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Evaluation of the AID carbapenemase line probe assay for rapid detection and identification of carbapenemase genes in Gram-negative bacilli

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Objectives: This study evaluated the AID carbapenemase line probe assay (LPA) for the detection and identification of carbapenem resistance genes in Enterobacteriaceae and other Gram-negative bacilli (GNB) using bacterial cultures and DNA extracts directly from patient urine samples.

Methods: The AID carbapenemase LPA detects 13 different carbapenemase genes. Test probe accuracy was verified for using clinical Enterobacteriaceae isolates harbouring bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{GIM}, bla_{AIM}, bla_{SPM}, bla_{IMP} and bla_{OXA-48} and a well-characterized set of *Escherichia coli* DH5α strains transformed with the vector plasmid pUC57-*kan* harbouring bla_{BIC}, bla_{SIM}, bla_{DIM}, bla_{IMI-3}, bla_{IMI-1} and bla_{NMC-A}. Sensitivity and specificity was determined by testing 151 clinical GNB strains previously characterized for the production of carbapenemase activity and carbapenemase genes. Direct detection of carbapenemase genes using the LPA was determined using 299 clinical urine specimens. Analytical sensitivity for detection in urine was determined by testing serial dilutions of bla_{KPC} and bla_{NDM} in clinical *Klebsiella pneumoniae* strains.

Results: All carbapenemase gene probes showed 100% accuracy without cross-reactions. Sensitivity and specificity of the LPA using clinical isolates was 100% for each. Analytical sensitivity for detection of bla_{KPC} and bla_{NDM} in urine was 10^1–10^2 cfu. The LPA detected carbapenemase genes in 20 urines, which were confirmed in 12 samples by conventional multiplex PCR. Remarkably, 0 of the 20 urines grew carbapenemase-suspicious GNB applying EUCAST recommendations.

Conclusions: The AID carbapenemase LPA is an accurate, sensitive and easy-to-use test for the detection and identification of carbapenemase genes, which can readily be implemented in any diagnostic laboratory.

Introduction

Carbapenems are important antibiotics for the treatment of infections, particularly those caused by MDR Gram-negative bacilli (GNB). However, an increasing prevalence of carbapenem-resistant Enterobacteriaceae clinical strains is recorded worldwide.[1–4] The emergence and spread of carbapenemase-producing Enterobacteriaceae over the past decade represents a serious issue in the hospital environment.[5,6] In general, carbapenemases hydrolyse all classes of β-lactams and are resistant to commercially available β-lactamase inhibitors except for avibactam, which recently became commercially available and inhibits class A, class B and some class D carbapenemases.[7] Carbapenemase genes are usually located on plasmids, facilitating an efficient intra- and inter-species dissemination.[8] Carbapenemases are categorized into three classes: A (penicillinases), B (metallo-β-lactamases) and D (oxacillinases).[9,10] The currently most prevalent carbapenemases are OXA-48 (class D), *Klebsiella pneumoniae* carbapenemase (KPC) (class A, non-metallo) and the metallo-carbapenemases (class B) including IMP, VIM and New Delhi metallo (NDM) carbapenemases. In several Mediterranean countries, such as Italy, Turkey and Greece, carbapenemase-producing Enterobacteriaceae are considered to have become endemic.[4]

Rapid detection and accurate identification of carbapenemase-producing Enterobacteriaceae is of the utmost relevance for an efficient therapy, adequate hospital hygiene measurements and knowledge on epidemiological spread.[11] Currently carbapenemase activity is mostly tested for by phenotypic testing of bacterial isolates using diffusion or dilution antibiotic susceptibility testing methods for screening and confirmation.[12] Alternatively, the

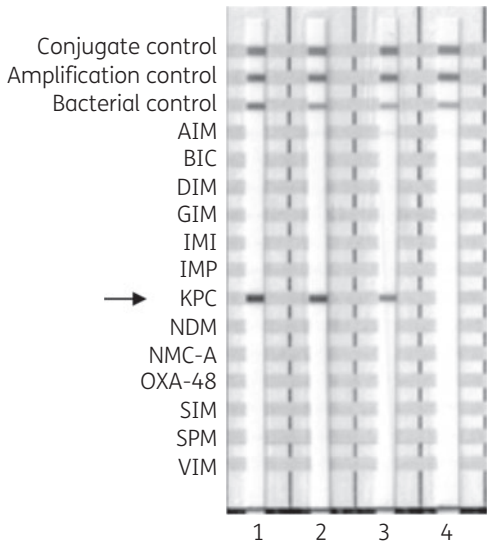

Figure 1. Analytical sensitivity of the AID carbapenemase LPA test for detection of a *K. pneumoniae* bla_{KPC} strain in urine. Urine was spiked with bacterial cells of a *K. pneumomiae* bla_{KPC} clinical strain and extracted using the Qiagen EZ1 extraction machine. Lanes 1, 2, 3 and 4: dilution series corresponding to amounts of DNA extracted from 10^4, 10^3, 10^2 and 10^1 cfu of *K. pneumomiae* bla_{KPC}, respectively.

