Rapid detection of MRSA in surveillance samples from high risk patients by two molecular techniques

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BILULU

ABSTRACT

Background: Performance of two commercial molecular techniques, both real-time multiplex PCR: **Xpert MRSA®,** (Cepheid) (Cepheid PCR) and **GeneOhm MRSA®,** (Becton Dickinson) (BD PCR) for the detection of MRSA in surveillance samples of high risk patients was evaluated and compared to culture technique.

Methods: High risk patients were sampled (April – June 2007) in 5 Belgian hospitals. Separate nasal, throat and perineum specimens were collected using double swabs next to routine swabs according to each hospital existing procedures. 236 Samples matched the inclusion criteria.

The first parts of each tri-set double swabs were used in the Cepheid PCR on day of reception. Results were available in almost one hour. The second parts of the double swabs were pooled in 600 µl physiologic solution and frozen. Reference culture was performed in 1 hospital using 100 µl for selective enrichment with plating on chromogenic agar after 24 hours. From the remaining fluid, 100 µl was used to carry out BD PCR in following manufacturer's instructions. The routine swabs were cultured in-house according to local procedures.

Results: 27 (11%) Samples showed inhibition when tested on Cepheid PCR, 1 (0.5%) on BD PCR. Cepheid PCR and BD PCR each identified 38/39 (97%) of the specimens culture positive for MRSA and 147/170 (87%) and 174/196 (89%) of the samples respectively negative for MRSA. Positive predictive value (PPV), 62% and 63.3%, and negative predictive value (NPV), 99.3% and 99.4% were comparable for both tests.

Conclusions: Rapid molecular assays proved to be a valuable tool in the organization of infection control policy for a MRSA high risk population. The excellent NPV allows efficient triage. Because of the low PPV, culture is still necessary to confirm. The number of invalid tests was rather high with Cepheid PCR in comparison to BD PCR.

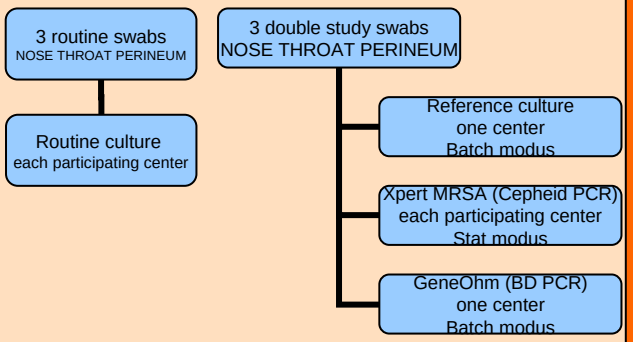
INTRODUCTION

Controlling MRSA is a primary focus of most Belgian hospital infection control programs. Currently, the standard surveillance method for detecting MRSA is culture, which is very laborious and time intensive. A rapid and more sensitive method for MRSA surveillance could represent a definite advantage for triage of colonized patients at an early stage. We evaluated the performance of two rapid real-time PCR methods in a routine MRSA screening program of high risk patients.

METHODS (1)

Specimen collection and processing

Patients at risk for MRSA carriage were sampled from April 2007 till June 2007 in 5 different Belgian hospitals. Separate nasal, throat and perineum swabs were collected using liquid Stuart double Copan® swabs. In addition, routine swabs were taken according to each hospital existing procedures. 236 Samples met the inclusion criteria. First parts of each triset of double swabs were vortexed consecutively in one tube of Elution Reagent (1.5ml) and processed in 1 Xpert MRSA test (Cepheid PCR) upon arrival in the lab. The second parts of the sets were each separately vortexed in 200 µl physiologic solution, pooled in one tube and frozen at -20°C till further testing.



METHODS (2)

Culture and MRSA identification

All samples were cultured in-house using chromogenic agar with or without enrichment according to local practices. Confirmation and identification of positive samples was performed according to the Belgian national guidelines (2003). A frozen aliquot of each sample was also sent to one center (Virga Jesse Hosp, Hasselt) for reference culture. 100 µL of the NaCl solution was inoculated into a staphylococcal enrichment broth (Tryptic Soy Broth containing 5% NaCl). After 24h incubation at 35°C in ambient air, the enrichment broth was subcultured on a selective MRSA-screening agar (MRSA-Select, Bio-Rad). The plates were incubated for 24 hours at 35°C and inspected for suspicious colonies. MRSA-negative cultures were incubated for another 24 hours and inspected again. The identification of the isolated MRSA strains was confirmed by biochemical analysis, slide and tube coagulase tests. Methicillin resistance was confirmed by disk diffusion with oxacillin and cefoxitin disks on MH II agar.

Molecular testing with GeneXpert (Cepheid PCR)

Molecular testing was performed on the GeneXpert® system (Cepheid) according to the manufacturer's instructions. The system combines sample preparation with real time PCR amplification and detection in almost one hour. Every participating hospital performed the analysis on their own instrument.

Molecular testing with GeneOhm (BD PCR)

Molecular testing was performed with the GeneOhm® MRSA test (Becton Dickinson) on a Corbett Rotorgene® (Westburg) real-time PCR instrument according to manufacturers instructions. All tests were performed in batch modus in one center. (Sint-Lucas Hosp., Ghent)

Figure 1: Xpert MRSA cartridge, reagents and the 4-site GeneXpert system

Figure 2: BD GeneOhm MRSA reagents and Corbett Rotorgene real-time PCR instrument

RESULTS

Table 1	Ref culture +	Ref culture -	Total (N=236)
Cepheid PCR +	38	23	61
Cepheid PCR -	1	147	148
Total	39	170	209
Invalids	1	26	27

Table 2	Ref culture +	Ref culture -	Total (N=236)
BD PCR +	38	22	60
BD PCR -	1	174	175
Total	39	196	235
Invalids	-	1	1

Table 1-2: Comparison of Cepheid and BD PCR to reference culture

Table 3	Cepheid PCR	BD PCR
Sensitivity (%)	97,4	97,4
Specificity (%)	86,5	88,8
NPV (%)	99,3	99,4
PPV (%)	62,3	63,3

Table 3: sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of Cepheid and BD PCR compared to reference culture

DISCUSSION

Both molecular tests show acceptable sensitivities and specificities compared to reference culture. Negative predictive value is excellent. Positive predictive value is rather low. Results of reference culture could have been hampered by the freeze-thawing step in the procedure. When molecular methods are compared to routine results PPV's increase from 62,3 to 80,3% for Cepheid PCR and from 63,3 to 77% for BD PCR. Freezing and thawing may have hampered the recovery of MRSA bacteria and may have influenced the differences in inhibition ratios seen in both molecular tests.

Cepheid PCR is quick and easy to perform in a routine laboratory setting, hands on time for BD PCR is larger. The latter test method is more complicated and more skilled technicians are needed

Both tests are very expensive and not reimbursed in most countries.

CONCLUSIONS

1. Rapid molecular assays proved to be a valuable tool in the organization of infection control policies for an MRSA high risk population in a hospital. The excellent NPV allows efficient triage.
2. Because of the easy use Cepheid PCR can be performed throughout working hours in the routine hospital lab. BD PCR requires specialized technicians but batch processing once or twice a day also yields acceptable turn around times.
3. The number of invalid results with Cepheid PCR was rather high compared to BD PCR.
4. Because of the low PPV, culture is still necessary to confirm.

Further studies to investigate if molecular techniques can be used as the first choice in primary MRSA screening are warranted.