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Evaluation of the BD MAX Check-Points CPO assay for the detection of carbapenemase producers directly from rectal swabs

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ABSTRACT

A novel real time multiplex PCR assay, BD MAX Check-Points CPO, was evaluated to detect carbapenemase-producing organisms in clinical settings on the BD MAX system. A total of 175 well-characterized isolates (including 123 carbapenemase-producers) and 128 rectal swab specimens (including 83 positives) of patients considered at “high-risk” for carriage of carbapenemase-producers were included. Bacterial suspensions were used to spike “true negative” rectal swabs to mimic a clinical sample. Fifty microliters of sample, containing either the spiked or the patient’s samples were processed. The BD MAX Check-Points CPO assay detected carbapenemases KPC, VIM/IMP, NDM, and OXA-48-like producers with a high sensitivity and specificity of 97.1 % and 98.8 %, respectively. Very rare variants of the IMP-type (IMP-11, -13, -14) and one rare and distantly related OXA-48 variant (OXA-535), remained undetected. With patients’ rectal swabs, sensitivity and specificity were 92.8% and 97.8%, respectively. Failure of detection was due to weak inoculum. The time to result was short: ca. 2 h30 for 12 samples (including extraction and PCR). The automated sample-in results-out platform is efficient, quick, and a easy to use tool for the detection of the main five carbapenemases. The lack of distinction between producers of VIM and IMP may be limiting in countries where these enzymes are widespread as in Asia, but not in France where IMP-producers are extremely rare.

INTRODUCTION

The global spread of carbapenemase-producing organisms (CPO; Enterobacteriaceae *Pseudomonas* and *Acinetobacter* species) is a major public health issue worldwide ¹⁻⁵. The most clinically-relevant carbapenemases belong to several Ambler classes of β -lactamases that differ by amino-acid sequence, structural features, and biochemical properties: class A (KPC-type), class B, i-e metallo- β -lactamases (MBLs such as IMP-, VIM- and NDM-types) or carbapenem-hydrolyzing class D β -lactamases (CHDL) such as OXA-48-like in Enterobacteriaceae, and OXA-23, OXA-40, OXA-58, and OXA-143 in *Acinetobacter* sp¹⁻⁵. The geographical distribution of CPEs is variable, but the same five main determinants are usually found at variable prevalence. KPC-producers have spread most extensively throughout the world, mostly in the United States, Greece, Italy, and Israel⁶⁻⁹. Metallo- β -lactamases, NDM enzymes initially associated with the Indian subcontinent, VIM with Italy, and IMP-1 with Japan, are now scattered around the world ⁷. OXA-48 carbapenemase initially reported in Turkey in 2001 ¹⁰ has subsequently spread in the Middle East, North Africa, Europe, and North America, whereas the closely related OXA-163 and OXA-181 variants have been reported in South America and India, respectively ¹¹⁻¹³.

Rapid identification of patients colonized with CPOs is mandatory to implement proper infection control measures to avoid further spread of these deadly bacteria to other hospitalized patients. Screening protocols are mainly based on cultures of rectal swab specimens on selective media ^{14,15}, followed by phenotypic tests to confirm a carbapenem-hydrolyzing activity, such as the Carba NP test ¹⁶, the rapid carbapenem inactivation method (rCIM) ¹⁷, lateral flow immunoassay ¹⁸, imipenem hydrolysis detected by MALDI-TOF¹⁹, BYG test ²⁰, and β -Carba ²¹ or disk diffusion synergy tests (DDST) for detection of MBLs and KPCs (eg, meropenem disks alone and meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid, or cloxacillin). High level temocillin resistance is a useful phenotypic trait for OXA-48 detection ²². Molecular based techniques such as PCR, and whole genome sequencing remain the gold standard for the precise identification of carbapenemase genes ²³. Culture of rectal swab

specimens followed by confirmation testing is long, and often not compatible with rapid implementation of reinforced hygiene measures.

