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ESBL detection directly from urine samples with the novel isothermal amplification-based eazyplex® SuperBug CRE assay

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INTRODUCTION

The prevalence of multiresistant extended-spectrum beta-lactamase (ESBL)- producing *Enterobacteriaceae* has dramatically increased over the past years, particularly in urine samples. Direct and rapid detection of ESBL in urine of patients with suspected urosepsis would be of great clinical benefit. The eazyplex® SuperBug CRE assay (Amplex Biosystems, Germany) developed for use on Genie® II instrument (Optigene, UK) is based on isothermal amplification technique. Besides the most common carbapenemases KPC, NDM, VIM, OXA-48 and OXA-181, this assay detects the ESBL types CTX-M-1 and CTX-M-9 group comprising >90% of all ESBL strains in our hospital. Although primarily developed for use with rectal swabs and bacterial cultures, we evaluated this assay directly on urine samples.

MATERIALS AND METHODS

20 consecutive *Enterobacteriaceae* isolates from urine with suspected ESBL-production (based on their antimicrobial resistance profile in Vitek 2; bioMérieux) were tested with eazyplex® according to manufacturer's instructions. Shortly, a fraction of bacterial colony was picked with one microliter inoculation loop and resuspended in 500 µl of RALF buffer (resuspension and lysis buffer). The suspension was incubated at 99° C for 2 minutes enabling lysis of bacterial cells. 25 µl of suspension were pipetted in each of the 8 tubes of the test strip containing lyophilized ready-to-use mixture for isothermal amplification. ESBL production was confirmed by use of conventional phenotypic methods.

Simultaneously, the corresponding urine samples were tested following our newly developed protocol for direct testing from urine samples. Shortly, 1.5 ml of urine was centrifuged at 13000 rpm for 2 minutes, the supernatant discarded and 2 µl of the semifluid pellet resuspended in 500 µl of RALF buffer (if no or very little pellet present, 25 µl of the remaining fluid were taken). Further procedure was identical to the one described for bacterial isolates. To determine the analytical sensitivity, a series of ten-fold dilutions of a CTX-M-1-group-producing clinical isolate (*E. coli* 701541/14) in 0.9% NaCl solution was tested following urine protocol.

RESULTS

Table 1. Performance of eazyplex® SuperBug CRE on 20 bacterial isolates and corresponding urine samples

				eazyplex® SuperBug CRE			
				Bacterial isolate		Urine	
No.	Species	Phenotype	Quantity in urine culture/ml	ESBL type	Time (min:sec)	ESBL type	Time (min:sec)
1	*E. coli*	ESBL	10E6	CTX-M-1 group	04:30	CTX-M-1 group	05:45
2	*E. coli*	ESBL	10E6	CTX-M-1 group	04:45	CTX-M-1 group	05:30
3	*E. coli*	ESBL	10E5	CTX-M-1 group	04:30	CTX-M-1 group	08:45
4	*E. coli*	ESBL	10E4	CTX-M-1 group	04:45	CTX-M-1 group	09:00
5	*E. coli*	ESBL	10E6	CTX-M-1 group	05:00	CTX-M-1 group	05:15
6	*E. coli*	ESBL	10E4	CTX-M-1 group	04:45	CTX-M-1 group	11:00
7	*E. coli*	ESBL	10E4	CTX-M-1 group	04:30	CTX-M-1 group	10:45
8	*E. coli*	ESBL	10E4	CTX-M-1 group	04:54	CTX-M-1 group	08:45
9	*E. coli*	ESBL	10E6	CTX-M-1 group	05:15	CTX-M-1 group	07:30
10	*E. coli*	ESBL	10E6	CTX-M-1 group	04:45	CTX-M-1 group	07:00
11	*E. coli*	ESBL	10E6	CTX-M-1 group	04:30	CTX-M-1 group	06:45
12	*K. pneumoniae*	ESBL	10E6	CTX-M-1 group	05:00	CTX-M-1 group	06:30
13	*E. coli*	ESBL	10E6	CTX-M-9 group	05:45	CTX-M-9 group	06:30
14	*E. coli*	ESBL	10E5	CTX-M-9 group	06:00	CTX-M-9 group	12:45
15	*E. coli*	ESBL	10E6	CTX-M-9 group	05:15	CTX-M-9 group	05:45
16	*E. coli*	ESBL	10E5	CTX-M-9 group	04:45	CTX-M-9 group	08:15
17	*E. coli*	ESBL	10E4	CTX-M-9 group	05:30	CTX-M-9 group	12:30
18	*E. coli*	AmpC	10E5	negative		negative	
19	*E. coli*	AmpC	10E5	negative		negative	
20	*E. cloacae*	AmpC	10E5	negative		negative	

Table 2. Determination of analytical sensitivity by using a CTX-M-1-group-producing *E. coli*

No. of bacterial cells/ml	Time (min:sec)	Result
10E5	8:45	positive
10E4	11:00	positive
10E3	16:00	positive
10E2	0	negative

Out of 20 urine samples tested, 17 grew ESBL-producing *Enterobacteriaceae* (16 *E. coli*, 1 *K. pneumoniae*). eazyplex® correctly detected ESBL-encoding genes in all 17 ESBL-positive isolates and in the corresponding urine samples (12 CTX-M-1 and 5 CTX-M-9 group). The remaining 3 culture isolates and corresponding urine samples tested ESBL-negative in eazyplex® and phenotypic tests confirmed AmpC production (2 *E. coli*, 1 *E. cloacae*). Analytical sensitivity for testing with our newly developed urine protocole is between 10^3 and 10^2 bacterial cells/ml. Hands-on time for urine samples and culture isolates was approx. 3 minutes. The turnaround time was 7-16 minutes.

DISCUSSION / CONCLUSIONS

Our pilot study demonstrated that the eazyplex® isothermal amplification assay is able to detect the most common CTX-M ESBL types directly from urine samples within 16 minutes. These rapid results might be critical for the initiation of an adequate therapy in patients with urosepsis. Additionally, this test can be used for detection of the most common ESBL and carbapenemase genes in positive blood cultures, bacterial isolates and directly from rectal swabs.