

## Evaluation of methods for the detection of nasal carriage of *Staphylococcus aureus*

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In the present study we investigate the optimal methodology for determination of the nasal carriage rate of *Staphylococcus aureus*. Tests were performed on 91 healthy laboratory staff. The reproducibility of different sampling, transportation, storage and culture methods was examined. We compared sterile dry cotton wool swabs with sterile dry cotton wool swabs impregnated with charcoal and 5% blood agar plates with mannitol salt agar plates after different incubation periods. Finally, we investigated the detection rate for *S. aureus* following direct plating compared to storage in Stuart's transport medium for 7 days. There were no differences in isolation rates from the right or left nostril using either cotton or charcoal swabs. Charcoal swabs gave an increased isolation rate as compared to cotton swabs, and incubation in broth enrichment medium containing 6.5% NaCl also increased the isolation rate. Storage in Stuart's transport medium for 7 days gave an increase in isolation rate as compared to direct plating on blood agar. With mannitol salt agar plates the increase in isolation rate when incubation was performed for from 2 to 4, 2 to 7, and 4 to 7 days was 5.9%, 16.7%, and 11.5%, respectively. For the detection of *S. aureus* nasal carriers we find the use of charcoal swabs and Stuart's transport medium combined with cultivation on mannitol salt agar for 7 days to be the optimal method.

Key words: *Staphylococcus aureus*; nasal carriage; detection; evaluation of methods.

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Investigations performed 30 to 50 years ago demonstrated that shortly after hospitalization many patients became colonized with *S. aureus*, and carrier rates of from 40% to around 90% were reported (11, 18). The colonizing strains were often "hospital staphylococci", differing in phage type pattern and antibiotic resistance from the strains found outside the hospital (15). Present investigations in Denmark have shown

that during recent years the strains causing infections in hospitalized patients are similar to the strains isolated from patients with infections outside the hospital with respect to phage type and antibiotic resistance (15). This might indicate that today in the majority of hospital-acquired infections the patients are infected with their own strain. There are, however, no recent studies illustrating whether the colonization rate and/or the colonizing strains differ in patients-staff inside and outside the hospital.

In order to study *S. aureus* carrier rates it is necessary to use a well-documented method and the present investigation was undertaken to

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identify optimal methods for the identification of nasal carriage of *S. aureus*.

## MATERIALS AND METHODS

The healthy normal subjects who consented to take part in this study comprised 77 female and 14 male staff members. Median age was 43 years (range 21-67 years). Nasal samples were collected by the same person over a 2 month period; a maximum of 16 samples were obtained from most of the staff members.

### *Swabs and swabbing technique*

Samples were obtained from the right or left anterior nares using sterile dry cotton wool swabs (Dansu A/S, Ganløse, Denmark) or sterile dry cotton wool swabs impregnated with charcoal (prepared from dry cotton wool swabs by boiling for 10 min in NaCl buffer, pH 7.38, and containing 5.2 mg active coal per swab (Sigma, St. Louis, USA)). The entire anterior nares was swabbed by twice rubbing around the inside of the nostril while applying an even pressure and rotating the swab without interruption.

### *Media*

Stuart's transport medium was prepared according to *Stuart et al.* except that the agar concentration was higher than the 0.3% Bacto agar used in their study (10). Broth enrichment culture medium with 6.5% NaCl: beef extract 400 g, glucose 0.3 g, NaCl 65 g, Na<sub>2</sub>HPO<sub>4</sub> 2.0 g, peptone 10.0 g, distilled water 1.0 l, final pH 7.4. Mannitol salt agar was prepared as described by *Chapman* (5). 5% horse blood agar: MgSO<sub>4</sub> 0.1 g, MnCl<sub>2</sub> 0.0067 g, Na<sub>2</sub>HPO<sub>4</sub> 8.0 g, casein hydrolysate 5.0 g, yeast extract 3.0 g, peptone 5.0 g, KCl 6.67 g, detergent 0.01 g, agar 10 g, horse blood 50 ml, cysteine HCl 0.05 g, sodium pyruvate 2.0 g, distilled water 1 l, final pH 7.4, dissolved and autoclaved at 121°C for 10 min. Agar plates: distilled water 1 l, beef extract 400 g, peptone 10 g, NaCl 3 g, Na<sub>2</sub>HPO<sub>4</sub> 2 g, fatty acid 0.01 g, glucose 0.3 g, agar 10 g, final pH 7.4, dissolved and autoclaved at 121°C for 15 min.

### *Culture method*

Swabs were either directly inoculated onto 5% blood agar or mannitol salt agar, or placed in broth enrichment medium with 6.5% NaCl or Stuart's transport medium. Broth enrichment medium was incubated at 37°C for 18 h, and Stuart's transport medium was stored at 4°C for 7 days before inoculation onto solid medium was performed. The plates were incubated at 37°C overnight, and then at room temperature (to stimulate pigment formation) for up to 6 days.

