Broad-range PCR compared to GenoType BacIdent for the detection of pathogens directly from clinical specimens

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Introduction:

Infectious diseases significantly contribute to morbidity and mortality worldwide [1]. Bacterial infections of primarily sterile or near-sterile sites are a frequently encountered clinical condition. They include (pleuro-) pneumonia, meningitis, peritonitis, bone/joint infection and others. Rapid initiation of appropriate antimicrobial therapy improves patients' outcome [2]. Molecular methods promise rapid and accurate detection of pathogens and allow early targeted therapy.

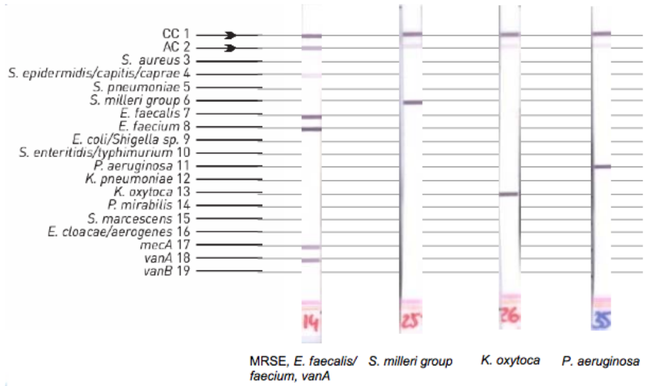
Objectives:

To compare the usefulness of GenoType BacIdent to detect pathogenic bacteria directly in samples from primarily sterile sites.

Methods:

The GenoType Bac Ident system uses a DNA-strip technique to detect 14 bacteria with simultaneous detection of mecA, vanA and vanB genes designed primarily to detect blood culture pathogens. We were interested to see if the method was sensitive enough to detect bacteria directly from clinical specimens from primarily sterile sites.

All suitable bacteriology specimens were subjected to routine culture, broad- range PCR (targeting 16s/18s sequences) and GenoType. The persons performing each test were blinded to the results of the other tests. The specimens included were: aspirate 133 (65 %), tissue biopsy 52 (25 %) and cerebrospinal fluid (CSF) 21 (10 %).



Results:

A total of 206 specimens were investigated between November 2013 and June 2014. GenoType detected a pathogen in 72 (35 %) specimens; culture was positive in 64 (31 %); and the PCR was positive in 41 (20 %) specimens. A total of 35 (17 %) specimens were positive in both GenoType and PCR and 118 (57 %) negative in both.

Seven specimens (3 %) were positive in the PCR and negative in the GenoType and 38 (18 %) positive in GenoType and negative in the PCR. The sensitivity and specificity for GenoType in comparison to culture was 80 % and 83 % respectively. The GenoType results corresponded to culture in 48 positive specimens and 114 negative specimens. Culture was positive in 12 specimens negative in Genotype and in 24 specimens the GenoType was positive with a negative culture result. The sensitivity and specificity of PCR compared to culture was 50 % and 94 % respectively. Of the discrepant results; in 30 specimens GenoType detected a single pathogen not detected by PCR and in a further 19 specimens more than one pathogen, of which only one pathogen (10) or no pathogen (9) was detected by PCR. In 7 specimens PCR detected a pathogen not detected by GenoType and in 8 specimens the amplification in GenoType was inhibited. In 10 specimens a pathogen was detected by PCR, which was not included in the GenoType panel. Of 24 culture negative and GenoType positive specimens, 19 were also negative in the PCR.

Culture	BacIdent		total (inh)	PCR		total
	pos	neg		pos	neg	
pos	48	12	60	32	32	64
neg	24	114	138	9	133	142
total (inh)	72	126	198 (8)	41	165	206

Conclusion:

The GenoType method is more sensitive in comparison to a broad-range PCR in detecting the 14 bacteria targeted when compared to culture. A limitation of the method is certainly the small range of bacteria targeted, however considering that the technique is designed to identify cultured pathogens, the sensitivity is surprisingly high. A larger multi-centre study may help identify specific pathogens missed by GenoType. The study also demonstrates the relative lack of sensitivity of broad-range PCR using universal 16s and 18s sequences compared to targeted PCR.

References

1.WHO. *Health statistics 2014.*
2.Funk, D.J. and A. Kumar. *Antimicrobial therapy for life-threatening infections: speed is life*. Crit Care Clin, 2011. 27(1): p. 53-76.

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