Evaluation of the eazyplex SuperBug CRE assay for the rapid detection of CTX-M genes as resistance markers in positive blood cultures

J. Rödel [1], B. Edel [1], S. Stoll [1], W. Pfister [1]

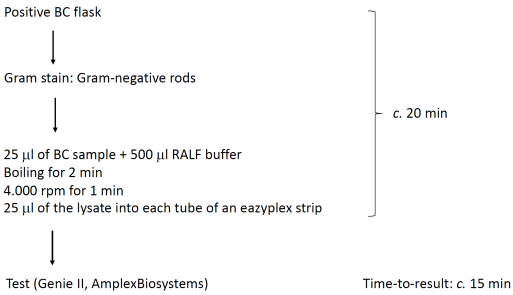
[1] Institut für Medizinische Mikrobiologie, Universitätsklinikum Jena, Erlanger Allee 101, 07747 Jena

Background

- Sepsis is a leading cause of death in intensive care units. Fast diagnosis of bloodstream infections including information on antibiotic resistance is crucial for the early initiation of a targeted therapy (1).
- Recently, multiplex PCR assay conducted directly from patient blood samples have been proposed to improve the time-to-result and sensitivity, compared to blood cultures (BCs) (2). However, high costs and too much hands-on time are not in line with the need of a fast and easy-to-perform assay that is usable in daily routine diagnostics.
- As an alternative solution the potential of BC diagnosis can be improved by application of rapid molecular biological tests to identify bacterial species and antibiotic resistance markers directly from positive BC flasks (3).
- In this study the eazyplex SuperBug CRE assay (AmplexDiagnostics, Gars-Bahnhof, Germany) was evaluated for the rapid identification of CTX-M β-lactamases from samples of BC flasks with growth of Gram-negative bacteria.

Methods

- Clinical samples: BCs submitted as part of routine patient care, March-July 2014
- BC system: BACTEC FX (BD Diagnostics, Heidelberg, Germany)
- Phenotypic antibiotic susceptibility testing (AST): Vitek 2 (bioMerieux, Nürtingen, Germany)
- **eazyplex SuperBug CRE (AmplexDiagnostics) Testing:**



- The eazyplex SuperBug CRE is a rapid NAAT based on isothermal amplification.
- Targets: CTX-M1-group, CTX-M9-group, OXA-48, VIM, KPC, NDM.

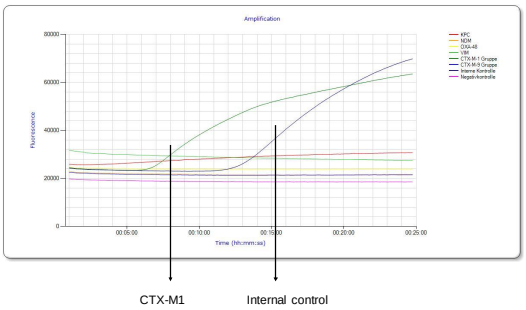
Results

- In all cases of *E. coli* and *K. pneumoniae* infections BCs that were tested positive for CTX-M1 by the SuperBug CRE were confirmed as ESBL strains by AST. For *K. pneumoniae* all CTX-M1 results were concordant with isolation of an 3MRGN strain. From one BC flask that were tested negative for CTX-M an *E. coli* strain with ESBL resistance was isolated. However, this strain was sensitive against ciprofloxacin (Table).
- *K. pneumoniae* strains with a negative CTX-M signal in the BC showed no single resistances against piperacillin/tazobactam or ciprofloxacin. In contrast such resistances were observed in non-ESBL strains of *E. coli* (Table).
- For the preparation of BC samples for tesing with the SuperBug CRE a minimum of hands-on time is required.
- The overall mean time to result of the isothermal amplification reaction was only 9 min for CTX-M and 12 min for the internal control (Table, Fig.).

Table. Identification of CTX-M genes from positive BCs by the SuperBug CRE test in comparison to AST

Species (n)	eazyplex SuperBug CRE			Time to positivity of BCs	Phenotypic AST			
	Time-to-result (min) Mean value (S.D.)				ESBL (n)	3MRGN (n)	Single resistances (n)	
	CTX-M1	CTX-M1 signal	Internal control				Pip/Taz	Cip
E. coli (10)	-	-	10,7 (3,7)	6,3 (6)	1/10	0	3	7
E. coli (8)	+	8,2 (3,1)	12,6 (4,5)	8 (7,7)	8/8	5/8	-	-
K. pneumoniae (2)	-	-	8,9	2,1	0/2	0/2	0	0
K. pneumoniae (4)	+	9,4 (3,6)	14,5 (5,7)	31,4 (29,7)	4/4	4/4	-	-

Fig. Isothermal amplification result by the SuperBug CRE



Conclusions

- The SuperBug CRE is a simple easy-to perform assay for the accurate detection of β-lactam resistance genes directly from positive BCs.
- The application of SuperBug CRE provides important resistance information one day earlier than the phenotypic AST.
- Under consideration of local resistance patterns a treatment recommendation based on the SuperBug CRE result for the implementation of a targeted antibiotic therapy in sepsis patients would be possible.
- To be usable in routine BC diagnosis the SuperBug CRE assay has to be combined with an appropriate tool for species identification directly from positive BCs.

References:

(1) Dellinger R.P. et al. 2013 Crit. Care Med. 41:580

(2) Fitting C et al. 2012. PLoS ONE 7:e38916

(3) Mancini N et al. 2014. J. Clin. Microbiol. 52:1242