Molecular methods are now available for detecting carbapenemase genes directly from clinical specimens in less than an hour. Several assays are commercially available. Some require DNA extraction from rectal swabs specimen prior to amplification such as the PCR-based Amplidiag CARBA+MCR assay (Mobidiag, Paris, France)²⁴ or the Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands)²⁵, whereas others do not require DNA extraction such as Hyplex SuperBug ID (Amplex Biosystems GmbH, Gießen, Germany)²⁶. Others are fully automated, such as CRE ELITE MGB kits on InGenius platform (Elitech, Les Ulis, France)²⁷ and GeneXpert Carba-R assay (Cepheid, Sunnyvale, CA)^{28,29}. Most of these assays target the ‘big 5’ carbapenemases: KPC, OXA-48-like, NDM, VIM, and IMP, representing more than 99% of the carbapenemases produced by Enterobacteriaceae in countries such as France⁷.

The BD MAX (BD Diagnostic Systems, Le Pont de Claix, France) is a fully automated system that combines extraction and real-time PCR. Here, we have evaluated the performance of the BD MAX Checkpoint CPO assay for the qualitative detection of the most widespread carbapenemase *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{OXA-48} genes directly from rectal swab specimens. The assay is designed for Enterobacterales as well as for non-fermenters such as *Pseudomonas* sp. and *Acinetobacter* sp.

Materials and Methods

Spiking of CPE negative rectal swabs

A total of 175 well-characterized isolates (including 123 carbapenemase-producers, CPs) were included. These CP isolates were as follows: Enterobacteriaceae (n=106) *Shewanella bicestris* (n=1), *Pseudomonas* sp. (n=11), and *Acinetobacter* sp. (n=5) (Table 1). The β -lactamase content of these isolates had been characterized by end-point PCR followed by Sanger sequencing²⁴ or by whole genome sequencing^{30,31}. Fifty-two non-CP or non-targeted carbapenemase-producers were also included as controls. “Non-targeted” carbapenemases” are

carbapenemases different from those detected by the BD MAX Check-Points CPO assay eg, IMI, NMCA, Sme, GES, FRI, LMB, TMB, GIM, AIM, SPM, DIM, OXA-198, OXA-23, OXA-40, and OXA-58 (Table 2).

Fresh overnight cultures on MH agar of the 175 well-characterized bacteria to be tested were used to prepare a bacterial suspension of 0.5 McFarland (inoculum of approximately 10^8 CFU/mL). These bacterial suspensions were then serially-diluted in sterile water, and with a final dilution into a negative rectal swab specimen (1 mL of transport media of true negative swab specimen, eSwab, Copan, Fisher Scientific, Illkirch, France) to obtain a concentration close to the limit of detection of each target, as recommended by the manufacturer to mimic true clinical samples. Thus, the final dilution starting from the 0.5 McFarland was 1/10 for non-CP, 1/1000 for KPC-producers, 1/5000 for VIM-producers, 1/7000 for IMP-producers, 1/400 for NDM-producers, and 1/5000 for OXA-48-producers.

Clinical prospective study on rectal swabs

The BD MAX Check-Points CPO assay was evaluated on 128 rectal swabs (including 83 positive as revealed either by positive culture on chromogenic selective media or GeneXpert Carba (Cepheid, Sunnyvale, CA) positive PCR of patients considered at “high-risk” for CP carriage according to the French national guidelines, eg, patients directly admitted from abroad, or previously hospitalized in areas with high prevalence of CPEs (require one screening upon admission) and contact patients of known carriers (require at least three screenings performed each a week apart). “High prevalence” corresponds to countries where some CPE are considered as endemic such as Greece, Israel, Latine America, and the US for KPC-producers, Asia, UK, and the United Arab Emirates for NDM-1, and Mediterranean countries for OXA-48 producers^{7,32}. Known carriers hospitalized were screened each week as a standard hygiene procedure, to follow their colonization/decolonization. The study included 45 negative samples (as revealed by negative culture on chromogenic selective media and by GeneXpert Carba PCR), as controls (Table 3). RESIST-4 O.K.N.V *K-Set* (Coris BioConcept, Gembloux, Belgium), single

immunochromatography test for quadruple and independent identification of OXA-48-like, KPC-like, NDM-like, and VIM-like carbapenemases was routinely used on colonies grown on ChromID CARBA SMART for rapid identification of carbapenemase-producers.

Fifty microliter from patient's rectal swab specimen were introduced into BD MAX sample tube buffer, mixed by vortexing, and processed by the BD MAX system, as recommended by the manufacturer. Total handling time was ca. 15 min for 12 samples simultaneously, including setting up of the BD MAX system. Thus, time to result time was 2h30 (including 15 min preparation time and 2 h 15 minutes running time for the automated extraction and amplification on the BD MAX system).

