American Society for Microbiology General Meeting, Philadelphia, PA (USA), May 2009

Enhanced Recovery of Low-Inoculum Methicillin-Resistant *Staphylococcus aureus* (MRSA) by the Novel Flocked ESwab Compared to a Conventional Swab, the M40 Transystem

C-056

Shaker E. Farhat[1]*, George Lim[1], Rudylor Malonzo[1], Bhavisha Shingala[1], Andrew E. Simor[1,2,3]

*E-mail: shaker@alpha-it.com

[1]Alpha Laboratories Inc., Toronto, ON; [2]Sunnybrook Health Sciences Centre, Toronto, ON; [3]University of Toronto, Toronto, ON, CANADA

ABSTRACT

Background: Colonization with MRSA is a risk factor for subsequent infection, and is associated with horizontal transmission. Culture of a nasal swab is the most widely employed and cost-efficient method for MRSA screening. Unlike most conventional agar-gel based swabs, the newly introduced ESwab (E; Copan Diagnostics Inc., Murrieta, CA) is a nylon-flocked swab transported in Amies liquid, with a potentially high organism release capability. The objective of this study was to compare the recovery of moderate-to-low inoculum MRSA by two swab systems using three methods: direct swab plating by a conventional swab (M40 Transystem [M]; Copan), direct swab plating by E, and plating of 100 µL of E tube liquid (EL).

Methods: Triplicate sets of E and M swabs were seeded with 100 µL aliquots of 21 MRSA strains, including community-associated (*n* = 10), hospital-acquired (*n* = 10), and a control strain (*S. aureus* ATCC 43300), suspended in sterile saline solution and serially diluted to inoculum concentrations ranging from 1.5 x 10^5 (10^5) to 1.5 x 10^1 (10^1) CFU/ml. Using CLSI M40A Roll-Plate Method, the seeded E and M swabs were stored at 20-25 °C, and plated on blood agar (BA) at 0, 6, and 24 hr. Additionally, 100 µL aliquots of EL were plated at same timepoints on BA. Viability was compared to the 0 hr count for each strain-swab combination.

Results: All 21 MRSA strains were adequately recovered from all swabs seeded with 10^5, 10^4, or 10^3 CFU/ml, regardless of swab type, plating timepoint, or plating procedure. However, of the 21 strains diluted to10^2 CFU/ml inoculum concentration, only 10 (48 %), 9 (43 %), and 9 (43 %) were recovered from M, 16 (76%), 16 (76 %), and 15 (71 %) from E, and 17 (81 %), 17 (81 %), and 16 (76 %) from EL, at 0, 6, and 24 h, respectively. At 10^1 CFU/ml concentration, 2 (10 %), 2 (10 %), and 1 (5 %) were recovered from M, 9 (43 %), 9 (43 %), and 8 (38 %) from E, and 11 (52 %), 11 (52 %), and 10 (48 %) from EL, at 0, 6, and 24 h, respectively.

Conclusion: The ESwab and its liquid transport medium are equivalent to the M40 transystem for recovery of MRSA at standard inocula ≥ 10^3 CFU/ml; however, these flocked ESwabs were better able to recover MRSA when present in lower numbers.

INTRODUCTION

One of the critical steps for effective laboratory diagnosis of infection or microbial colonization is adequate sampling and transport of specimens. There have been continuous efforts to develop and evaluate new and improved swab transport systems and to enhance their performance under various conditions.[1,2,4-9]

Colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) is a risk factor for subsequent infection and is associated with horizontal transmission, length of hospitalization, and overall burden of disease.[11,12] The use of a swab is the most widely employed method for detection of MRSA wound infections, and nasal swabs are widely used for detection of MRSA colonization. The ability of a swab to recover MRSA if present in a patient sample is crucial to prevent the reporting of a false-negative result in an MRSA-colonized or infected patient.

Among newly introduced transport media is the elution swab (ESwab; Copan Diagnostics, Inc., Murrieta, CA), a unique, patented, nylon-flocked swab, which once placed in its modified Amies liquid transport medium, it enables the elution of the entire sample immediately, resulting in a significantly improved release of bacteria. The ESwab has been shown in several studies to be equivalent or superior to conventional swabs,[1,2,4,5,13,14] and its utility for MRSA nasal screening has been recently demonstrated.[10] However, no studies to date have determined its ability, compared to a conventional swab, to release organisms when present in very low quantity.[13]

In this study, the efficacy of two swab systems to recover MRSA as low as 10 CFU/mL, was assessed by direct swab plating using a conventional swab (M40 Transystem [M]; Copan) compared to the ESwab (E), versus plating of 100 µL of the ESwab tube liquid (EL).