### *Identification of S. aureus*

Colonies were isolated and checked by Gram staining. All Gram-positive organisms were tested for catalase production after pure cultivation on agar plates, and colonies suspected by colony morphology of being staphylococci were tested for coagulase production using the citrate-plasma tube technique.

### *Design*

A pilot experiment with 10 consecutive swabs from both nostrils of three well-known carriers was performed to evaluate the effect of continuous swabbing on the bacterial contents of the nostrils.

A series of experiments were subsequently performed to evaluate a) isolation rates from the two nostrils, b) comparison of cotton and charcoal swabs, c) recovery using Stuart's transport medium, d) use of broth enrichment medium with NaCl, e) comparison of 5% blood agar with mannitol salt agar, and f) comparison of mannitol salt agar at different times of incubation. When comparing different swabs or swabs from different nostrils, the order of sampling was changed in half the cases, e.g. swabbing from the right nostril using charcoal followed by cotton, and the reverse order for the left nostril. In each experiment swabs from all persons available on that day were collected without any other selection criteria being applied.

### *Statistical analysis*

Because the data were not mutually independent we used the two-tailed McNemar test.

## RESULTS

### *Consecutive swabs from well-known carriers*

By taking 10 consecutive swabs from three well-known carriers it was shown that *S. aureus* could be detected from all 10 both using cotton and charcoal swabs (data not shown).

### *Comparison of isolation rates from right and/or left nostril using cotton or charcoal swabs*

Using cotton swabs *S. aureus* was isolated from both the right and left nostril of 14/70 subjects (20%), from only the right nostril of 2/70 (2.9%), and from only the left nostril of 1/70 (1.4%) ( $P=1.00$ ). Using charcoal swabs *S. aureus* was isolated from both the right and left nostril of 22/76 subjects (28.9%), from only the right nostril of 2/76 (2.6%), and from only the left nostril of 3/76 (3.9%) ( $P=1.00$ ). There were no significant differences in isolation rates from the right or left nostril whether cotton or charcoal swabs were used.

This was also the case if the data from the two experiments were pooled (4 vs 4;  $p=0.72$ ). The increased isolation rates when taking swabs from both nostrils were only 3 of 70 (4.3%) with cotton swabs, and 5 of 76 (6.6%) using charcoal. The order of swabbing did not influence the results (data not shown).

#### Comparison of isolation rates using cotton or charcoal swabs

In the right nostril *S. aureus* was isolated from both cotton and charcoal swabs in 10/59 subjects (16.9%), with only cotton in 2/59 (3.4%), and with only charcoal in 9/59 (15.3%) ( $P=0.07$ ). In the left nostril *S. aureus* was isolated from both cotton and charcoal swabs in 11/59 subjects (18.6%), with only cotton in 1/59 (1.7%), and with only charcoal in 8/59 (13.6%) ( $P=0.11$ ). Charcoal swabs gave a non-significantly increased isolation rate both from the right and left nostril as compared with cotton swabs (9 vs 2 and 8 vs 1, respectively). However, if data from the two experiments were pooled, charcoal was significantly better than cotton (17 vs 3;  $p<0.01$ ). Thus, the isolation rates were 19/59 (32.2%) and 19/59 (32.2%) for charcoal swabs as compared to 12/59 (20.3%) and 12/59 (20.3%) for cotton swabs. The order of swabbing did not influence the results (data not shown).

#### Comparison of direct plating with storage in Stuart's transport medium

Using cotton swabs *S. aureus* was isolated on 5% blood agar both by direct plating and after storage in Stuart's transport medium in 15/70 subjects (21.4%), by direct plating in 0/70, and only from Stuart's medium in 5/70 (7.1%) ( $P=0.07$ ). Using charcoal swabs *S. aureus* was isolated on 5% blood agar both by direct plating and after storage in Stuart's transport medium for 7 days in 24/76 subjects (31.6%), by direct plating in 0/76, and only from Stuart's medium in 5/76 (6.6%) ( $P=0.07$ ). Storage in Stuart's transport medium for 7 days in both experiments gave a non-significant increase in the isolation rate as compared to direct plating (5 vs 0 with both swabs). If the results from the two experiments were pooled, an increased isolation rate was obtained using Stuart's transport medium (10 vs 0,  $p=0.01$ ). For charcoal swabs the iso-

lation rate was then 29/76 (38.2%) after storage as compared to 24/76 (31.6%) without storage. For cotton swabs the isolation rate was 20/70 (28.6%) as compared to 15/70 (21.4%).

