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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



Direct 16S sequencing The 3130xl Genetic Analyzer and

MicroSeq system (Applied Biosystems) was applied on culture-positive samples.



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



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

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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.

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Sample	Cultivation	Direct sequencing and RipSeq Mixed	Clone library	Real-time PCR
1 (an)	Citrobacter freundii	1: C.freundii/gillenii/youngae/	31 clones: 5 C. freundii, 3 E. faecalis, 3 V. dispar, 20	Positive for E. faecalis
	Enterococcus faecalis	Enterobacter asburiae	uncultured bacteria (n.n.: V. dispar, V. parvula and C.	
		2: E. faecalis	freundii)	
		3: Veillonella parvula		
1 (ae)	C. freundii	1: C. freundii/gillenii	Clone library not constructed	Positive for E. faecalis
	E. faecalis	2: E. faecalis	,	
2 (ae)	E. faecium	1: E. faecium	32 clones: 10 E. faecium, 9 Enterococcus sp., 13	Positive for Staphylococcus sp.
	CoNS		uncultured bacteria (n.n.: E. faecium)	
3 (ae)	Klebsiella oxytoca	1: Enterobacter cloacae/ludwigii/	20 clones: 2 Klebsiella sp., 7 Staphylococcus sp., 11	Positive for S. aureus
	Staphylococcus aureus	K. oxytoca	uncultured bacteria (n.n.: S. aureus)	
	Micrococcus sp.	2: S. aureus	· · · · ·	
3 (an)	K. oxytoca	1: C. youngae/E. cloacae/ludwigii/	22 clones: 1 Klebsiella sp., 1 Staphylococcus sp., 11	Positive for S. aureus
	S. aureus	K. oxytoca	uncultured bacteria (n.n.: S. aureus)	
		2: S. aureus	, , , , , , , , , , , , , , , , , , , ,	
4 (ae)	E. cloacae	1: Aeromonas hydrophila	23 clones: 1 A. veronii, 1 Enterobacter sp., 6 Bacillus sp.,	Positive for Staphylococcus sp.
	Acinetobacter baumanii	2: E. cloacae/hormachei	6 B. cereus, 3 B. anthracis, 6 uncultured bacteria (n.n.: E.	
	S. epidermidis	Zi zi dioddde, normaener	hormaechei and E. cloacae)	
	Bacillus cereus		inormacener and ar diodedacy	
4 (an)	E. cloacae	1: V. dispar/parvula	22 clones: 2 Enterobacter sp., 2 A. hydrophila, 5	Positive for Staphylococcus sp.
	A. baumanii	2: E. coli/Shigella boydii	Aeromonas sp., 13 uncultured bacteria (n.n.: A.	Control of Stap (1) (Cooccas Sp.
	7t. Saamami	2. E. cony strigenta boyan	hydrophila, E. hormaechei and E. cloacae)	
5 (an)	E. coli	1: B. anthracis/cereus/thuringiensis	39 clones: 1 Escherichia sp., 6 Clostridium	Positive for K. pneumoniae
	K. pneumonia	2: E. cloacae/hormachei/E. coli/albertii/	clostridioforme, 9 Veillonella sp., 5 V. dispar, 1	Negative for E. faecalis
	E. faecalis	S. boydii/dysenteriae/sonnei	Enterococcus sp., 1 E. faecalis, 1 Clostridium sp., 15	ivegative for E. faccans
	L. Idecalis	3: Kluyvera ascorbata	uncultured bacteria (n.n.: V. parvula and C.	
		3. Mayvera ascorbata	clostridioforme)	
6 (ae)	Streptococcus mitis	1: S. mitis/genomosp.	40 clones: 1 S. parasanguis, 4 G. haemolysans, 3	Not analyzed
	Gemella haemolysans	C1/oralis/parasanguinis/sp.	Staphylococcaceae sp., 32 uncultured bacteria (n.n.: S.	ivot analyzed
	Gerriella Haerilorysans	2: S. oligofermentans	mitis, G. haemolysans, Granulicatella adiacens, S.	
		3: Streptococcus sp. (oral taxon 056)	parasanguis, Actinomyces sp.)	
6 (an)	S. mitis	1: S. mitis	Clone library not constructed	Not analyzed
o (an)	G. haemolysans	2: Abiotrophia para-adiacens	Clottle library flot constructed	ivot analyzeu
	S. salivarius	3: G. haemolysans		
7 (an)	Propionibacterium acnes	1: P. acnes	Clone library not constructed	Negative for S. aureus and
/ (all)	Propionibacterium acries	2: S. aureus	Clottle library flot constructed	Staphylococcus sp.
0 (0.0)	S. epidermidis		Clana library not constructed	Positive for Staphylococcus sp.
8 (an)	Candida albicans	1: S. epidermidis	Clone library not constructed	'''
9 (ae)	S. aureus	1: S. aureus	Clana library not constructed	Negative for C. albicans. Positive for S. aureus
J (ae)			Clone library not constructed	rositive for 5. dureus
10 (an)	Hemolytic streptococci grp. B	2: S. agalactiae	Clara library and an administrati	Desirius fault ausaussa'
	K. pneumoniae	1: K. pneumoniae	Clone library not constructed	Positive for K. pneumoniae
	E. faecium	1.0 111 / 11		
11 (ae)	S. oralis	1: S. mitis/oralis	Clone library not constructed	Not analyzed
	CoNS	2: S. epidermidis		