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Prospective evaluation of the Amplidiag® CarbaR+VRE assay for direct screening of carbapenemase producing Gram-negative bacilli from rectal swabs

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Abstract

This prospective study evaluated the ability of the qPCR Amplidiag® CarbaR+VRE assay to detect Carbapenemase-producing Gram-negative bacilli (CP-GNB) directly on 1,830 rectal swabs extracted using the fully automated platform Amplidiag® Easy instrument.

The Amplidiag® CarbaR+VRE assay gave a positive signal for 94 rectal swabs, whereas only 70 grew with CP-GNB on chromogenic media including 4 VIM-producing *P. aeruginosa*, 8 OXA-23-producing *A. baumannii* and 58 carbapenemase-producing *Enterobacteriaceae*. All the CP-GNB culture positive were detected by the Amplidiag® CarbaR+VRE assay. Twenty-four qPCR positive and culture negative samples were further investigated using targeted PCRs and subsequent DNA sequencing. Seventeen and seven of these were positive and negative with PCR/DNA sequencing, respectively. Taken together, the Amplidiag® CarbaR+VRE could detect carbapenemases directly from rectal swabs in 3h30 using a fully automated platform and showed high biological performances (sensitivity, specificity, negative and positive predictive values were 100, 98.6, 100 and 74.5%, respectively).

1. Introduction

Over the last decade, increasing resistance to carbapenems, the favored last resort drugs for treating multidrug resistant (MDR) Gram-negative bacterial (GNB) infections, is a serious threat to global health. Resistance is largely attributed to carbapenemases that are capable of hydrolyzing most β -lactams (including carbapenems) and are carried by diverse transferable mobile genetic elements (plasmids, transposons and integrons) (1,2). These elements usually carry multiple additional resistance genes that confer resistance to most non- β -lactam antibiotics and therefore greatly reduce treatment options. Consequently, infections caused by carbapenemase-producing Gram-negative bacilli (CP-GNB) are difficult to treat, associated with high mortality rates and costly to healthcare facilities (3).

OXA-48, NDM, KPC, IMP and VIM are the most common carbapenemases among carbapenemase-producing *Enterobacteriaceae* (CPE) with great geographic differences in the prevalence (4, 5). Currently, OXA-48 and its variants (OXA-48-like) are the most clinically-relevant carbapenemases in some Western European countries (France, Belgium and Spain) and are frequently found in *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* spp. (5).

Many hospital outbreaks of CP-GNB have been reported in several countries (5). Consequently, guidelines for the prevention and control of CP-GNB based on early active screening of asymptomatic carriers at hospital admission have been implemented in many settings (6-8). The early identification of colonized patients with CP-GNB combined with quarantine of these patients is the cornerstone of the prevention of outbreaks with CP-GNB (“search and isolate” strategy). The current process for detecting CP-GNB using rectal swabs is usually performed with chromogenic culture media containing antibiotics. However, culture-based methods for screening are time-consuming and require at least 18 h incubation to obtain growing bacteria, and additional time (going from a few minutes to several hours) to

confirm the presence of a CP-GNB. Moreover, some of these media are unable to detect OXA-48 and its variants because of low level hydrolytic activity against carbapenems (9-12). Therefore, molecular methods using multiplex real-time PCR (qPCR) assays have been recently developed to rapidly detect the most frequent carbapenemase genes from rectal swabs with shorter turnaround times (13-15). The aim of this study was to investigate the performance of the qPCR Amplidiag[®] CarbaR+VRE assay (Mobidiag, Paris, France), designed to simultaneously detect the most prevalent carbapenemases in *Enterobacteriaceae* (OXA-48-, NDM-, KPC-, VIM- and IMP-likes) and in *A. baumannii* (OXA-23, OXA-40, OXA-58, and OXA-51 with an upstream inserted *ISAbaI*), *vanA* and *vanB* genes directly from rectal swabs and, to assess the workability of this qPCR screening method in the context of a routine diagnostic laboratory that weekly processes around 400 rectal swabs. We focused the evaluation of the Amplidiag[®] CarbaR+VRE assay on the detection of the most common carbapenemases among GNB because Lille hospital is particularly impacted by CP-GNB outbreaks especially with CPE outbreaks while it is not the case for vancomycin-resistant enterococci (VRE). Consequently, the systematic screening for fecal carriage of VRE is not implemented in our hospital.

