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ARTICLE

The role of broth enrichment in *Staphylococcus aureus* cultivation and transmission from the throat to newborn infants: results from the Swedish hygiene intervention and transmission of *S. aureus* study

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Abstract *Staphylococcus aureus* is detected by direct plating, whereas incubation in enrichment broth prior to plating to increase the proportion of positive samples has not been fully evaluated. *S. aureus* throat colonization has been suggested to be more common than colonization of the anterior nares, but no data are available on the transmission of *S. aureus* from the throat. Swab samples were collected from the anterior nares and umbilicus from newborn infants (n=168), anterior nares, throat, skin lesions, and vagina from parents (n=332), and anterior nares, throat, and skin lesions from healthcare workers (n=231) at three maternity wards. *spa* typing was used to elucidate the transmission routes of *S. aureus*. The use of enrichment broth prior to plating increased the proportion of positive samples by 46 %. The prevalence of *S. aureus* colonization in adults was 58 %. Throat colonization (47 %) was significantly more common than colonization in any of the other screened sites (p<0.001). In total, 103 out of 168 (61 %) newborn infants were colonized during their hospital stay. Overall, 124 *S. aureus* transmissions to newborn infants were detected. Although we detected an increased risk of transmission from the nares as compared to the throat, with an odds ratio of 4.8 [95 % confidence interval (CI) 1.8–12.7], we detected a transmission rate of 7 % from the throat. We show that *S. aureus* throat colonization is more common than colonization in any of the other sites among the parents and staff. We also show evidence of transmission from the throat.

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Introduction

The clinical spectrum of diseases caused by *Staphylococcus aureus* ranges from skin- and soft-tissue infections to life-threatening invasive infections, but it is also a commensal of the human flora. Swedish guidelines for culturing samples from skin- and soft-tissue infections indicate direct plating onto solid medium, i.e., blood-, hematin-, and CLED-agar, incubated aerobically, anaerobically, and in carbon dioxide [1]. The incubation of swabs in enrichment broth prior to plating has been suggested to increase the sensitivity of *S. aureus* detection [2, 3]. Using enrichment broth with methicillin in combination with polymerase chain reaction (PCR) increased the recovery of methicillin-resistant *S. aureus* (MRSA) by 35 %, compared to direct plating on selective medium [4]. The use of broth (without the addition of methicillin) prior to plating has, however, not been fully evaluated regarding increased sensitivity of methicillin-sensitive *S. aureus* (MSSA) detection. However, a recent report showed increased sensitivity from broth enrichment when used for multisite sampling of pregnant women and their neonates [5].

Approximately 30 % of the population is colonized with *S. aureus* in the anterior nares [6], and nasal colonization poses a threat for endogenous infection [7, 8]. Several studies have, therefore, focused on the eradication of nasal carriage of *S. aureus* to minimize the risk of endogenous infections [9–12]. The transmission of *S. aureus* from hospital staff and/or fellow patients has previously been proposed as an explanation for infections with strains not observed in the anterior nares of the patient [13]. Recent studies have indicated that throat colonization might be even more common than nasal colonization [2, 14]. Therefore, the importance of screening for *S. aureus* carriage in the throat, as well as the

inclusion of the throat in eradication schemes, has been discussed [2, 14, 15]. To our knowledge, no data are available on the transmission of *S. aureus* from the throat.

In this study, we aimed to clarify the effect on *S. aureus* detection by using an enrichment broth prior to plating compared to direct plating. Also, we determined the prevalence of *S. aureus* throat colonization and the importance of throat colonization in the transmission of *S. aureus* from healthcare workers (HCWs) and parents to newborn infants.

Materials and methods

Enrichment broth and plating of *S. aureus* samples

Swab samples were collected from the anterior nares and umbilicus from newborn infants and from the anterior nares, throat, skin lesions, and vagina (only mothers) from adults (Table 1). Swabs were streaked on BBL™ CHROMagar™ Staph aureus medium (Becton Dickinson, Franklin Lakes, NJ) and subsequently submerged into 5 mL of enrichment broth, according to Nilsson and Ripa [3], except for the addition of phenyl red. Plates and broths were incubated for 18–24 h at 35 °C (broth on gentle agitation). Colonies suspected to be *S. aureus* from direct plating were verified by DNase testing and replated onto BBL™ CHROMagar™ Staph aureus medium (Becton Dickinson). From samples that did not grow any *S. aureus* in direct plating, 10 μL of broth was plated onto BBL™ CHROMagar™ Staph aureus medium (Becton Dickinson) and incubated at 35 C for 18–24 h. *S. aureus* verification was performed as described above.

