Evaluation of a rapid molecular assay (EAZYPLEX® SuperBug) for the detection of genes encoding the prevalent carbapenemases in Isolates of the family *Enterobacteriaceae*

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Objective

To Evaluate a rapid commercial molecular assay (Eazyplex® SuperBug; ESB), based on real-time isothermal amplification, targeting genes encoding KPC, VIM, NDM and OXA-48 enzymes and extended-spectrum β-lactamase (ESBL) of the CTX-M variety in *Enterobacteriaceae*.

Methods

- Consecutive carbapenem-non-susceptible isolates of species of the family *Enterobacteriaceae,* recovered from various clinical specimens over a period of 6 months (April to September 2014) were chosen for the study.
- The presence of carbapenamase was tested for by the Hodge-test and combined disk tests containing imipenem and meropenem plus 3-aminophenyl-boronic acid or EDTA.
- PCR and sequencing were performed to detect the presence of various resistance genes.
- ESB qualitative assay was performed according to manufacturer's protocol. A positive result was defined as fluorescence signal above 10,000 and an amplification rate >0.007. Tests which gave equivocal results were repeated.

Results

- The combination of PCR and sequencing results was used as reference standard. A total of 88 clinical isolates with elevated MIC (>1µg/ml) to a carbapenem was studied. **See Table 1.** Of these, a carbapenemase was present in 49 (55.7%) and CTX-M type ESBL in 55 (62.5%).
- No false-negative results were observed using the ESB assay for NDM and VIM; the sensitivity and specificity were 100% and 100%, and 100% and 100%, respectively. Both positive and negative predictive values (PPV and NPV) were 100% each. **See Table 2**
- However, for detection of OXA-48, we encountered 5 isolates that gave repeatedly negative results while PCR sequencing was initially positive. Repeat of PCR sequencing was negative for these isolates. Thus, the sensitivity and specificity were 100% and 100%, respectively. ESB assay detected KPC (KPC-2 by sequencing) in 1 isolate. CTX-M-1 and CTX-M-9 were detected in 54 and 1 isolates, respectively but were all identified as CTX-M-15 by sequencing. **See Table 3**

Table 1: Number of CRE harboring a carbapenemase by Eazyplex SuperBug Assay and PCR sequencing

Organism (n)	ESBL & Carbapenemase detected									
	CTX-M (n=55)		NDM (n=24)		OXA-48 (n=16)		VIM (n=8)		KPC (n=1)	
	ESB	PCR	ESB	PCR	ESB	PCR	ESB	PCR	ESB	PCR
K. pneumoniae (46)	35	35	15	15	12	12	8	8	1	1**
E. coli (26)	16	16	5	5	2	2	0	0	0	0
E. cloacae (7)	2	2	3	3	2	2	0	0	0	0
E. aerogenes (1)	0	0	0	0	0	0	0	0	0	0
M. morganii (6)	1	1	1	1	0	0	0	0	0	0
S. marcescens (1)	0	0	0	0	0	0	0	0	0	0
Salmonella spp. (1)	1	1	0	0	0	0	0	0	0	0
Total	55*	55	24	24	16	16	8	8	1	1

ESB = Eazyplex Superbug assay; *CTX-M-1 =54; CTX-M-9 =1: All were CTX-M-15 by PCR sequencing; **ESB = KPC; KPC-2 by PCR sequenc ing

Table 2: Sensitivity and specificity of Eazyplex SuperBug Assay

S.No	Category	CTX-M (in %)	OXA (in %)	NDM (in %)	VIM (in %)	Sum total (in %)
1	Sensitivity	100.00	100.00	100.00	100.00	100.00
2	Specificity	100.00	100.00	100.00	100.00	100.00
3	Positive Predictive Value (PPV)	86.48	100.00	100	87.5	93.5
4	Negative Predictive Value(NPV)	95.65	100.00	100	100	98.91
5	Disease prevalence	61.66	24.41	25.42	11.86	37.19

Table 3: **Sensitivity and specificity of Eazyplex SuperBug Assay for detection of individual genes**

Gene	Category	Percentage
CTX-M	Sensitivity	100.00
	Specificity	100.00
KPC	Sensitivity	100.00
	Specificity	100.00
NDM	Sensitivity	100.00
	Specificity	100.00
OXA-48 family without 181	Sensitivity	100.00
	Specificity	100.00
VIM	Sensitivity	100.00
	Specificity	100.00

Conclusions

- The test system is a freeze dried ready to use amplification reaction which can be stored direct at workplace.
- During the amplification the results are shown in Real-Time.
- It demonstrated excellent sensitivity and specificity for the detection of NDM, VIM, OXA-48 and KPC. Thus, it shows very good results for the detection of the most prevalent carbapenemases produced in the family of *Enterobacteriaceae*.
- It also has an added advantage of being rapid – 20 min from sample-to-result.

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