2. Material and methods

2.1 Settings and surveillance samples

This prospective study was conducted between November 2017 and February 2018 at the teaching hospital of Lille (2,965 beds). During this 4-month period, a total of 1,830 consecutive rectal swabs from 1,301 patients were collected according to hospital's CP-GNB standard infection control program. A mean of 1.40 rectal swab (range 1-7) were obtained per patient (one from 955 patients, 2 from 219 patients, 3 from 78 patients, 4 from 28 patients, 5 from 7 patients, 6 from 4 patients and 7 from 3 patients). Briefly, active screening for rectal

carriage of carbapenem-resistant organisms was performed among patients i) admitted to high-risk facilities for acquisition of such organisms (medical and surgical intensive care, burn, hematology and long-term care units), ii) admitted to healthcare facilities with known CP-GNB outbreak and iii) transferred from another hospital or from countries with high CP-GNB prevalence or identified as CP-GNB contact or with past history of CP-GNB colonization (6). These patients were preemptively isolated with additional contact precautions (staff wear gowns, single-room isolation, dedicated equipment) while waiting the fecal swab screening results. Sample collection was performed as follows: a single rectal swab in Amies medium (Transwab®, Sigma, Fallavier, France) were obtained on admission and then once a week until the patient's discharge. Within 24 h, the samples were directly plated (without pre-enrichment step in brain heart broth) on each side of the chromogenic chromID® CARBA SMART agar medium (bioMérieux®, Marcy l'Etoile, France) with the PREVI®Isola automated seeder system (bioMérieux) and analyzed by the multiplex real-time PCR Amplidiag® CarbaR+VRE assay, as recommended by the manufacturer.

2.2 Culture-based screening for CPE and drug susceptibility testing

Suspected carbapenem-resistant colonies were identified using MALDI-TOF mass spectrometry (Brüker Biotyper, Wissenbourg, France) and were submitted to susceptibility testing using the automated system Vitek 2 (BioMérieux). MICs of carbapenems were determined with E-tests (BioMérieux) and interpreted according the guidelines of the Committee for Antibigram of the French Society of Microbiology (CA-SFM) / European Committee on Antimicrobial Susceptibility Testing (EUCAST) and carbapenemase activity was detected by the β -carba™ test (BioRad, Marnes la Coquette, France). Specific rapid (15 min.) detection of OXA-48 and related enzymes, KPC and NDM carbapenemases production was carried out using immunochromatographic assay (ICT) Resist-3 O.K.N. K-SeT (Coris

Bioconcept, Gembloux, Belgium) because these 3 carbapenemases are predominant in *Enterobacteriaceae* isolated from our hospital. The Xpert Carba-R V2[®] assay (Cepheid, Maurens Scopont, France) was used for the detection of genes coding for VIM and IMP enzymes or for samples positive for growth on chromID[®] CARBA SMART medium but negative with ICT assay. *Bla*_{OXA-23} gene detection was performed using an in-house PCR directly on colonies of *Acinetobacter* spp. by using the following in-house designed primers OXA-23F (forward), 5'-TTCTGGTTGTACGGTTCAGCA-3' and OXA-23R (reverse) 5'-ATATCCATTGCCCAACCAGTC-3'.