Throat colonization and transmission

This study was performed at the three delivery wards in Jönköping County (Sweden) in 2009 and 2010. Parents arriving at any of the delivery wards were asked to participate. Families were excluded if a caesarean section was performed or if the newborn infant had to be treated at the intensive care unit. Written informed consent was collected from all parents, which was also valid for their underaged (<18 years old) children. Oral information regarding the study was given to staff. The results from cultures were communicated to participants, if desired, by the responsible scientists.

The sampling scheme for parents (n=332; 167 mothers, 165 fathers), staff members (n=231; 91 % females), infants (n=168; 83 girls, 85 boys), visiting siblings (n=33), and adult visitors (n=2) has been described previously [16]. Briefly, staff members were sampled at the start of their shifts and parents were sampled upon arrival at the delivery ward. A vaginal sample was collected from the mothers at the first vaginal examination. Staff members and parents were sampled once. Infants were sampled from the anterior nares and umbilicus at 2 h postpartum and subsequently every 24 h until discharge.

S. aureus culture and verification was performed as described above.

spa typing was performed on one colony from each *S. aureus*-positive sample (either from direct plating or plating after enrichment broth), as described previously [16]. PCR products were purified and sequenced by GATC Biotech AG (Konstanz, Germany) and a *spa* type was assigned to each isolate using the Ridom StaphType Software (v1.5.21, Ridom GmbH, Würzburg, Germany), as described previously [17]. Based upon repeat pattern (BURP) cluster analysis was performed as described previously, using default settings [18].

Three possible transmission sources were evaluated. Primarily, the infant's own family, meaning that, if the infant's *spa* type was detected among its own family members, they were considered the source of transmission, even if the *spa* type was also present in staff and/or family members of other newborn infants. Secondarily, the staff and/or family members of other newborn infants, simultaneously cared for at the department, were considered the source of transmission if the infant's *spa* type was found among those but not among its own family. Finally, if the infant's *spa* type could not be detected either among its own family, members of staff, another family, or in the environment, the source was considered unknown.

Table 1 Samples positive for *Staphylococcus aureus* by direct plating and additional proportion of *S. aureus*-positive samples after broth enrichment

	No. of samples	Samples positive (by direct plating, *n*)	Additional positive samples after broth enrichment, *n*	Additional proportion of positive samples (%)
Vagina	157	3	8	267
Nares (infants)	524	38	47	124
Throat	500	121	121	100
Skin lesions	62	9	3	33
Nares (adults)	597	162	24	15
Umbilicus	531	155	22	14
All sites	2,371	488	225	46

Table 2 Prevalence (%) of *S. aureus* in the various sites sampled

	Culture site					
	Anterior nares	Throat	Vagina	Skin lesions	Umbilicus	Total
Mothers	32	40	7	42		51
Fathers	45	60		23		70
Female HCWs	31	43		5		53
Male HCWs	32	37				58
Female adults	32	42	7	18		53
Male adults	43	58		23		69
All adults	36	47	7	20		58
Female infants	43				53	61
Male infants	40				53	61
All infants	42				53	61
All populations*	37	47	7	20	53	59

*Also including visiting relatives and siblings

Statistical analysis

The χ^2 test and Fisher's exact test were used for comparison between groups. A p-value<0.05 was considered statistically significant.

Ethical approval

The study was approved by the regional ethical board at Linköping University (M80-07), August 8th 2007.

Results

Additive effect on *S. aureus* yield by the use of enrichment broth

The use of enrichment broth prior to plating increased the proportion of *S. aureus*-positive samples by 46 % (Table 1). The largest additive effect was seen in vaginal samples and in samples from the nares of infants and the throats of adults (Table 1).

S. aureus colonization

A total of 2,434 samples were collected from 766 individuals, resulting in 788 *S. aureus* isolates, corresponding to 32 % of the samples being positive for *S. aureus*.

The colonization rates are shown in Table 2. The overall prevalence of *S. aureus* colonization in adults was 58 %. Skin lesions (n=60) were only sampled from adults and 20 % of them contained *S. aureus*. Adult males were significantly more often colonized than females (69 % and 53 %, respectively, p=0.0002). In total, 61 % of the newborn infants were colonized during hospitalization and no gender-associated difference was observed. At the age of 2 h, 10 % and 8 % of the newborn infants were colonized in the anterior nares and the umbilicus, respectively. Most of the observed colonizations occurred during the following 22 h of life in the umbilicus (Fig. 1).

