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Direct sequencing and RipSeq interpretation as a tool for identification of polymicrobial bacteremia

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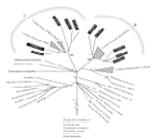
BACKGROUND AND AIM

Direct sequencing has become an important supplementary tool for identification of microorganisms in culture-negative infections. However, the combination of broad-range PCR and direct sequencing is not compatible with polymicrobial samples, as it gives mixed sequencing chromatograms. The commercially available tool RipSeq Mixed separates chromatograms resulting from up to three different species.
In a previous study, 293 blood samples were examined by cultivation based methods and direct sequencing for comparison. For 15 samples direct sequencing was invalid despite that one or more species were identified by cultivation.
In this study the chromatograms of these 15 samples were analyzed using RipSeq Mixed to see if this would affect the outcome of direct sequencing.

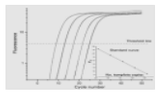
MATERIALS & METHODS

Cultivation
Aerobic and anaerobic blood cultivation was performed using the BACTEC 9240 system.

Direct 16S sequencing
The 3130xl Genetic Analyzer and MicroSeq system (Applied Biosystems) was applied on culture-positive samples.



Clone library
Establishment of 16S rRNA gene clone library and subsequent sequencing.



Quantitative PCR (qPCR)
Species specific determination of gene copy numbers.

CONCLUSION

- Analysis of sequencing chromatograms with RipSeq Mixed revealed DNA from 1-3 different bacterial species in all 15 samples where direct sequencing was initially invalid.
- RipSeq Mixed thereby improved the performance of direct sequencing considerably.
- Generally there is a risk of detecting clinically irrelevant DNA residing in the sample when applying DNA based methods. To make sure that only active microorganisms are detected, the less stabile RNA could be targeted instead of DNA. However, this study is based on culture-positive samples and therefore the findings are assumed to result from active bacteria.

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RESULTS

RipSeq analysis of the sequencing chromatograms revealed bacterial DNA in all 15 samples and for 12 samples (80%) DNA from at least two different species was identified. The results of the RipSeq analysis corresponded well with the cultivation data and the clone libraries, and real-time PCR confirmed several of the findings. However for some samples, bacteria were identified by one method that could not be confirmed by any of the other methods.

Table 1: Fifteen blood samples found to be polymicrobial by cultivation but negative by direct sequencing were analyzed by Ripseq Mixed. The RipSeq algorithm assigns a similarity score to each result, and results below 99.3% identity were left out. The RipSeq results are listed according to the score. For selected samples clone libraries were constructed and real-time PCR performed. "(an)" and "(ae)" indicate anaerobic and aerobic blood cultivation, respectively. "CoNS": Coagulase-negative staphylococci. "n.n.": nearest neighbor to the BLAST hits referred to as uncultured bacteria.

Sample	Cultivation	Direct sequencing and RipSeq Mixed	Clone library	Real-time PCR
1 (an)	Citrobacter freundii Enterococcus faecalis	1: C.freundii/gillenii/youngae/ Enterobacter asburiae 2: E. faecalis 3: Veillonella parvula	31 clones: 5 C. freundii, 3 E. faecalis, 3 V. dispar, 20 uncultured bacteria (n.n.: V. dispar, V. parvula and C. freundii)	Positive for E. faecalis
1 (ae)	C. freundii E. faecalis	1: C. freundii/gillenii 2: E. faecalis	Clone library not constructed	Positive for E. faecalis
2 (ae)	E. faecium CoNS	1: E. faecium	32 clones: 10 E. faecium, 9 Enterococcus sp., 13 uncultured bacteria (n.n.: E. faecium)	Positive for Staphylococcus sp.
3 (ae)	Klebsiella oxytoca Staphylococcus aureus Micrococcus sp.	1: Enterobacter cloacae/ludwigii/ K. oxytoca 2: S. aureus	20 clones: 2 Klebsiella sp., 7 Staphylococcus sp., 11 uncultured bacteria (n.n.: S. aureus)	Positive for S. aureus
3 (an)	K. oxytoca S. aureus	1: C. youngae/E. cloacae/ludwigii/ K. oxytoca 2: S. aureus	22 clones: 1 Klebsiella sp., 1 Staphylococcus sp., 11 uncultured bacteria (n.n.: S. aureus)	Positive for S. aureus
4 (ae)	E. cloacae Acinetobacter baumanii S. epidermidis Bacillus cereus	1: Aeromonas hydrophila 2: E. cloacae/hormachei	23 clones: 1 A. veronii, 1 Enterobacter sp., 6 Bacillus sp., 6 B. cereus, 3 B. anthracis, 6 uncultured bacteria (n.n.: E. hormaechei and E. cloacae)	Positive for Staphylococcus sp.
4 (an)	E. cloacae A. baumanii	1: V. dispar/parvula 2: E. coli/Shigella boydii	22 clones: 2 Enterobacter sp., 2 A. hydrophila, 5 Aeromonas sp., 13 uncultured bacteria (n.n.: A. hydrophila, E. hormaechei and E. cloacae)	Positive for Staphylococcus sp.
5 (an)	E. coli K. pneumonia E. faecalis	1: B. anthracis/cereus/thuringiensis 2: E. cloacae/hormachei/E. coli/albertii/ S. boydii/dysenteriae/sonnei 3: Kluyvera ascorbata	39 clones: 1 Escherichia sp., 6 Clostridium clostridioforme, 9 Veillonella sp., 5 V. dispar, 1 Enterococcus sp., 1 E. faecalis, 1 Clostridium sp., 15 uncultured bacteria (n.n.: V. parvula and C. clostridioforme)	Positive for K. pneumoniae Negative for E. faecalis
6 (ae)	Streptococcus mitis Gemella haemolysans	1: S. mitis/genomosp. C1/oralis/parasanguinis/sp. 2: S. oligofermentans 3: Streptococcus sp. (oral taxon 056)	40 clones: 1 S. parasanguis, 4 G. haemolysans, 3 Staphylococcaceae sp., 32 uncultured bacteria (n.n.: S. mitis, G. haemolysans, Granulicatella adiacens, S. parasanguis, Actinomyces sp.)	Not analyzed
6 (an)	S. mitis G. haemolysans S. salivarius	1: S. mitis 2: Abiotrophia para-adiacens 3: G. haemolysans	Clone library not constructed	Not analyzed
7 (an)	Propionibacterium acnes	1: P. acnes 2: S. aureus	Clone library not constructed	Negative for S. aureus and Staphylococcus sp.
8 (an)	S. epidermidis Candida albicans	1: S. epidermidis	Clone library not constructed	Positive for Staphylococcus sp. Negative for C. albicans.
9 (ae)	S. aureus Hemolytic streptococci grp. B	1: S. aureus 2: S. agalactiae	Clone library not constructed	Positive for S. aureus
10 (an)	K. pneumoniae E. faecium	1: K. pneumoniae	Clone library not constructed	Positive for K. pneumoniae
11 (ae)	S. oralis CoNS	1: S. mitis/oralis 2: S. epidermidis	Clone library not constructed	Not analyzed