Detection of MRSA by the BD Genome IDI MRSA Assay (BMA) from Flocked Swabs transported in Liquid Stuart's

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ABSTRACT (revised)

Background: MRSA screening by culture requires 48-72 hours compared to 1 hr for the BMA. At the Ottawa Hospital, nasal and rectal swabs are pooled in a selective broth prior to testing. To improve turnaround times (TAT) we evaluated the performance of the BMA using a flocked swab and a liquid Stuart's (LS) transport system. **Method**: Flocked swabs dipped in a 0.5 McFarland saline suspension of CMRSA-2, -7 and -10 clinical isolates were placed in the LS and incubated at room temperature (RT) for 1 hr, 2 hr, and overnight. To simulate a rectal swab, CMRSA-2 an inoculated swab was dipped in a MRSA negative stool and incubated in LS at RT for 1hr, 2 hr and overnight. For the simulated nasal swab, nares of a negative volunteer were swabbed prior to dipping in a CMRSA-2 suspension and incubated 1hr, 2hr and overnight at RT. For BMA testing, swabs were vortexed in the LS and a 50ul aliquot was transferred to the sample diluents buffer and processed according to manufacturer`s instructions. All runs were performed in triplicate. Serial dilutions of 5 different C-MRSA clinical isolates were used in order to determine the limit of detection for the BMA in both LS and 0.45 % sodium chloride solution (NaCl). Duplicate runs were performed using both the BMA kit (mechanical) lysis and achromopeptidase lysis procedures. **Results:** All 0.5 McFarland inoculated flocked swabs transported in LS including simulated rectal and nasal specimens were detected by BMA. Using both LS and NaCl as the transport medium the BMA had a limit of detection of 10^4 CFU/mL. The BMA runs using the achromopeptidase lysis procedure were able to reach the same limit of sensitivity using NaCl but not LS as the transport medium. **Conclusion:** Flocked swabs transported in LS have the potential to be used with the BMA for screening of MRSA colonized patients. Achromopeptidase lysis procedure did not perform as well as the BMA kit lysis procedure using LS as the transport medium. Pooling of nasal and rectal specimens collected on flocked swabs and transported in LS could be used with the BMA for direct testing eliminating the use of the selective broth and potentially reducing TAT.

BACKGROUND

At The Ottawa Hospital (TOH) methicillin-resistant *S. aureus* (MRSA) screening swabs are sent to the Microbiology laboratory where a Real-time Polymerase Chain Reaction (PCR) assay is used to identify patients who are colonized. The BD Genome MRSA assay (Becton Dickinson, MA) (BMA) consists of primers which are specific for sequences of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) which are unique to MRSA. Rectal and nasal swabs are pooled into a selective enrichment broth and incubated at 35^{0}C overnight prior to extraction for use in BMA. Flocked swabs (see Figure 1) transported in liquid Stuart's (LS) have been used for some time for the molecular testing of both viral and bacterial pathogens. The LS transport media can be used directly for DNA extraction for use in the BMA. Given the possibility of reduced TAT using this system we sought to study the performance and limit of sensitivity of the BMA using simulated flocked swab specimens and known concentrations of various MRSA strains transported in LS and a sterile saline solution.

METHODS

Flocked swabs (Starplex Scientific Inc., Etobicoke, ON Canada) dipped in a 0.5 McFarland saline suspensions of CMRSA-2, CMRSA-7 and CMRSA-10 clinical isolates were placed in the LS and incubated at room temperature (RT) for 1 hr, 2 hr, and overnight. To simulate a rectal swab, a CMRSA-2 inoculated swab was dipped in a MRSA negative stool and incubated in LS at RT for 1hr, 2 hr and overnight. For the simulated nasal swab, nares of a negative volunteer were swabbed prior to dipping in a CMRSA-2 suspension and incubated 1hr, 2hr and overnight at RT. For BMA testing, swabs were vortexed in the LS and a 50ul aliquot was transferred to the sample buffer and processed according to manufacturer`s instructions. All runs were performed in triplicate. Serial dilutions of 6 different CMRSA clinical isolates were used in order to determine the limit of detection for the BMA in both LS and 0.45 % sodium chloride solution (NaCl). Duplicate runs were performed using both the BMA kit (mechanical) lysis and achromopeptidase lysis procedures described previously[1,2]. Accuracy of serial dilutions was confirmed via colony counts

RESULTS

- All 0.5 McFarland inoculated flocked swabs transported in LS including simulated rectal and nasal specimens were detected by BMA.
- Colony counts revealed that the median number of CFU/mL was 89% (range = 44-144%) of the estimated CFU/mL.
- Using both LS and NaCl as the transport medium the BMA had a limit of detection of 10^4 CFU/mL (see Tables 1 and 2).
- The BMA runs using the achromopeptidase lysis procedure were able to reach the same limit of sensitivity using NaCl but not LS as the transport medium.

Table 1: BMA performance using sterile Liquid Stuart's as the transport liquid

CMRSA Clinical Isolate	BMA Kit 10^4	BMA Kit 10^3	Achromopeptidase 10^4	Achromopeptidase 10^3
1	+	-	-	-
2	+	+	+	-
7	+	-	-	-
8	+	-	-	-
9	+	-	-	-
10	+	-	-	-

Table 2: BMA performance using sterile 0.45 % NaCl as the transport liquid

CMRSA Clinical Isolate	BMA Kit 10^4	BMA Kit 10^3	Achromopeptidase 10^4	Achromopeptidase 10^3
1	+	-	+	+
2	+	+	+	+
7	+	-	+	+
8	+	+	+	+
9	+	-	+	-
10	+	-	+	-

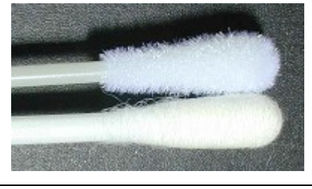
DISCUSSION

- The reduced number of counted colonies compared to estimated bacterial concentrations could be due to variability in the dilution process and/or clumping of bacteria to form individual colony forming units
- Certain CMRSA strains (e.g. CMRSA 2) appeared to be detected at lower concentrations using the BMA extraction method regardless of the transport solution.
- Achromopeptidase (a potent bacteriolytic enzyme) has been used for lysis previously with the BMA[2] but not in conjunction with LS transport media.
- The cause of the apparently reduced sensitivity of the achromopeptidase lysis procedure in LS when compared to NaCl is unclear but include possible inhibition of the enzymatic activity by component(s) of the LS and/or change in viscosity of the lysis solution.
- It is uncertain whether or not the difference in sensitivity observed between the two lysis methods when using LS will have clinical relevance.

CONCLUSION

Flocked swabs transported in LS have the potential to be used with the BMA for screening of MRSA colonized patients. Achromopeptidase lysis procedure did not perform as well as the BMA kit lysis procedure using LS as the transport medium. Pooling of nasal and rectal specimens collected on flocked swabs and transported in LS could be used with the BMA for direct testing eliminating the use of the selective broth and potentially reducing TAT. Further evaluations are on-going to determine the feasibility and performance of the BMA from nasal and rectal specimens collected on flocked swabs and pooled in LS.

Figure 1: Flocked swab (top), traditional swab (bottom)



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