Real-time PCR assay with the BD MAX Check-Points CPO assay

The BD MAX Check-Points CPO assay is a mono-reagent, ready-to use, fully automated *in vitro* diagnostic multiplex real-time PCR assay to get an optimal detection of the five main groups of carbapenemase genes, i) *bla*_{KPC-like} class A carbapenemase genes, ii) *bla*_{NDM}, iii) *bla*_{VIM}, *bla*_{IMP} MBL class B carbapenemase genes, and iv) *bla*_{OXA-48-like} class D carbapenemase genes. Extraction is performed in the automate in unitary reagent cartridge containing all liquid reagents and disposable tips required for DNA extraction and dehydrated pellets containing magnetic beads of DNA affinity, protease reagents, and a multiplex real-time PCR Master Mix containing primers and TaqMan probes specific for carbapenemase genes and for control of sample processing (Table 2). An internal control is included at the extraction step to control the absence of PCR inhibitors in the eluted DNA.

Analysis was performed with the software included in the BD MAX system. Threshold cycle (C_T) values were shown for each channel of detection and the resulting content was clearly stated as positive or negative for each gene or gene couple (*bla*_{VIM}, *bla*_{IMP}).

Statistical analysis

The sensitivity and specificity value of the BD MAX Check-Points CPO assay were calculated with their respective confidence interval 95% (95%CI) using the free software available on the website for statistical computation (<http://vassarstats.net/>). Both GeneXpert and culture plus enrichment methods were taken-into-account for the statistical analysis of the performance of the BD MAX Check-Points CPO assay.

Results

Retrospective evaluation on a collection of well-characterized bacterial isolates

During this retrospective evaluation, the BD MAX Check-Points CPO assay was used as recommended by the manufacturer, on fresh overnight grown bacterial colonies that were diluted into negative rectal swabs to mimic true clinical samples. Among the carbapenemase producers, no lack of sensitivity was observed between Enterobacteriaceae, *Pseudomonas* sp. or *Acinetobacter* sp. (Table 1). The BD MAX Check-Points CPO assay efficiently detected 118/123 CPOs tested. The five CPOs that were not detected expressed three IMP-variants (ie., *Serratia marcescens* IMP-11, two *P. aeruginosa* IMP-13, and *A. baumannii* IMP-14) and an OXA-48-variant (OXA-535 from *Shewanella bicestria*). In multiple carbapenemase-producers, all resistance determinants were correctly identified (Table 1). All non-carbapenemase producers gave a negative result except OXA-163 and OXA-405 (Table 1), two OXA-48 variants lacking significant carbapenemase activity.

Using this strain collection (n=123), the overall sensitivity and specificity for the detection of the 5-main carbapenemases were 95.1% [95% confidence interval (95%CI) = 89.2% to 98.0 %] and 96.1% [95%CI = 85.7% to 99.3 %]. However, as claimed by the manufacturer, other rare carbapenemases such as GES-like carbapenemases (here GES-5), FRI-1, IMI, GIM-1, etc and *Acinetobacter* sp. carbapenemases (OXA-23, OXA-40, OXA-58, etc) were not detected (Table 1).

Prospective evaluation of the BD MAX Check-Points CPO assay on rectal swabs collected in the hygiene units of the hospital

During three months 83 positive and 45 negative rectal swabs were collected prospectively. Positivity of the swabs was defined as a growth of Enterobacteriaceae on ChromID CARBA SMART (bioMérieux, La Balme-les-Grottes, France) plates followed by confirmation of carbapenemase production using RESIST-4 O.K.N.V K-*SeT* and subsequent in house-PCR/sequencing. The BD MAX Check-Points CPO assay detected all KPC-, NDM-, VIM-, IMP-, and OXA-48–producers in clinical rectal swabs (Table 3). The most commonly found carbapenemase was OXA-48 (n=55) followed by NDM (n=19), and KPC-2 (n=7). The samples containing both *bla*_{OXA-48} and *bla*_{KPC-like} genes or *bla*_{OXA-48} and *bla*_{NDM} genes were equally well detected. Five samples (three NDM and two OXA-48–like) identified as positive by culture with only a few colonies growing on the ChromID CARBA SMART plate, and by GeneXpert Carba PCR were not detected by the BD MAX Check-Points CPO assay. Conversely, four samples containing non-cultured CPs were efficiently identified by the GeneXpert carba PCR and the by BD MAX Check-Points CPO assay. The overall sensitivity was then 92.8 % (CI95=84.3% to 97.0%). One non-cultured CP was only identified by the GeneXpert Carba system and another one only by the BD MAX Check-Points CPO assay (Table 3). Among the negative samples tested, the specificity was 97.8 % (CI95=86.8% to 99.9%) (Table 3).