METHODS

Using the CLSI- M40A Roll-Plate Method, swabs were seeded in triplicate with 100 µL aliquots of 21 MRSA strains, comprising a control strain (*S. aureus* ATCC 43300) and 20 clinical isolates, including 10 community-associated (CA-MRSA) and 10 hospital-associated (HA-MRSA) strains, which had been previously characterized for Panton-Valentine leukocidin (PVL). In this study, all of the CA-MRSA isolates were PVL positive and the HA-MRSA isolates were PVL negative.

Each isolate was suspended in sterile saline and the suspensions were serially diluted from 1.5 x 10^8 CFU/mL (0.5 McFarland Std.), into 1:10 to 1:10,000,000 suspensions, with final inoculum concentrations ranging from 10^1-10^5 CFU/mL, which were used to seed the swabs.[9]

The seeded swabs were stored in room temperature, and were plated in accordance with CLSI- M40A Roll-Plate Method onto 5% sheep blood agar (BA) at 0, 6, and 24 hr. The initial plating was performed about 30 minutes post swab inoculation to allow reasonable time for the organism suspension to equilibrate onto the swab. Using a sterile pipette, a suspension of 100 µL of the ESwab liquid (EL) left in the ESwab transport device was inoculated onto a BA plate, and was spread using a sterile spreader.

Plates were promptly incubated and read at 24 hr. Viability was compared to the zero hour count for each strain-swab combination for the concentrations indicated in accordance with CLSI-M40.

Figure 1: Recovery of MRSA strains in 1.5x10^3 (10^3) to 1.5x10^5 (10^5) CFU/mL concentrations by direct plating of the conventional swab M40 Transystem (M), *vs* the ESwab (E) *vs* 100 µL of ESwab tube liquid (EL)

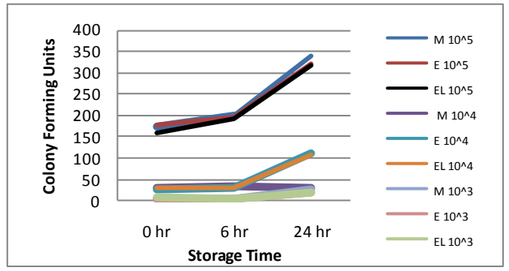

Figure 2: Number of MRSA strains in 1.5 x 10^2 CFU/mL concentration, recovered by direct plating of the conventional swab M40 Transystem (M), *vs* the ESwab (E), *vs* 100 µL of ESwab tube liquid (EL)

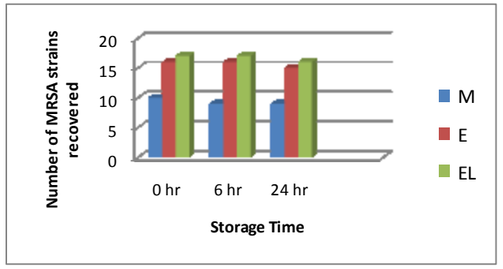

Figure 3: Number of MRSA strains in 1.5 x 10^1 CFU/mL concentration, recovered by direct plating of the conventional swab M40 Transystem (M), vs the ESwab (E), and 100 µL of ESwab tube liquid (EL)

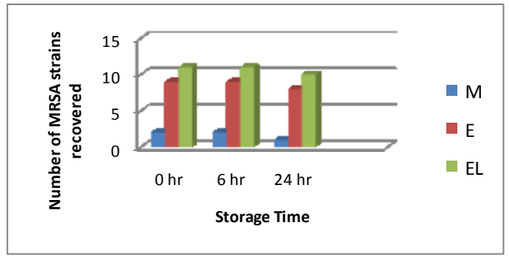

Figure 4: The conventional swab M40 Transystem *vs* the ESwab

RESULTS & DISCUSSION

In this study we sought to determine if the ESwab had an advantage over its conventional counterpart in picking up MRSA even when present at very low levels. The efficacy of recovering any amount of MRSA is of pivotal importance and has clinical ramifications for patient colonization, infection control, and for containing the spread of MRSA.

