

Evaluation of the Copan ESwab transport system for the detection of methicillin-resistant *Staphylococcus aureus*: a laboratory and clinical study

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as an important pathogen. Successful detection starts with the selection of an appropriate specimen collection device. The Copan ESwab, a new swab, was compared with a commonly used Copan Venturi swab for the recovery of MRSA. In vitro assessment was performed according to the Clinical and Laboratory Standards Institute (CLSI) M40-A protocol. For the clinical evaluation, 24 patients with known MRSA carriage were included. The ESwab fulfilled the CLSI acceptance criteria for MRSA viability. In the clinical study, both swabs performed equally in qualitative terms of positive and negative. However, the in vitro and in vivo evaluation revealed at least 3.6-fold higher recovery of viable MRSA with the ESwab as compared with the Venturi swab. In conclusion, the ESwab may contribute to improve the quality of a MRSA screening protocol.

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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide healthcare-associated pathogen (National Nosocomial Infections Surveillance System, 2004; Tiemersma et al., 2004). An accurate diagnosis of an MRSA infection permits the initiation of appropriate therapy and control measures (Harbarth, 2006; Struelens and Denis, 2006). For MRSA detection, swabs are a frequently used sampling device. Recently, a new patented nylon flocked ESwab with liquid Amies medium has been developed (Drake et al., 2005; Van Horn et al., 2008). We assessed the ability of the ESwab to maintain MRSA viability to the Clinical and Laboratory Standards Institute (CLSI, 2007) M40-A guideline. In addition, the recovery of MRSA by the ESwab in patient samples was determined. In all the tested conditions, the traditional Venturi Transystem consisting of a Dacron

swab and an agar Amies transport medium was used as comparator.

2. Materials and methods

2.1. Tested swabs

The Venturi Transystem (Copan, Brescia, Italy) with Amies gel transport medium was compared with the ESwab (Copan) immersed in liquid Amies transport medium.

2.2. In vitro validation

A MRSA ATCC 43300 strain was used, and all incubations were performed on a 5% sheep blood agar (Becton Dickinson, Sparks, MD) according to the CLSI (2007) guideline.

2.2.1. Swab elution method

Three separate swabs were dipped in 100 µL of a 10⁷ colony-forming units (CFUs)/mL solution before transferring

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Table 1
Recovery of MRSA using the roll plate method

Transport system (incubation temperature)	Inoculum (CFU/swab) ^a	Mean no. ± SD of colonies (% recovery)		
		0h	24h	48h
ESwab (2–4 °C)	5000	<30	<30	<30
	50 000	215 ± 36	210 ± 30 (98)	275 ± 80 (128)
	500 000	>300	>300	>300
ESwab (20–25 °C)	5000	<30	40 ± 9 (186)	105 ± 17 (488)
	50 000	215 ± 36	>300	>300
	500 000	>300	>300	>300
Venturi (2–4 °C)	1000	<30	<30	<30
	10 000	<30	<30	<30
	100 000	104 ± 15	74 ± 12 (74)	63 ± 15 (61)
Venturi (20–25 °C)	1000	<30	<30	<30
	10 000	<30	<30	<30
	100 000	104 ± 15	101 ± 26 (97)	83 ± 17 (80)

^a CFU/swab calculated from viable count of the inoculating suspensions described in Materials and methods.

them to their transport medium for the following incubation times, 5 min (time 0), 24 h, 48 h, at the respective incubation temperatures of 20 to 25 °C and 2 to 4 °C. After incubation, the Venturi and ESwab were placed in 1-mL 0.85% physiological saline and the liquid transport medium, respectively, and diluted to 10, 100, 10³, 10⁴, and 10⁵ CFUs/mL. Of each dilution, 100-μL aliquots in duplicate were plated and incubated in an aerobic atmosphere at 37 °C during 24 h.