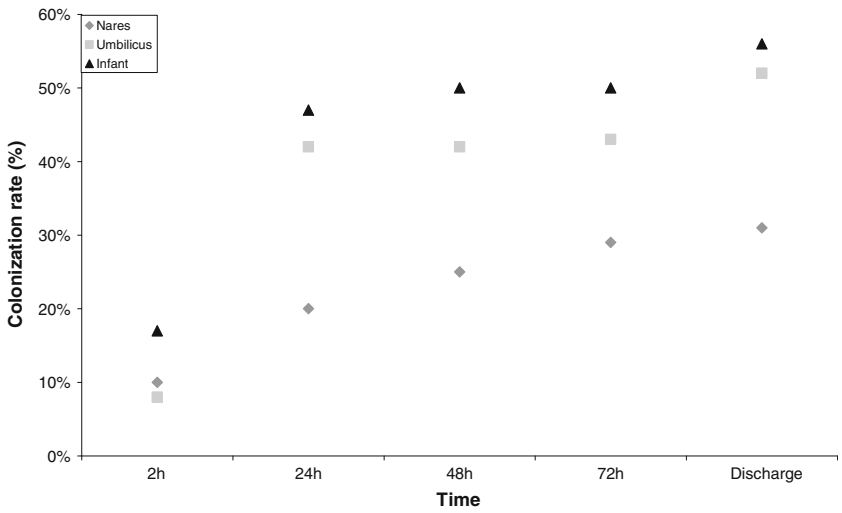

Fig. 1 Site-specific and overall prevalence of *Staphylococcus aureus* among newborn infants, from age 2 h to discharge

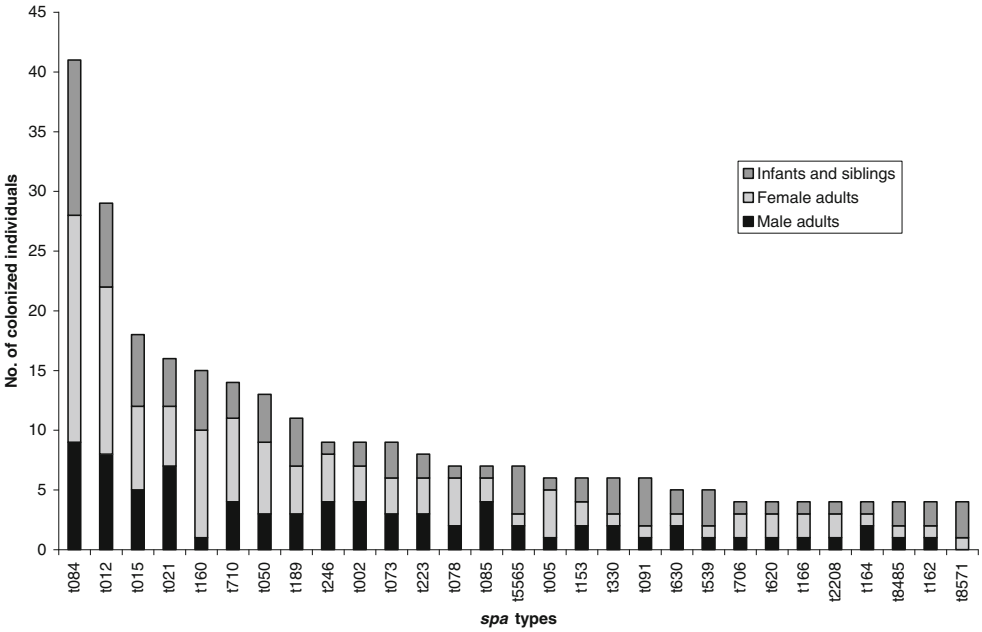

Fig. 2 *spa* type distribution among adults, infants, and siblings

Among adults, throat colonization (47 %) was significantly more common than colonization in any of the other sites sampled ($p<0.001$) (Table 2). This was also the case when dividing the adults into females and males ($p<0.001$). Infants were significantly more often colonized in the umbilicus (53 %) than the anterior nares (42 %, $p<0.05$).

spa typing and cluster analysis

In total, 170 different *spa* types were detected and 84 of these (49 %) were found in single individuals. The distribution of the most frequently isolated *spa* types is shown in Fig. 2, with *spa* type t084 being the most common (9 %, 74 of 788 isolates), followed by t012 (5 %). Among adults, no specific *spa* type was associated with female or male gender (Fig. 2), nor was there a difference in the anterior nares compared to the throat (data not shown). The *spa* type distribution showed a high diversity among both parents and staff; t160 was, however, more common among staff as compared to parents ($p=0.004$). We found 140 adults that were colonized in more than one site. Of these, 42 individuals (30 %) were colonized with *S. aureus* of more than one *spa* type; thereby, they were defined as multiclonal individuals. Thirteen isolates (1.6 %) were considered non-typable. BURP cluster analysis revealed 19 clusters and nine singletons (12 *spa* types shorter than five repeats were detected and excluded from the analysis). The largest cluster was *spa* CC 015, including 53 (31 %) of all *spa* types and 297 (38 %) of all isolates, followed by *spa* CC 084 (including 8 % of *spa* types and 14 % of isolates), *spa* CC 349 (8 % of *spa* types and 4 % of isolates), and *spa* CC 002 (6 % of *spa* types and 6 % of isolates).