Discussion

The BD MAX Check-Points CPO assay has been tested on i) a collection of 175 characterized isolates expressing various β -lactamases, including 52 non-producers or not-targeted carbapenemase-producers, and ii) 128 rectal swabs, including 83-positive specimens. As this assay is meant to detect CPOs directly on rectal swabs, carbapenemase producing *P. aeruginosa* and *Acinetobacter baumannii* isolates that may also colonize the digestive tract of patients were also included. BD MAX Check-Points CPO assay presents a sample-to-result of less than 2h30 minutes for 12 samples processed simultaneously.

Although most carbapenemase variants were efficiently detected by the BD MAX Check-Points CPO assay, four from our collection were not detected. OXA-535-producing *Shewanella bicestrii* is the progenitor of OXA-436, a distantly related OXA-48 variant responsible of an outbreak associated with several enterobacterial species in Denmark^{31,33}. IMP-13 and IMP-14 are distantly related IMP variants rarely identified in Enterobacteriaceae that pose identification problems with different assays such as GeneXpert Carba (Cepheid) and also with immunochromatographic assays for rapid detection of carbapenemases^{18,34}. Moreover OXA-163 and OXA-405, two OXA-48 variants that lack carbapenemase activity but that present strong expanded-spectrum hydrolytic activity were falsely detected by the BD MAX Check-Points CPO assay. These variants are not carbapenemases, but their nucleotide sequence is too similar to the *bla*_{OXA-48} gene to be distinguished by most molecular detection assays^{24,27,29}.

Accordingly, when extrapolating these results to the global French epidemiology, as represented by the carbapenemase genes identified by the French National Reference Center (F-NRC) for Carbapenem-resistant Enterobacteriaceae, excellent specificity and sensitivity might be expected for the detection of CPEs. Between 2012 to 2018 among the 9,468 CPE isolates sent to the F-RC for CPEs, the performances of the BD MAX Check-Points CPO assay would have 99.26 % sensitivity and 99.99 % specificity with 1 OXA-405-producer being falsely identified as CPE and 69 non-targeted CPEs would have been missed (54 IMI; 1 FRI-1; 2 GES; 10 OXA-23; 2 TMB-1; and 1 SME-4) (Data from the F-NRC; http://www.cnr-resistance-antibiotiques.fr/ressources/pages/Rapport_CNR_2017_VF_2.pdf, last accessed 08/28/19, and unpublished results). Similarly, when extrapolating our results to the global French epidemiology of CP *P. aeruginosa*, the BD MAX Check-Points CPO assay would have been able to detect 93% of CP *P. aeruginosa* isolates (148 VIM-, 15 IMP-, one KPC-, and three NDM-producers), missing only two DIM- and 10 GES-producers (Data from the F-NRC).

Taken together, our results obtained with the BD MAX Check-Points CPO assay on our collection of isolates, and when extrapolated to the global French epidemiology of CPOs, we

would expect (in the best way) 99.26 % sensitivity for CPE detection, 93% for CP *Pseudomonas* sp., but only 12.5 % for CP-*Acinetobacter* sp, as in that species OXA-23 accounts for the most prevalent carbapenemase.