2.2.2. Roll plate method

One hundred microliters of 10⁴, 10⁵, and 10⁶ CFUs/mL MRSA solutions were used to inoculate the swabs; after immersion, they were replaced in their respective transport media during 5 min (time 0), 24 h, and 48 h and at both 20 to 25 °C and 2 to 4 °C, each in triplicate. The ESwab was vortexed during 15 s before plating. Culture was performed by streaking the swabs over the entire agar. The final CFU is the average of the 3 plates, counted in triplicate, after 24 h of incubation.

2.3. Fluid absorption test

The tested swabs were weighed before and after immersion in brain heart infusion (BHI) (Becton Dickinson) and after plating.

2.4. In vivo part

Eighteen patients known to carry MRSA were sampled by both tested swabs, alternating each swab as the first applied, at the same site. In addition, 6 patients harboring MRSA were sampled with the Venturi swab as the first swab used. The delay between sampling and culture was 3 h maximum. The ESwab and Venturi swab were vortexed 15 s in the transport medium and 0.85% saline, respectively, before culture. Both swabs were streaked on a MRSA-ID plate (MRSA CHROMagar; bioMérieux, Marcy l'Etoile, France),

which was incubated at 35 °C for 24 h. After plating, both swabs were immersed in enrichment BHI with 6.5% NaCl (Becton-Dickinson). If green colonies were present on the MRSA-ID, they were plated on a *S. aureus* ID plate (SA select; Bio-Rad) to confirm *S. aureus*. If no growth was present, the enrichment medium was checked for turbidity, and if present, a MRSA-ID was plated. In the absence of turbidity, the enrichment was incubated for another 24 h at 35 °C before inoculating a MRSA-ID.

2.5. Calculations

Differences were calculated with the nonparametric Mann–Whitney *U* test using GraphPad Prism version 2.0 (GraphPad Software, CA).

3. Results

3.1. In vitro study

3.1.1. Fluid absorption test

The ESwab absorbed 1.15 times more BHI broth suspension (137 ± 9.6 mg, mean ± SD), as compared with the Venturi swab (119 ± 9.1 mg) ($P > 0.05$, $n = 6$). The ESwab released 1.95 times (47 ± 4.6 mg) more liquid than the Venturi swab (24 ± 4.7 mg) ($P < 0.05$, $n = 6$).

3.1.2. Viability and overgrowth with the roll plate and swab elution method

As per the CLSI guideline, we selected the inoculum that provided plate counts between 30 and 300 CFUs. The ESwab showed no decrease in viability at refrigerator temperature with a recovery of 98% and 128% with the roll plate method (Table 1) and of 98% and 172% with the swab elution method (Table 2) at 24 and 48 h of incubation, respectively. At room temperature, even a tendency to overgrowth was observed with an increase in viability to 186% and 488% with the roll plate method (Table 1) and to

Table 2
Recovery of MRSA using the swab elution method

Transport system (incubation temperature)	Inoculum (CFU/swab) ^a	Mean no. ± SD of colonies (% recovery)		
		0h	24h	48h
ESwab (2–4 °C)	100	<30	<30	<30
	1000	64 ± 10	63 ± 10 (98)	110 ± 40 (172)
	10 000	>300	>300	>300
ESwab (20–25 °C)	100	<30	<30	<30
	1000	64 ± 10	128 ± 22 (200)	300 ± 48 (468)
	10 000	>300	>300	>300
Venturi (2–4 °C)	100	<30	<30	<30
	1000	<30	<30	<30
	10 000	72 ± 14	75 ± 9 (104)	69 ± 13 (95)
Venturi (20–25 °C)	100	<30	<30	<30
	1000	<30	<30	<30
	10 000	72 ± 14	66 ± 3 (91)	74 ± 6 (103)

^a CFU/swab calculated from viable count of the inoculating suspensions described in Materials and methods.

200% and 468% with the swab elution method (Table 2) at 24 and 48 h, respectively, but all conditions fulfilled the CLSI acceptance criteria.