2.3 Direct detection of carbapenemase genes in rectal swabs with Amplidiag[®] CarbaR+VRE assay

Briefly, on the day of the sample collection, 300 µL of Transwab[®] liquid was suspended in eNat[™] tubes (Copan Italia SpA, Brescia, Italy) and incubated for 30 min at room temperature. Then, the eNat[™] tubes were loaded on the fully automated platform Amplidiag[®] Easy for DNA extraction and PCR plate setup according to the manufacturer's instructions (Mobidiag, Helsinki, Finland). Forty-eight samples could be extracted in 2 hours and subsequently analyzed in one PCR run. The assay panel Amplidiag[®] CarbaR+VRE presented in Table 1 is a qPCR designed to detect carbapenemase genes *bla*_{OXA-48} like, *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM} as well as undifferentiated *Acinetobacter* oxacillinases: OXA-23, OXA-40, OXA-58 and OXA-51 with upstream inserted *IS*AbaI (*bla*_{AcOXA}), *vanA* and *vanB* genes (16, 17). Internal amplification control, positive and negative PCR controls are included in each PCR plate to provide assurance that clinical specimens have been successfully amplified. Total run time was 3h30 for DNA extraction (2 h) and for 45 cycles of amplification (1h30) as recommended by the manufacturer. Run files were automatically

exported from the PCR software to be interpreted by the provided Amplidiag® Analyzer software (Mobidiag).

2.4 Data analysis

A qPCR positive result was considered when a Ct value of < 45 was detected for at least one carbapenemase gene. True positive results were defined as a positive carbapenemase gene signal on direct qPCR assay if any of the carbapenemase targets were positive and were identical to that gene detected on colonies growing on the selective media. True negative results were defined as a negative carbapenemase gene signal on direct qPCR assay if all of the carbapenemase targets were negative and the selective culture did not grow with CPE. Discrepant results are classified as a result obtained using direct Amplidiag® CarbaR+VRE assay that did not agree with the reference-culture method on the same sample. The corresponding DNAs of discrepant rectal swabs were sent to Mobidiag in Finland for result confirmation. They were reanalyzed by simplex PCR on extracted DNA from specimens with the following PCR cycling parameters (1 min. at 95°C, followed by 45 cycles of 15 sec. at 95°C and 1 min. at 60°C). Then, direct DNA sequencing of target genes was performed using the same amplification primer set used in the Amplidiag® CarbaR+VRE assay (Patent N°EP2430188B1).

2.5 Statistical analysis

Sensitivity, specificity, negative and positive predictive values (NPV and PPV) of Amplidiag® CarbaR+ VRE assay were calculated for all samples tested by using the presence of CP-GNB on chromogenic culture as the reference standard. We applied the Youden index [(sensitivity + specificity)-1] to determine an optimal cycle threshold (Ct) cut-off of qPCR for

discriminating the true positives (18). The difference in mean Ct between true- and false-positive samples was analyzed using Student's t-test.

3. Results

3.1 Comparison of Amplidiag® CarbaR+VRE assay with culture

During the four-month study period, the presence of CP-GNB and carbapenemase genes were detected in 70 (3.8%) and 94 (5.1%) of the 1,830 rectal swabs using reference-culture method and qPCR Amplidiag® CarbaR+VRE, respectively (Figure 1). It should be noted that 4 and 8 rectal swabs grew with VIM-producing *Pseudomonas aeruginosa* and OXA-23-producing *Acinetobacter baumannii*, respectively on ChromID® CARBA SMART medium. Among the 58 rectal swabs culture positive with CPE, 19 yielded more than one microorganism, especially among the OXA-48 producers (Table 2). *K. pneumoniae* was the most frequently (n=34) isolated *Enterobacteriaceae* followed by *Escherichia coli* (n=17) and *Enterobacter cloacae* (n=9). Here, the most prevalent carbapenemase among *Enterobacteriaceae* screened by the both methods were OXA-48, followed by KPC and NDM (Tables 2 and 3). Metallo-β-lactamases VIM and IMP were not detected in *Enterobacteriaceae* isolates grown on CARBA SMART medium (Tables 2 and 3). With a Ct cut-off value of < 45, the qPCR Amplidiag® CarbaR+VRE detected all the 70 carbapenemase culture positive samples (sensitivity, 100%).