Comparison of C_T values between the retrospective and the prospective study showed a marked difference in C_T values particularly between colonies (with C_T values <25) and rectal specimen (C_T values included in an interval of 20 to 35). The manufacturer did not indicate the limit of detection chosen for the interpretation by the software, but it can be speculated that this C_T value is around 40. Discrepant results between culture, GeneXpert, and BD MAX Check-Points CPO assay have been observed during this study. To maximize the chances to detect all CP-carriers, molecular and culture-based methods (including o/n enrichment) have been implemented in our hospital for the screening of patients considered as “high-risk” of colonization with carbapenemase producers. For this study, to test the performance of the BD MAX, among the “negative samples”, a BD MAX positive result that was not correlated with positive culture and/or a positive GeneXpert was considered false positive, thus giving a specificity of 97.8 % (CI95=86.8% to 99.9%). These culture negative, OXA-48 PCR positivity might be explained by the presence of *Shewanella* species (natural progenitor of OXA-48-like enzymes) or OXA-48 variants, such as OXA-244 that do not grow on neither side of the ChromID CARBA SMART medium³⁵. Thus, OXA-244 detection relies only on molecular tests directly from rectal swabs³⁵. In France OXA-244-producing *E. coli* are still rare but their prevalence increased between 2012 and 2018 from 0% to 1.9% (51 isolates), respectively. It is very likely that this number is an underestimate of the real prevalence, likely the result of under-detection, and silent spread of this resistant determinant (unpublished data).

Conclusion

Our study demonstrated that the BD MAX Check-Points CPO kit is well adapted to the detection of CPEs harboring one or several of the five main carbapenemases or of their variants, especially those encountered in France, which reflects the epidemiology of CPE in many

European countries. In addition, this assay is fully automated, with reduced hands on time and works directly on clinical samples, such as blood cultures and rectal swabs, with a time to results of less than three hours. As for other molecular assays, mutation and/or polymorphisms in the primer/probe binding region of the targeted gene may lead to false negative results, as it is the case with IMP-13-like enzymes. These variants are very rare among Enterobacteriaceae.

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Table 1. Global performances of the BD MAX Check-Points CPO kit on spiked “negative rectal swabs”.

Strains	β -lactamase content	BD MAX results (C _T values)			
		KPC	VIM/IMP	OXA-48	NDM
Non carbapenemase producers/ Non targeted carbapenemase producers (52)					
	Overexpressed cephalosporinase, ESBL				
<i>Enterobacteriaceae</i> (32) <i>Pseudomonas</i> sp.	IMI, NMCA, Sme, GES, FRI, LMB, TMB,	-*	-	-	-
(12) <i>Acinetobacter</i> sp.(8)	GIM, AIM, SPM, DIM, OXA-198, OXA-23, OXA-40, OXA-58				
<i>K. pneumoniae</i> (1), <i>S. marcescens</i> (1)	OXA-163, -405	-	-	23-31	-
Single carbapenemase producers (113)					
<i>Enterobacteriaceae</i> (23), <i>Pseudomonas</i> sp. (2)	KPC-2, -3, -5, -6, -7,-14, -28	25-38	-	-	-
<i>Enterobacteriaceae</i> (17), <i>Pseudomonas</i> sp. (2), <i>Acinetobacter</i> sp.(2)	NDM-1, NDM-4, NDM-5, NDM-7, NDM-9	-	-	-	32-39
<i>Enterobacteriaceae</i> (11), <i>Pseudomonas</i> sp. (3), <i>Acinetobacter</i> sp.(1)	VIM-1, VIM-2, VIM-4, VIM-19	-	29-35	-	-

Enterobacteriaceae (8), <i>Pseudomonas</i> sp. (2), <i>Acinetobacter</i> sp.(1)	IMP-1, IMP-8, IMP-10, IMP-11	-	29-33	-	-
Enterobacteriaceae (1)	IMP-11	-	-	-	-
<i>Pseudomonas</i> sp. (2)	IMP-13	-	-	-	-
<i>Acinetobacter</i> sp.(1)	IMP-14	-	-	-	-
Enterobacteriaceae (36)	OXA-48, -162, -181, -204, -232, -244, -370, - 372, -517, -519	-	28-34	-	-
<i>Shewanella bicestrii</i> (1)	OXA-535	-	-	-	-
<hr/> Multiple Carbapenemase producers (10) <hr/>					
Enterobacteriaceae (4)	NDM-1/ NDM-5 + OXA-48/OXA-181/OXA- 232	-	-	26-29	36-39
Enterobacteriaceae (1)	NDM-1 + VIM-2	-	33	-	39
Enterobacteriaceae (1)	OXA-181 + NDM-5 + VIM-1	-	32	31	37
Enterobacteriaceae (2)	OXA-48 + VIM-1/VIM-4	-	30-34	29-32	-

Enterobacteriaceae (1)	NDM-7+KPC-4	40	-	-	41
Enterobacteriaceae (1)	OXA-48, KPC-28	39	-	31	-

Sensitivity : 97.1 % [94.7 % to 99.6 %] and specificity : 98.8 % [97.3 % to 100 %] for targeted gene detection.

