were similar in performance and are suitable for the pre-operative identification of nasal carriers of *S. aureus*.

Background

Staphylococcus aureus is the leading causative micro-organism of post-operative infections. Post-operative wound infections are associated with increased morbidity, mortality and length of hospital stay. In 2003, the annual economic burden of *S. aureus* infections in surgical patients was estimated at \$12.3 billion.¹ The nasal carriage of *S. aureus* is an important risk factor for the development of infections in surgical patients (CDC Guidelines and Review 1996). Approximately 30% of all people are nasal carriers of *S. aureus*. In patients with a post-operative wound infection caused by *S. aureus*, the strain cultured from the wound is mostly identical to the patients' nasal strain.² Since the 1960s, numerous reports have appeared that focused on antimicrobial therapy for the nasal carriage of *S. aureus* and preventing post-operative infections. In most studies, mupirocin ointment was used to eradicate *S. aureus* from the nose. A meta-analysis showed that perioperative treatment of proven carriers with mupirocin resulted in a significant reduction of post-operative infections with *S. aureus*.³ In patients that do not carry *S. aureus*, no effect was found.

In the Amphibia hospital, both patients undergoing thoracotomy and patients undergoing orthopaedic surgery are screened pre-operatively for nasal carriage of *S. aureus*. Patients identified as a nasal carrier of *S. aureus* are offered intranasal mupirocin treatment pre-operatively, aiming to reduce the risk of developing a post-operative wound infection. Until recently, conventional culture of nasal swabs were used to identify *S. aureus* carriers. However, conventional culture takes at least 18-24 hr to produce preliminary results. For logistic reasons, a faster test is preferable, since the treatment of *S. aureus* carriers is started the day before surgery. Recent developments have led to improved and easy-to-handle polymerase chain reaction (PCR) techniques that enable the detection of *S. aureus* within 2 h. Currently available techniques include the Roche *Staphylococcus* Kit on Lightcycler (Roche; RSA) and the Becton Dickinson (San Diego, CA) GeneOhm StaphSR assay on Smartcycler (Cepheid; BDSA).

In this comparative prospective study, we tested these PCR-based methods (RSA and BDSA) against conventional culture and evaluated their applicability in daily practice.

Methods

During the months November 2007 to January 2008, all patients scheduled for cardiothoracic or orthopaedic surgery were included in the study. On the day of admittance to the hospital, two nasal swabs were taken: one conventional and one extra double-headed swab. The order in which the two samples were taken was left to chance. A double-headed swab (BBL Culture Swab, Becton Dickinson, San Diego, CA, USA) was

used in order to minimise the number of swabs taken from the patient: it consists of a double conventional swab that allows for a single swabbing action, but can be separated into two conventional swabs. The conventional single-headed swab (BBL Culture Swab, Becton Dickinson, San Diego, CA, USA) was tested with the Roche Staphylococcus Kit on Lightcycler 2.0 (Roche, Basel, Switzerland; RSA). After dividing both heads, one head of the double-headed swab was tested with the Becton Dickinson (San Diego, CA, USA) GeneOhm StaphSR assay on Smartcycler (Cepheid, Sunnyvale, CA, USA; BDSA) and the other head was used for semi-quantitative culture. All swabs were processed within 4 h after sampling.

Semi-quantitative culture was performed by preparing two bacterial suspensions in broth enrichment medium (Mueller Hinton with 6.5% NaCl [MH broth]): suspension I by vigorously vortexing the swab in 2.5 ml of MH broth and suspension II by inoculating 50 µl of suspension I in 5.0 ml MH broth. From both suspensions, 250 µl was inoculated on a selective *S. aureus* agar plate (SSA, BioRad, Hercules, CA, USA), respectively plate A and B. On this chromogenic SSA plate, *S. aureus* appears as pink colonies. Both the SSA plates and suspension I were incubated for 18–24 h at 36 °C (5% CO₂). After 24 h, the SSA plates were inspected for colonies suspected of *S. aureus* and 10 µl of suspension I was subcultured on an SSA (plate C) and incubated for 18–24 h at 36 °C (5% CO₂). On inspection of the SSA plates (both direct and from the pre-incubated suspension I), all colonies suspected of *S. aureus* (pink colonies) were tested with a tube coagulase test (coagulase plasma, Becton Dickinson, San Diego, CA, USA). *S. aureus* strains were defined by pink colonies with a positive coagulase test. The number of colonies on the SSA plates was converted into a semi-quantitative measure according to table 1.

The other half of the double-headed swab was used for analysis with the BDSA. Following the manufacturer's guidance on adapted extraction, the swab was incubated

Table 1. Method of the semi-quantitative culture.