Twenty-four samples were negative by culture but yielded a positive qPCR result for at least one targeted carbapenemase gene: *bla*_{OXA-48} (n=13), *bla*_{KPC} (n=1), *bla*_{NDM} (n= 3), *bla*_{VIM} (n=5) and *bla*_{IMP} (n=2) and were classified as false positive results (specificity, 98.6%). The NPV value was excellent (100%) but the PPV value was lower (74.5%). Finally,

concordant results were found in 1,806 (98.6%) rectal swabs (70 positives and 1,736 negatives).

3.2 Discrepant results

The samples corresponding to the 24 discrepant results were reanalyzed by simplex PCR plus DNA sequencing and were reinoculated by manual direct plating of rectal swab on chromogenic medium. The status of these samples was unchanged and the subsequent culture was negative for CPE. Unfortunately, subsequent rectal swabs of the corresponding patients could not be performed because all these patients but one was discharged from hospital. Seventeen of these 24 samples (71%) were confirmed positive for a carbapenemase-encoding gene using the simplex PCR followed by DNA sequencing, and thus could correspond to true positives (Table 4). Of these 17 samples, three samples were collected from three patients with a past history of CPE carriage (2 OXA-48 and one NDM producers) and 5 were collected in wards in which outbreaks of OXA-48 producers were ongoing. The 24 qPCR positive and culture negative samples showed a significantly higher Ct values (mean Ct of 38.0 ± 4.4) than true-positives (mean Ct of 28.8 ± 5.6), $P < 0.0001$. The remaining 7 qPCR positive and culture negative samples could not be confirmed using simplex PCR and might indeed correspond to true negatives. Ct values for these 7 samples were significantly higher to those of 17 samples that could be confirmed by simplex PCR (41.0 ± 3.1 vs 36.5 ± 4.3 , $P = 0.008$). It is conceivable that these 7 samples were misclassified due to a non-optimal Ct cut-off. A slight decrease of the Ct cut-off value to ≤ 42 would improve the PPV (74.5% vs 76.9%) while sensitivity, specificity and Youden index (100%, 98.8% and 0.99, respectively) would be very high. Finally, implementation of qPCR allowed the detection of 17 additional samples positive for carbapenemase genes as compared to our culture-based method.

3.3 Use of qPCR Amplidiag® CarbaR+VRE in routine laboratory

The implementation of automated qPCR has proven to be well-suited for the routine of our laboratory which weekly processes up to 400 to 500 rectal swabs for rapid screening of CP-GNB among high risk patients. Indeed, the Amplidiag® Easy platform automates the nucleic acid extraction and PCR plates set up, enabling 48 samples screening in 3 h 30. The only manual handling is the transfer of the ready PCR plates into the thermocycler. When a first set of extraction was ready to run qPCR, another set of 48 samples could be automatically extracted. In practice, 2 to 3 series of 48 rectal swabs could be processed per day with a time to results of 3h30 vs 29 h for culture-based methods. In addition, the average costs of processing both positive and negative screens was quite similar between culture reference method (~ 9 €) and Amplidiag® CarbaR+VRE assay (11 €). This cost included both laboratory reagents and staff time to manage rectal swabs sent to laboratory.

4. Discussion

Since CP-GNB outbreak situations are recognized in our hospital (mainly OXA-48 producing *Enterobacteriaceae*), the workload due to active screening of rectal carriage of CP-GNB has significantly increased. Thus, the number of rectal swabs received in our lab has been multiplied five-fold between 2014 and 2017. Even with an automated streaking instrument, plating and reading plates are time-consuming and uses up significant space in our 35°C incubators. Recently, commercial real-time multiplex assays have been developed as screening tool for direct detection of carbapenemase genes in rectal swab (19-22). Here, we evaluated the performance of the qPCR Amplidiag® CarbaR+VRE with the automated Amplidiag® Easy platform. The most obvious advantage of qPCR over culture is the reduced time-to-results to define the patient's carbapenemase status as compared to culture-based

methods. In addition, the staff training requirements were minimal because specific competence in molecular biology was unnecessary. The training period for the technical staff was one day, including a description of the Amplidiag® Easy instrument, the software and handling.