The viability with the Venturi swab showed a time- and temperature-dependent decrease, with a recovery at 48 h incubation at room and refrigerator temperature of 80% and 61%, respectively, with the roll plate method (Table 1), whereas viability was maintained with the swab elution method in all tested conditions (Table 2). The most remarkable finding is the 4- and 9-fold higher initial recovery at time 0 (0 h) of the ESwab as compared with the Venturi swab with the roll plate (Table 1) and swab elution method (Table 2), respectively.

3.2. Clinical study

3.2.1. Detection of MRSA in samples from known MRSA carriers

If we would report the test result qualitatively as positive or negative, the Venturi and the ESwab performed equally and 17 of the 24 included patients were found positive by both swabs. In the samples from the remaining 7 patients, MRSA could not be recovered, neither with the Venturi nor with the ESwab. From the 16 samples that were found positive without the enrichment procedure, 12 samples were within the countable limits of 30 to 300 on the MRSA plate. We separated the data from these 12 samples according to which swab was applied as first for sampling. In case the ESwab or the Venturi swab was used as the first sample, the recovery with the ESwab was 3.6- to 6-fold higher as compared with the Venturi system (data not shown). For the remaining 4 samples with more than 300 CFUs, we remarked a more pronounced massive growth in the ESwab than in the Venturi swab, with 2 of the 4 samples the Venturi as the first swab used for sampling.

4. Discussion

A crucial step for MRSA detection remains an adequate specimen collection and transport system to the laboratory. Cotton, Dacron, and rayon swabs only absorb bacteria onto their surface, thereby shutting them in their fiber matrix (Osterblad et al., 2003). Copan provides a new nylon-tipped ESwab, which was shown recently to meet the swab elution method acceptance criteria of the CLSI protocol (Van Horn et al., 2008). We included the roll plate method of the CLSI guideline in our validation for the detection of MRSA because it is the best reflection of the standard procedure in a laboratory. The clinical performance of the ESwab was also evaluated because it is the only approach that takes into account preanalytic variables.

In the roll plate and the swab elution method, the ESwab had a 4- and 9-times higher recovery, respectively, of *S. aureus* at time 0 as the Venturi swab. Accordingly, to reach a similar detectable growth as with the ESwab, the

inoculum concentration of the Venturi swab had to be 4- to 9-fold higher. A higher absorption and release capacity of the ESwab as compared with the Venturi swab may account for this difference. Another contributing factor might be the transport medium itself. The Venturi swab contains a gelatinous transport medium that may retain part of the inoculum. The ESwab, on the contrary, is immersed in liquid, which facilitates elution of the sample by capillary hydraulics. The clinical performance of both swabs to detect MRSA in patients was assessed using a standard MRSA screening protocol. Both swabs performed equally in qualitative terms of positive and negative, but for those samples that could be judged quantitatively, the ESwab had a 3.6- to 6-fold higher recovery than the Venturi swab.

The transport of clinical samples necessitates that the viability of microorganisms is preserved during a time span of at least 48 h. Another variable in transportation is the storage temperature. Some authors recommend to store swabs at 4 °C to overcome overgrowth (Human and Jones, 2005; Tyede and Hoiby, 1992); the opposite can also be encountered by loss of viability. Transportation times of 24 and 48 h were included at both ambient and refrigerated temperature in our evaluation protocol. The ESwab could retain viability during a 48-h time span at both investigated temperatures. At room temperature, a tendency to overgrowth was observed. However, in case of MRSA screening, overgrowth should be recognized as advantageous. The Venturi swab had a 30% to 40% decrease in recovery with the roll plate method at 4 °C after 24 and 48 h of incubation, respectively. In the other tested conditions, the swab could maintain a good recovery.

The most important finding in this study is that the recovery of MRSA by the ESwab in all tested conditions exceeded that of the Venturi swab by a factor of at least 3.6.

In conclusion, the higher recovery of the ESwab makes it an alternative to the traditional Venturi swab for MRSA detection.

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