Growth on the highest ranking plate	Number of colonies on SSA plates	Estimated number of CFU in original swab	Semi-quantity
No growth on all plates	NA	0	Neg
Growth on plate C only	Any number	1-10	0
Plate A	1-10 colonies	10-100	1
	11-100 colonies	100-1000	2
	>100 colonies	>1000	3
Plate B	1-10 colonies	1000-10.000	4
	11-100 colonies	10.000-100.000	5
	>100 colonies	>100.000	6

NA=not applicable, CFU=colony forming units

Two bacterial suspensions in broth enrichment medium were made: suspension I by vigorously vortexing the swab in 2.5 ml MH-broth and a subsequent 1:100 dilution resulting in suspension II. From both suspensions 250 µl was inoculated on a selective *S. aureus* agar plate (SSA), respectively plate A and B. After 24 hours incubation suspension I was subcultured on a SSA plate (Plate C).

in 200 µl of achromopeptidase lysis solution (15 min at 37°C, followed by 5 min at 99°C).⁴ Subsequently, the lysate was cooled to 4°C, and 3 µl of the lysate was tested with the BDSA using a Smartcycler instrument (Cepheid Innovation). The BDSA on the Smartcycler allows for random access processing of a low number of samples (maximum of 16 per machine) by inserting a completely closed cartridge. The decisional algorithm for the BDSA is embedded in the Smartcycler software and automatically produces the results of the test: 'reactive' (*S. aureus* present), 'positive' (meticillin-resistant *S. aureus* [MRSA] present), 'negative' or 'unresolved'. The test can differentiate between meticillin-susceptible *S. aureus* (MSSA) and MRSA and shows this on the monitor by the text 'positive' or 'POS,' respectively. The test result is 'unresolved' in case of inhibitory specimen or reagent failure.

The conventional (single) swab was analysed with RSA. The swab was soaked with 100 µl of 0.9% NaCl and filtrated through a Roche S.E.T.S. II inner tube. Thirty microlitres of the filtrate was mixed with 100 µl of S.E.T.S. II neutralisation buffer and beads. After incubation (2 min at 95°C), the mixture was shaken in a MagNA Lyser (Roche, Basel, Switzerland) for 30 s and centrifuged. Five microlitres of the supernatant was run in the RSA using the Lightcycler 2.0 real-time PCR instrument (Roche, Basel, Switzerland) according to the manufacturer's instructions. The Lightcycler allows for higher volume throughput (maximum of 32 tests per run) and is a batch-based PCR technique. First, staphylococcus DNA is amplified with species-specific primers derived from the ITS regions, resulting in an amplicon independent from any staphylococcal subspecies. Subsequently, two probes hybridise to the amplicon, resulting in fluorescence resonance energy transfer (FRET). Finally, the result interpretation in positive samples is based on melting curve analysis: *S. aureus*- positive samples are differentiated from samples with coagulase-negative Staphylococcus species by demonstrating a unique melting peak at about 62°C in samples containing *S. aureus* DNA. Samples showing no Staphylococcus DNA amplification were either interpreted as 'negative' or 'invalid' (e.g. inhibition of amplification), depending on the internal control being positive or negative, respectively.

The gold standard was defined as follows: (1) a positive culture result was considered to be a true-positive patient; (2) if the culture result was negative and both molecular techniques were positive, the patient was also considered to be a true-positive; (3) a negative culture result in combination with one negative PCR result was considered to be a true-negative patient (with one false-positive PCR). If the PCR gave an indeterminate result, this would be interpreted as a negative result. All results were archived in a single datasheet and statistically analysed using SPSS (version 15.0, SPSS Inc.).

Results

In total, 404 consecutive patients were tested. In 111 (27.5%) patients, the carriage of *S. aureus* was detected according to the gold standard. In two cases, a negative culture was found in combination with both PCR results being positive (two false-negative cultures). Figure 1 displays the semi-quantitative culture results of the true- positive patients and the spread of RSA- and BDSA- positive cases. In eight cases, both PCR methods gave a negative result in combination with a positive culture (double false-negative PCR); in seven of these cases, the semi-quantitative measure was 1–10 colony-forming units (CFU) and in one case 10–100 CFU. In eight cases, the BDSA tested negative, while the RSA and culture both established a positive result. In seven cases, this was associated with a count of 10–100 CFU or lower. Similarly, the RSA was negative in 12 cases, while the BDSA and the culture gave a positive result. In ten cases, this was found in combination with a count of 10–100 CFU or lower. Both the RSA and the BDSA gave an 'indeterminate' result in one case, and in both cases, this was associated with a true- negative patient. In cases with a low bacterial carriage, the conventional culture with broth enrichment seems to be more sensitive than the molecular-based techniques.