Transmission of *S. aureus*

In total, 124 *S. aureus* transmissions to newborn infants were detected. The majority (57 %, 71 of 124) of these originated from a family member of the colonized infant, 21 from the mother, 19 from the father, and, for the remaining 31, the origin could be the mother, father, or another relative. In 28 %, the source of transmission was either a staff member or a parent of other newborn infants. In 16 %, no source was identified. Infants with colonized parents had a significantly increased odds ratio (OR) [OR_{MH} of 2.8, 95 % confidence interval (CI) 1.8–4.5, adjusted for gender of adult] for colonization than infants of non-colonized parents ($p<0.001$). The rate of transmission from the anterior nares and the throat was compared and from 34 % of nares-only carriers and from 7 % of throat-only carriers, transmission occurred. The highest rate of transmission (61 %) occurred from multiple-site carriers (Table 3). Thus, there is an increased

Table 3 Colonization state of possible transmitters and transmissions from these individuals

Colonization state	Number	Transmissions	
		n	%
Multiple-site*	102	62	61
Nares only	44	15	34
Throat only	99	7	7
Total	325	124	37

*Individuals colonized with *S. aureus* of the same *spa* type in more than one site

risk of transmission from the nares as compared to the throat (OR=4.8, 95 % CI 1.8–12.7).

Discussion

In this study, we show that the incubation of swabs in enrichment broth prior to plating increased the proportion of *S. aureus*-positive samples by 46 %. We also show that *S. aureus* throat colonization is more common than nasal colonization, which is in accordance with recent studies [2, 14]. Furthermore, this study also indicates the transmission of *S. aureus* originating from the throat.

We found the *S. aureus* colonization rate to be higher in all sites sampled when using enrichment broth prior to plating, with at least a doubling in positive samples from the anterior nares of infants, the throat of all populations, and vaginal samples from mothers. Thus, to determine the true rate of *S. aureus* colonization incubation in enrichment broth prior to plating seems necessary.

In this study, the highest *S. aureus* colonization rate was seen in the throat, which supports the suggestion to also include throat samples in *S. aureus* screening programs [2].

Several studies have focused on the eradication of *S. aureus* nasal colonization to prevent endogenous infections [9–11]. One study suggested that infections with strains not observed in the anterior nares of the patient might originate from hospital staff and/or fellow patients [13]. In this study, we show that the transmission potential of the anterior nares is five times higher than that of the throat. This is based on the rate of transmission when the anterior nares or the throat is the only potential source. However, as throat colonization is high and the fact that we show that transmission from the throat does occur, the eradication of throat colonization might be necessary to prevent endogenous *S. aureus* infections and nosocomial spread. This was also suggested in a recent review article [19].

We show that transmission mainly originates from the parents of the colonized infant, independent of whether it was the mother or the father who was colonized. Infants of colonized parents also had an increased risk of colonization compared to infants of non-colonized parents. One limitation of our study is that we only study transmissions to newborn infants and, therefore, we cannot draw firm conclusions regarding transmissions to adults. Furthermore, the role of broth enrichment in MRSA screening programs may differ from our data; however, increased MRSA detection rates by the use of broth enrichment has been shown previously [4].

As 91 % of the HCWs are females, similar colonization rates for HCWs and mothers was expected. Colonization was more frequent in adult males as compared to adult females, which has been described previously [16]. This gender-associated difference in colonization was not observed for newborn infants. Most of the infants that were colonized before discharge were so in the umbilicus, whereas nasal colonization was much lower. Lebon et al. showed a dramatic decrease in *S. aureus* colonization rates among infants, starting at 52 % at 1.5 months of age, dropping to only 13 % at 14 months of age [20]. We could, in this study, see that nasal colonization in infants starts already at birth, reaching 56 % at discharge, which usually occurred at 24 to 48 h of age.

The most common *spa* type in this study, t084, has recently been described as a newcomer in our geographical area [16], and it is also common in Northern Norway [21, 22]. We could not find a gender-specific *spa* type among adults, which has been described previously [21]. We show that *spa* type t160 was significantly associated with staff, as compared to a Norwegian study where t012 and t015 were associated with staff [22]. Multiclonal colonization occurred in 30 % of the colonized adults. Multiclonal colonization, in both individuals and sites, could have an impact on antibiotic treatment, given simultaneous colonization with susceptible and resistant strains, and impair epidemiological conclusions. This question would, therefore, need further focus. The two largest *spa* clusters in our study contained more than one-third of all the *spa* types and more than half of the isolates. On the contrary, almost half of the *spa* types were only detected in single individuals.

In conclusion, we show that the use of enrichment broth for the incubation of swabs prior to plating has a high additive effect on the proportion of *S. aureus*-positive samples and that the transmission of *S. aureus* from the throat is relevant.

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Conflict of interest The authors declare that they have no conflict of interest.

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