#### Comparison of direct plating using broth enrichment medium with NaCl

An increased isolation rate was obtained if the cotton swabs were first incubated in broth enrichment medium with NaCl before plating (9 vs 0; Table 1); while the use of broth enrichment medium with NaCl and charcoal swabs gave only a minor non-significantly increased isolation rate as compared with direct plating (3 vs 0; Table 1). The pooled data from both experiments showed an increased isolation rate when using NaCl (12 vs 0;  $P=0.01$ ).

#### Isolation rates for *S. aureus* grown on 5% blood agar plates for 2 days or mannitol salt agar plates for 2 days

Using charcoal swabs *S. aureus* was isolated from the right nostril both by direct plating on 5% blood agar and on mannitol salt agar in 11/59 subjects (11.5%), by only direct plating on 5% blood in 2/59 (2.1%), and only on mannitol salt agar in 1/59 ( $P=1.00$ ). This indicates that after cultivation for 2 days there are no major differences in isolation rates for the two media. When incubation on 5% blood agar plates was extended for more than 2 days the plates showed heavy overgrowth, and therefore the results of further incubation were investigated only for the selective mannitol salt agar medium.

TABLE 1. Number of samples with (+) or without (-) growth of *S. aureus* by direct cultivation or inoculation in broth enrichment medium with NaCl using either cotton swabs or charcoal swabs. Swabs were cultivated on 5% blood agar for 2 days.

		Cotton (n=59)		Charcoal (n=59)	
		NaCl		NaCl	
		+	-	+	-
Direct cultivation	+	7	0	19	0
	-	9 <sup>a</sup>	43	3 <sup>b</sup>	37

<sup>a</sup> Increased isolation rate after NaCl ( $p<0.01$ )

<sup>b</sup>  $p=0.25$ ; NaCl vs direct cultivation.

TABLE 2. Number of samples with (+) or without (-) growth of *S. aureus* using charcoal and direct cultivation on mannitol salt agar plates at different days of incubation. Day 2 vs day 4 ( $n=85$ ). Day 2 or day 4 vs day 7 ( $n=96$ ).

		4 days		7 days	
		+	-	+	-
2 days	+	16	0	16	0
	-	5 <sup>a</sup>	64	16 <sup>b</sup>	64
4 days	+		21	0	
	-			11 <sup>c</sup>	64

<sup>a</sup>  $p=0.07$ ; mannitol salt agar day 2 vs mannitol salt agar day 4

<sup>b</sup>  $p<0.001$ ; mannitol salt agar day 2 vs mannitol salt agar day 7

<sup>c</sup>  $p<0.001$ ; mannitol salt agar day 4 vs mannitol salt agar day 7.

#### Comparison of incubation on mannitol salt agar plates for 2, 4, and 7 days

In Table 2 two experiments are shown where the isolation rate is compared 2 and 4 days after incubation on mannitol salt agar, and incubation after both 2 and 4 days is compared with 7 days of incubation. Incubation for 4 days showed an increased isolation rate (5 vs 0; Table 2) as compared to for 2 days. Thus the increase in isolation rate was 5.9% (5/85) when incubation from 2 to 4 days was performed. When 2 or 4 days was compared to 7 days of incubation, there was in both cases a significantly increased isolation rate for 7 days of incubation (16 vs 0 or 11 vs 0; Table 2). The isolation rate increased by 16.7% (16/96) between 2 and 7 days of incubation, and 11.5% (11/96) between 4 and 7 days of incubation (Table 2, 3).

Among the 91 subjects we detected 145 *S. aureus* strains during the period (unpublished results).

## DISCUSSION

An important consideration in studies like the present is whether it is possible to take more than one swab from the nose without the first swab having a major impact on the results of additional swabbing. We found that successive swabbing up to 10 times from the same nostril did not lower the detection rate of *S. aureus*. These findings are in accordance with those of Solberg (19) who performed five consecutive (immediately after each other) nasal cultures from 10 permanent carriers. Within wide limits the results obtained were representative of the number of *S. aureus* in the vestibulum nasi (19). Also, we did not find any difference between isolation rates when a given swab was used either first or second. In the present study there were no differences in isolation rates from the two nostrils, and by means of cultivation from both nostrils it is only possible to achieve a marginal increase in isolation rate (Table 3).