In the present study, the qPCR Amplidiag® CarbaR+VRE assay demonstrated excellent sensitivity and NPV values (100%) and a specificity value close to 100%. In contrast, the PPV value was lower as reported in many studies performing molecular methods (13, 21, 23). Here, almost 30% of carbapenemase-positive results by qPCR were not confirmed by selective culture. The confirmation of the presence of carbapenemase genes by simplex PCR and DNA sequencing in most of the 24 false-positives suggested that these false-positives could be actually true-positives. A first explanation of this high number of false-positive results could be a better sensitivity of qPCR than culture. In the present study, this could be due to the direct plating of rectal swab on chromogenic media without pre-enrichment in broth which increases the rate of detection of MDR bacteria from rectal swabs (24, 25). We did not deliberately perform this enrichment step in order to be close to our routine conditions for the screening rectal swabs. Indeed, enrichment of culture entails a greater amount of work and thereby is not suited for the processing of weekly 450 rectal swabs. Moreover, this step delays individual culture results by one day which might be a disadvantage in the wards where patients are preemptively isolated with enhanced precaution contact measures.

Second explanation could be that, some variants of carbapenemases may be only detectable by qPCR and not on ChromID® CARBA SMART medium. As an example, variants OXA-244 (an OXA-48 variant with a low carbapenemase activity) does not grow well on the ChromID® CARBA SMART medium that we routinely use, whereas it can be detected by the PCR Amplidiag® CarbaR+VRE (26). It is therefore not excluded that a

number of false-positives OXA-48 may be related to the presence of this variant. A third explanation could be that the high rate of false positives may also result from the detection of carbapenemase genes present in other bacterial species from the transient digestive flora such as *Shewanella* spp., which has been accounted for the spread of OXA-48 in *Enterobacteriaceae*, or VIM and IMP- producing *P. aeruginosa* or *P. putida* (27, 28). The subsequent negative simplex PCR results in 7 of the 24 qPCR false-positives may result from sample degradation during freezing or laboratory errors. However, it should be noted that all these samples except two, yielded a low signal (Ct ranged from 40 to 44) and could be misclassified with the manufacturer's Ct value of < 45 . Studies conducted with automated qPCR have shown that decreasing the Ct value below that manufacturer's value is more suitable to reduce misclassification (false positive and false negative) (13). Oueslati *et al.* (16), used a Ct cut-off value of ≤ 35 by using the Amplidiag® CarbaR+VRE assay on bacterial colonies. Here, the PPV slightly increased without compromising the sensitivity with a higher Ct value (≤ 42). However, the selection of this cut-off value cannot be recommended because it has been set based on our local hospital epidemiology. Further studies are needed to assess the performance of Amplidiag® assay due to differences in prevalence of different types of carbapenemases in hospitals and geographical regions. In particular, the performance of the Amplidiag® CarbaR+VRE assay should be evaluated in settings with high prevalence of carbapenemase-producing non-fermenters.

Due to the high sensitivity and NPV values of qPCR, negative results can accurately rule out the presence of a colonizing CP-GNB on the day of admission. On the other hand, the PPV value $< 90\%$ did not allow patients to be clearly identified as positive CP-GNB carriers when qPCR was positive. The use of a molecular method alone seems unsuitable to define a positive patient in a low-prevalence area and therefore to implement recommended costly hygiene measures (isolation in single-bed room with designated nursing staff, staff