* -, no amplification.

Table 2. Properties of the dyes and channels used in the BD MAX Check-Points CPO kits.

Channel	Gene target	Excitation filter cwl (nm)	Detection filter cwl (nm)
1	<i>bla</i> _{KPC}	475	520
2	<i>bla</i> _{VIM} / <i>bla</i> _{IMP}	530	565
3	<i>bla</i> _{OXA-48-like}	585	630
4	<i>bla</i> _{NDM}	630	665
5	Internal Control	680	715

Table 3. Performance of the BD MAX Check-Points CPO on rectal swabs.

	BD MAX results (C _T values)					
	GeneXpert Carba/ RESIST-4 O.K.N.V (Coris)	BDMax	KPC	VIM/IMP	OXA-48	NDM
	Sequencing result					
Species isolated on ChromID CARBA SMART medium (n=83)						
<i>K. pneumoniae</i> (5)	KPC-2/-3	KPC	20-31	-	-	-
<i>A. baumannii</i> (3)	NDM-1	NDM	-	-	-	37-42
<i>E. coli</i> (6)	NDM-5	NDM	-	-	-	22-35
<i>E. coli</i> NDM + <i>R. ornithinolytica</i> NDM (1)	NDM-5	NDM	-	-	-	26
<i>E. coli</i> NDM + <i>R. ornithinolytica</i> OXA-48 (1)	NDM-5 + OXA-48	OXA48 + NDM	-	-	22	26
<i>K. pneumoniae</i> (7)	NDM-1	NDM	-	-	-	22-35

<i>neg</i> (1)	NDM	NDM	-	-	-	32
<i>K. pneumoniae</i> (2)	NDM-1	NEG	-	-	-	-
<i>A. baumannii</i> (1)	NDM-1	NEG	-	-	-	-
<i>neg</i> (1)	VIM	VIM/IMP	-	33	-	-
<i>E. coli</i> (16)	OXA-48	OXA-48	-	-	13-30	-
<i>E. coli</i> (1)	OXA-48	OXA-48 + KPC	44	-	16	-
<i>E. coli</i> + <i>C. freundii</i> (1)	OXA-48	OXA-48 + KPC	39	-	17	-
<i>K. pneumoniae</i> (1)	OXA-48	OXA-48 + NDM	-	-	28	40
<i>E. coli</i> + <i>K. pneumoniae</i> (10)	OXA-48/OXA-181	OXA-48	-	-	15-27	-
<i>E. cloacae</i> (4)	OXA-48	OXA-48	-	-	15-18	-
<i>E. cloacae</i> + <i>K. varicola</i> (2)	OXA-48	OXA-48	-	-	20-21	-
<i>K. oxytoca</i> (1)	OXA-48	OXA-48	-	-	31	-
<i>C. freundii</i> (2)	OXA-48	OXA-48	-	-	17-20	-
<i>C. freundii</i> + <i>K. oxytoca</i> (1)	OXA-204	OXA-48	-	-	16	-

<i>E. coli</i> + <i>K. oxytoca</i> (1)	OXA-204	OXA-48			20	
<i>K. pneumoniae</i> (9)	OXA-48/OXA-181	OXA-48	-	-	16-30	-
neg (2)	OXA-48-like	OXA-48	-	-	18-23	
neg (1)	OXA-48-like	NEG	-	-	-	-
<i>E. coli</i> (1)	OXA-181	NEG	-	-	-	-
<i>K. pneumoniae</i> (1)	OXA-48	NEG	-	-	-	-

Negative samples (n=45)

neg (44)	neg	neg	-	-	-	-
neg (1)	neg	OXA-48	-	-	35	-

Sensitivity: 92.8 % (84.3 % to 97.0 %)

Specificity: 97.8 % (86.8 % to 99.9 %)

Error rate : 3.6 % (two KPC and one NDM positives in samples containing OXA-48 producers, as detected by culture)

NB : one *E. coli* GeneXpert NEG BD MAX detected.