The roll plate method was used because it resembled more closely actual laboratory practice.[14] As can be seen in Figure 1, viability of the MRSA strains, when compared to zero hour counts was maintained for each strain-swab combination in the10^5, 10^4, and 10^3 CFU/mL concentrations. Prolongation of swab storage was associated with an increase in CFU counts (Figure 1), an observation consistent with the organism robust and high proliferation rate,[15] which may contribute to its persistance in at-risk patients.[3]

While recovery of all strains in the 10^3 to 10^5 CFU/mL concentrations was achieved by all three methods (Figure 1), only 10 (48 %), 9 (43 %), and 9 (43 %) strains in the 10^2 CFU/ml inoculum concentration were recovered from the M40 swab, 16 (76%), 16 (76 %), and 15 (71 %) from the ESwab, and 17 (81 %), 17 (81 %), and 16 (76 %) from the ESwab tube liquid (100 µL) culture, at 0, 6, and 24 h, respectively (Figure 2). At 10^1 CFU/ml concentration, only 2 (10 %), 2 (10 %), and 1 (5 %) were recovered from the M40 swab, 9 (43 %), 9 (43 %), and 8 (38 %) from ESwab, and 11 (52 %), 11 (52 %), and 10 (48 %) from the ESwab tube liquid (100 µL) culture, at 0, 6, and 24 h, respectively (Figure 3).

There were no differences in recovery rates attributable to PVL status or MRSA source (CA-MRSA *vs* HA-MRSA) (data not shown). While the ESwab allowed less overgrowth in general as previously noted,[14] its superiority over the conventional swab was evident with low-level MRSA, and this was more accentuated by the enhanced recovery of MRSA through the use of 100 µL of the ESwab tube liquid. This advantage may be explained by the unique design of the ESwab flocked applicator, which draws the sample by high capillary action and absorbs it between its fibres, so that once the swab is placed in the transport medium or touches the surface of a culture plate, it immediately and completely elutes the sample and releases the organisms.

CONCLUSIONS

1. At standard inocula of ≥ 10^3 CFU/ml, the ESwab and its liquid transport medium are equivalent to the conventional M40 transystem for recovery of MRSA.
2. When MRSA is present in low concentrations at ≤ 10^2 CFU/ml, the ESwab is superior to the conventional M40 transystem in recovering the organism.
3. We recommend the use of the ESwab to maximize the likelihood of recovering MRSA when present in a low inoculum in specimens from patients colonised or infected by this organism. Its 1 ml of Amies liquid provides multiple aliquots that can be used or stored for additional testing when needed.

REFERENCES

1. Biggs C. 2007. *Gen. Meet. Am. Soc. Microbiol.*, Toronto, ON, Canada. C-365.
2. Coleman SS, et al. 2007. *Gen. Meet. Am. Soc. Microbiol.*, Toronto, ON, Canada. C-366.
3. Dasenbrook EC, et al. 2008. *Am. J. Respir. Crit. Care Med.* 178: 814-821.
4. Davidson J, et al. 2007. *Gen. Meet. Am. Soc. Microbiol.*, Toronto, ON, C-364.
5. DeFields M, et al. 2007. *Annu. Conf. Can. Assoc. Clin. Microbiol. Infect. Dis.*, Halifax, NS, Canada. P-19.
6. Farhat SE, et al. 2001. *J. Clin. Microbiol.* 39: 2958-2960.
7. Farhat SE, et al. 2002. *Gen. Meet. Am. Soc. Microbiol.*, Orlando, FL, USA. C-59.
8. Farhat SE, et al. 2008. *Gen. Meet. Am. Soc. Microbiol.*, Boston, MA, USA. C-171.
9. Sarina M, et al. 2007. *Gen. Meet. Am. Soc. Microbiol.*, Toronto, ON, Canada, C-362.
10. Silbert S, et al. 2007. *Gen. Meet. Am. Soc. Microbiol.*, Toronto, ON, Canada. C-368.
11. Simor AE, Daneman N. 2009. *Infect. Dis. Clin. North Am.* 23: 133-151.
12. Simor AE, et al. 2004. *Can. J. Infect. Dis.* 15: 39-48.
13. Van Horn KG, et al. 2008. *Diagn. Microbiol. Infect. Dis.* 62: 471-473.
14. Van Horn KG, et al. 2008. *J. Clin. Microbiol.* 46: 1655-1658.
15. Yoshida Y, et al. 2008. *Eur. Cong. Clin. Microbiol. Infect. Dis.*, Barcelona. Spain O-511.

ACKNOWLEDGMENTS

We thank Christine Watt and Nyog Inn Ng-Haing, Sunnybrook Health Sciences Centre, for kindly providing the MRSA clinical strains for this study, and Betty Premraj and Tommy Li, Alpha Laboratories, for excellent technical and poster layout assistance. This work was supported mainly by Copan Diagnostics Inc, and in part by Starplex Scientific Inc and Bio-Media Ltd.