reinforcement and sometimes interruption of admissions) (7, 29, 30). Some authors have proposed to consider qPCR-positive and culture negative patients as negative CP-GNB carriers, thus maintaining only standard contact precautions and continue to monitor these patients with subsequent rectal swabs (15). Another alternative would be to consider these patients as suspect CP-GNB carriers, thus maintaining or implementing contact precautions and systematically inoculate on chromogenic agar plates qPCR positive samples. Since results of the qPCR is available on the day the swab is received, plating can be done on the same day without delay. Thus, following plating, either the culture is positive and the patient is therefore definitely considered as a true positive, or the culture is negative and the patient remains under standard contact precautions until the next rectal sampling. With this algorithm and due to the low prevalence of CPEs in our hospital (<5%), the number of swabs plated would decrease in our laboratory and therefore so would the cost and the technical time. The small price difference observed between culture-based method and Amplidiag® assay (~ 2 €) could be compensated by the reduction of time to results and therefore by the reduction of duration of additional contact precautions among patients preemptively isolated.

Finally, a combination of qPCR and culture of all qPCR positive samples may be the ideal workflow especially during cross-sectional screening of contact patients connected with a CP-GNB case-positive patient. Indeed, qPCR provides faster time-to-results and culture of qPCR-positive samples enables isolation of pathogens for further characterization. However, continuous surveillance of carbapenemases epidemiology is required because the sensitivity of Amplidiag® CarbaR+VRE assay may decrease if allelic variations within target genes occur or if some new or rarer carbapenemases become more prevalent in institutions.

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312 **Declaration of interest**

313 The funder did not contributed with the design of the study analyzing and interpreting the
314 results nor with the decision to submit the work for publication. The authors declare no other
315 conflicts of interest

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Table 1 the current panel of gene targets detected by the Amplidiag® CarbaR+VRE assay

Target group of genes	Reported results
OXA-48 and OXA-48 like: OXA-48, OXA-162, OXA-181, OXA-204, OXA-232, OXA-244, OXA-245, OXA-405	OXA-48/181
KPC-1 to KPC-24	KPC
NDM-1 to NDM-16	NDM
VIM-1 to VIM-21, VIM-23 to VIM-46	VIM
IMP-1 to IMP-35, IMP-37, IMP-38, IMP-40 to IMP-45, IMP-47, IMP-48, IMP-51, IMP-53	IMP
Oxacillinase from Acinetobacter:	
- OXA-23 and OXA-23 like: OXA-23, OXA-27, OXA-49, OXA-73, OXA-102, OXA-103, OXA-105, OXA-133, OXA-134, OXA-146, OXA-165, through OXA-171, OXA-225, OXA-239	AcOXA
- OXA-24/40 and OXA-24/40 like: OXA-24, through OXA-26, OXA-40, OXA-72, OXA-139, OXA-160, OXA-207	
- OXA-51 and OXA-51 with upstream inserted ISAbal: OXA-51, OXA-64 through OXA-71, OXA-75 through OXA-80, OXA-82 through OXA-84, OXA-86, through OXA-95, OXA-98, through OXA-100, OXA-104, OXA-106 through OXA-113, OXA-115 through OXA-117, OXA-120 through OXA-128, OXA-130 through OXA-132, OXA-138, OXA-144, OXA-148 through OXA-150, OXA-172 through OXA-180, OXA-194 through OXA-197, OXA-200 through OXA-203, OXA-206, OXA-208, OXA-216, OXA-217, OXA-219, OXA-223, OXA-241, OXA-242, OXA-248 through OXA-250, OXA-254.	
- OXA-58 and OXA-58 like: OXA-58, OXA-96, OXA-97, OXA-164	
<i>VanA</i>	<i>vanA</i>
<i>VanB</i> : <i>vanB1</i> , <i>vanB2</i> and <i>vanB3</i>	<i>vanB</i>

Table 2 Distribution of carbapenemase types in all bacterial species identified in chromogenic culture medium