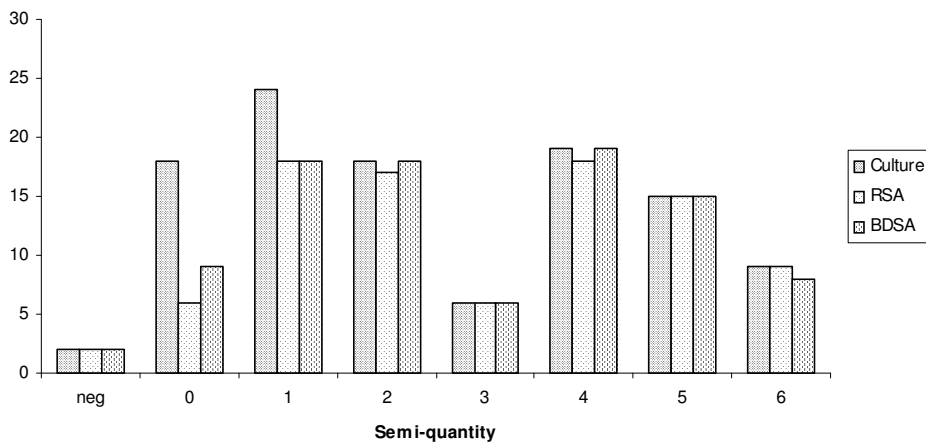


Figure 1. Semi-quantitative culture results (see Table 1) of the true-positive patients (n=111) and spread of RSA- and BDSA-positive cases.

Table 2 shows the overall results and sensitivity/specificity for all tests in comparison to the gold standard. Compared to the gold standard, both PCR tests gave a similar result. In samples with more than 100 CFU, the sensitivity of both tests was very high (97.0% for RSA and 98.5% for BDSA). In two cases, both molecular tests were positive while culture grew no *S. aureus*, and these were considered to be false-negative culture results.

Table 2. Overall results and sensitivity/specificity in comparison to the gold standard.

	Gold Standard	Culture	RSA	BDSA
Test result				
Negative	293	295	307	306
Positive	111 (27.5 %)	109 (27.0 %)	96 (23.8 %)	97 (24.0 %)
Undetermined		0	1	1
Sensitivity (95% CI)		98.2 % (93.0-99.7)	82.0 % (73.3-88.4)	85.6 % (77.3-91.3)
Specificity (95% CI)		100%*	98.3 % (95.8-99.4)	99.3 % (97.3-99.9)
PPV (95% CI)		100 % (95.8-100)	94.8 % (87.7-98.1)	97.9 % (92.0-99.6)
NPV (95% CI)		99.3 % (97.3-99.9)	93.5 % (90.0-95.9)	94.8 % (91.5-96.9)

CI=confidence interval, PPV=Positive predictive value, NPV=Negative predictive value, NS=not significant compared to gold standard, *specificity was 100% by definition.

Discussion

In this study, both the RSA and BDSA demonstrated similar sensitivity and specificity in comparison to the gold standard. Both techniques showed a relatively low sensitivity but excellent specificity. The lower sensitivity was associated with very low CFU counts; in most false-negative PCR results, only the broth enrichment medium gave a positive result, indicating a very low bacterial load in the original swab. It can be debated whether very low grade carriage is a risk factor for infection. Several reports have demonstrated that the risk of infection in carriers is limited to carriers with a high microbial load of *S. aureus*.⁵⁻⁷ If, indeed, low grade carriage is of no clinical significance and samples with low bacterial counts (less than 100 CFU) are considered as negative, the sensitivity of both tests increases remarkably from 82.0 and 85.6% to

97.0 and 98.5% in the RSA and BDSA, respectively. For the indication of pre-operative screening, the test characteristics are adequate.

The BDSA, being a random access technique, offers the advantage of direct handling of samples when they enter the laboratory. However, it provides relatively low capacity, since the maximum number of tests that can be run simultaneously is 16. By contrast, the RSA in combination with the Lightcycler enables the testing of higher numbers of samples (maximum of 32), but it is not random access. The BDSA has the advantages of random access, less hands-on time, easier handling, standardised interpretation and the testing of MRSA simultaneously.

In conclusion, both the RSA and the BDSA are suitable tests for the pre-operative screening of *S. aureus* in nasal swabs. They provide results in approximately 2 h. The decision for which tests to use will mainly be determined by the way the test fits into the workflow of the laboratory.

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