By comparing cotton and charcoal swabs and direct cultivation on 5% blood agar plates for 2 days we found a higher isolation rate when we used charcoal swabs instead of cotton swabs. Generally, wooden applicators covered by cotton wool are used to collect material for biological examination. However, certain wood materials are known to be toxic to some bacteria,

TABLE 3. Summary of differences in *S. aureus* isolation rates using different procedures

Procedure 1	vs	Procedure 2	Increase in isolation rates by procedure 2
Right nostril		Left nostril	None
One nostril		Both nostrils	4.3% <sup>a</sup> -6.6% <sup>b</sup>
Cotton swabs		Charcoal swabs	11.9%
Direct plating		Stuart's medium (7 days)	6.5% <sup>b</sup> -7.1% <sup>a</sup>
Direct plating		Broth enrichment medium	5.1% <sup>a</sup> -15.2% <sup>b</sup>
Blood agar (2 days)		Mannitol agar (2 days)	None
Mannitol agar (2 days)		Mannitol agar (4 days)	5.9%
Mannitol agar (2 days)		Mannitol agar (7 days)	16.7%
Mannitol agar (4 days)		Mannitol agar (7 days)	11.5%

<sup>a</sup> cotton swabs

<sup>b</sup> charcoal swabs.

especially the gonococcus (10). The higher isolation rates using charcoal swabs can be explained by the toxic effect of the swabs that can then be counteracted by boiling in buffer and impregnating in charcoal (10). Another reason could be the larger active surface of charcoal as compared to cotton swabs.

For both swabs the higher isolation rate using Stuart's transport medium may not be surprising as this medium contains glycerophosphate, which is a well-known nutrient factor for most bacteria. One explanation could be that some transfer of glycerophosphate from Stuart's transport medium to the solid media occurs with the charcoal swab, or that there is limited growth of *S. aureus* in Stuart's transport medium but not of the other bacteria normally found in the nostrils. Another explanation could be that other bacteria died while *S. aureus* survived, as it is more resistant to physical and chemical stress than are most other non-sporulating bacteria due to its solid cell wall.

Broth enrichment medium with NaCl almost completely inhibited the growth of Gram-negative rods, and also gave a significantly higher isolation rate with cotton swabs, but only a small non-significant increase with charcoal swabs. This method also possesses the advantage that the colonies are more clearly coloured and the production of coagulase is larger (4).

Most laboratories do not use broth enrichment medium for epidemiological surveillance (2). However, when surveying for methicillin-resistant *S. aureus* Cookson *et al.* (7) found that 62% of positive individuals and Sautter *et al.* (16) that 50% of positive individuals would not have been detected without the use of broth enrichment medium. Cookson used peptone water-moistened swabs.

The mannitol salt agar plates have been used and incubated for 5 days (11), but the further increase in the carrier rate with an increasing incubation time has not been described before. Using mannitol salt agar plates luxuriant *S. aureus* growth is seen, whereas coagulase-negative staphylococci exhibit poor growth. When there is a small inoculum of *S. aureus* in a mixed culture, this may have a pronounced effect on the detection rate. Further increased incubation at room temperature seems to have an effect on the chromogenesis, as differences between the porcelain-white staphylococci and the pig-

mented cultures are more pronounced on the mannitol salt agar plates, and these differences increase with increasing incubation period.

The importance of the higher detection rate with certain procedures in this study is at present unknown as it is uncertain whether a low number of colony-forming units of *S. aureus* is of any clinical significance.

It is, however, obvious that the procedure can considerably influence the number of *S. aureus* nasal carriers that will be detected (Table 3). A major increase in rates is obtained by the use of a charcoal swab instead of a cotton swab and by extension of the incubation period with mannitol salt plates, while the broth enrichment medium only gave a major increase when cotton swabs were used, and swabbing from both nostrils only gave a minor increase in rates (Table 3).

The method that should be used in a given situation necessarily depends on a range of parameters. First of all we need data on whether the amount of *S. aureus* in the nose correlates with the amount on the skin, the probability of spread to other persons, and the possibility of obtaining a positive culture with a given procedure. Recently there has been an increased interest in nasal carriage of *S. aureus* because the carriers act as a reservoir for methicillin-resistant *S. aureus* during outbreaks (3, 7), and also because the carrier state may be a target for prophylaxis (1, 6, 8, 14). In certain diseases or following certain operations nasal carriage may predispose to infections (9, 12, 13, 17, 21). It has furthermore been demonstrated that either oral rifampicin or nasal mupirocin lowered not only the carrier rate but also the infection rate of *S. aureus* in dialysis patients (1, 6). The finding that nasal application of mupirocin not only lowers nasal carriage rates but also hand carriage rates indicates that eradication of nasal *S. aureus* may in the future be of potential benefit in the prophylaxis of hospital-acquired infections (8, 14). The choice of procedure will, of course, also depend on the purpose of sampling, the cost benefits, and the speed with which an answer is required.

In conclusion, the procedure greatly influences the number of nasal carriers found in a given population, and the key points seem to be the swab material, the transport medium, the solid medium, and the incubation period.

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