Carbapenemase types (no)	Species(no)
OXA-48 (42)	<i>Escherichia coli</i> (9)
	<i>Klebsiella pneumoniae</i> (5)
	<i>Enterobacter cloacae</i> (4)
	<i>Citrobacter freundii</i> (3)
	<i>Klebsiella oxytoca</i> (2)
	<i>E. coli</i> + <i>K. pneumoniae</i> (5)
	<i>E. coli</i> + <i>K. oxytoca</i> (1)
	<i>K. pneumoniae</i> + <i>E. cloacae</i> (3)
	<i>K. pneumoniae</i> + <i>K. oxytoca</i> (3)
	<i>K. pneumoniae</i> + <i>C. freundii</i> (2)
	<i>K. oxytoca</i> + <i>C. freundii</i> (2)
	<i>E. coli</i> + <i>E. cloacae</i> (1)
	<i>E. coli</i> + <i>C. freundii</i> (1)
	<i>K. oxytoca</i> + <i>E. cloacae</i> (1)
KPC (10)	<i>K. pneumoniae</i> (10)
NDM (6)	<i>K. pneumoniae</i> (6)
VIM (4)	<i>P. aeruginosa</i> (4)
OXA-23 (8)	<i>A. baumannii</i> (8)

Table 3 Diagnostic performance of the Amplidiag® CarbaR+VRE assay as routine screening

tool for									direct
detection									of CP-
GNB in	Targets	TP	FP	TN	FN	Sensitivity	Specificity		rectal
swabs	OXA-48	42	13	1775	0	100	99.3		(Ct cut-
off value	KPC	10	1	1819	0	100	99.9		< 45).
	NDM	6	3	1821	0	100	99.8		
	VIM	4	5	1821	0	100	99.7		
	IMP	0	2	1828	0	-	99.9		

465	OXA _{ab}	8	0	1822	0	100	100
466	<u>TP: true positive, FP: false positive</u>						
	Overall	70	24	1736	0	100	98.6
467	<u>TN: true negative, FN: false negative</u>						

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Table 4 Discrepant results analysis and final interpretation using modified Ct value of ≤ 42 .

Samples	Target identified by		Discrepant analysis				
	reference-culture method	Amplidiag® CarbaR+VRE	Ct value of positive signal	Simplex PCR plus DNA sequencing	Final interpretation	Past history of CPE carriers	CPE outbreak in ward
1	None	NDM	33	NDM	True positive	No	No
2	None	NDM	32	NDM	True positive	Yes	OXA-48/181
3	None	NDM	37	NDM	True positive	No	No
4	None	OXA-48/181	32	OXA-48/181	True positive	Yes	OXA-48/181
5	None	OXA-48/181	38	OXA-48/181	True positive	Yes	OXA-48/181
6	None	OXA-48/181	39	OXA-48/181	True positive	No	OXA-48/181
7	None	OXA-48/181	40	OXA-48/181	True positive	No	No
8	None	OXA-48/181	42	OXA-48/181	True positive	No	OXA-48/181
9	None	OXA-48/181	42	OXA-48/181	True positive	No	OXA-48/181
10	None	OXA-48/181	37	OXA-48/181	True positive	No	OXA-48/181
11	None	OXA-48/181	40	OXA-48/181	True positive	No	No
12	None	OXA-48/181	38	OXA-48/181	True positive	No	No
13	None	OXA-48/181	38	OXA-48/181	True positive	No	OXA-48/181
14	None	VIM	38	VIM	True positive	No	OXA-48/181
15	None	VIM	33	VIM	True positive	No	No
16	None	VIM	29	VIM	True positive	No	No
17	None	VIM	30	VIM	True positive	No	No
18	None	IMP	40	No target gene	False positive	No	OXA-48/181
19	None	IMP	43	No target gene	False positive	No	OXA-48/181
20	None	KPC	36	No target gene	False positive	No	No
21	None	OXA-48/181	42	No target gene	False positive	No	OXA-48/181
22	None	OXA-48/181	44	No target gene	False positive	No	OXA-48/181
23	None	OXA-48/181	44	No target gene	False positive	No	OXA-48/181
24	None	VIM	38	No target gene	False positive	No	OXA-48/181

Figure 1. Results of CP-GNB culture and qPCR Amplidiag® CarbaR+VRE assay for 1830 rectal swabs screened between November 2017 and February 2018.