RAPIDEC® CARBA test can be used for biochemical identification of carbapenemase activity.[13,14] Phenotypic susceptibility testing for carbapenemases allows limited differentiation and identification of carbapenemase genes and it is complicated by both the frequent presence of multiple β-lactamases in a single bacterial strain, e.g. ESBLs, AmpCs and carbapenemases, and different levels of β-lactamase gene expression. Genotypic testing for the presence of carbapenemase genes is increasing since it allows simultaneous detection and accurate identification of different carbapenemase genes by multiplex PCR and is independent of culturing.[11] One of the first multiplex carbapenemase PCR test consisting of three endpoint multiplex PCRs was described in 2011.[15] Several other tests were developed, which are based on macroarray detection or RT–PCR, both requiring expensive laboratory equipment.[14,16–20]

Recently, a new line probe assay (LPA) was designed by Autoimmun Diagnostika (AID) GmbH (Germany), which detects 13 carbapenemase genes, among which are the most prevalent types encountered in Enterobacteriaceae (Figure 1), e.g. KPC, OXA-48, NDM and VIM. Genetic detection by LPA is based on reverse hybridization and requires limited technical resources in the clinical microbiology laboratory, thereby facilitating its implementation. LPAs are widely used in the clinical microbiology laboratory, for example for the detection of mutations associated with antibiotic resistance in the *Mycobacterium tuberculosis* complex, the detection of *mecA/mecC*-based methicillin resistance in MRSA or the detection of ESBL genes in Enterobacteriaceae.[21–25] The aim of this study was to evaluate the specificity and sensitivity of the AID carbapenemase LPA for detection of carbapenemase genes from cultures of clinical Enterobacteriaceae and other GNB isolates. This study also intended to evaluate its use for direct detection in clinical urine specimens.

Methods

Strains and urine samples

A collection of non-duplicate, non-outbreak, clinical GNB strains were used to determine sensitivity and specificity of the AID carbapenemase LPA. One hundred and forty-two strains were isolated from clinical specimens of patients from tertiary and secondary care hospitals in the Zurich metropolitan area (Switzerland, Europe) from 2011 to 2014. Previously, we have analysed these 142 isolates phenotypically and genotypically for the production of carbapenemase activity and carbapenemase genes.[12,26] In this clinical strain set, 32 of 142 isolates were carbapenemase positive. The remaining 110 clinical strains were phenotypically characterized as non-carbapenemase producers and genetically confirmed as carbapenemase-negative by multiplex PCR. Two additional clinical isolates carrying bla_{IMP} (*Acinetobacter baumannii*) and bla_{GIM} (*Pseudomonas aeruginosa*), respectively, were made available by the National Reference Centre for Gram-negative Hospital Pathogens, Bochum, Germany). Finally, seven clinical strains carrying bla_{IMP} (three *Enterobacter cloacae* complex isolates), bla_{SPM} (three *P. aeruginosa* isolates) and bla_{AIM} (one *P. aeruginosa* isolate) were sent to us by courtesy of Dr Mark Toleman. In summary, the tested collection of in total 151 strains consisted of 41 carbapenemase-positive strains (27%) and 110 carbapenemase-negative strains (73%).

A set of 299 urine samples from patients with a suspicion for urinary tract infection (UTI) were directly investigated for the presence of carbapenemase genes. The urine samples were also analysed for the presence of carbapenemase-producing GNB strains using standard culture methods and antibiotic susceptibility testing of culture isolates as described previously.[12,27] In brief, 1 μL of urine samples in sodium borate/formate containing BD Vacutainer (BD, Franklin Lakes, NJ, USA) was plated on to each of Columbia 5% sheep blood agar (bioMérieux SA, Marcy-l'Étoile, France), chromogenic UriSelect™ 4 agar (Bio-Rad Laboratories, Hercules, CA, USA) and Columbia colistin/nalidixic acid agar with 5% sheep blood (bioMérieux SA). Following manual inoculation with a quantitative streaking pattern agar plates were analysed after 1 and 2 days of incubation. The sample evaluation algorithm resembled the diagnostic approach as suggested by Cumitech guidelines.[28] Urine samples were clinically scored culture positive when a cfu count of $>10^4$/mL for potential pathogens was observed and the sample was not considered as contaminated, i.e. presence of not more than two different microorganisms. Meropenem disc diffusion from pure subcultures was used to phenotypically test for carbapenemase activity in GNB using the EUCAST screening cut-off of 25 mm.[12]

Synthetic PCR amplicons and transformation into Escherichia coli DH5α

Synthetic PCR amplicons were generated for carbapenemase genes that were not available to us from clinical isolates. Genes for bla_{BIC} (885 bp; GenBank accession number GQ260093.1), bla_{SIM} (741 bp; GenBank accession number GQ288397.1), bla_{DIM} (756 bp; GenBank accession number KC004136.2), bla_{IMI-1} (879 bp; GenBank accession number JX090311.1), bla_{IMI-3} (879 bp; GenBank accession number GU015024.1) and bla_{NMC-A} (879 bp; GenBank accession number Z21956.1) were synthesized and introduced into the multiple cloning site of plasmid pUC57-*kan* (GenScript, Piscataway, NJ, USA). Plasmids were transformed into *E. coli* DH5α cells using standard laboratory methods.[29]

AID carbapenemase LPA

The AID carbapenemase LPA contains probes for the detection of 13 carbapenemase-encoding genes (i.e. bla_{AIM}, bla_{BIC}, bla_{DIM}, bla_{GIM}, bla_{IMI}, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{NMC-A}, bla_{OXA-48}, bla_{SIM}, bla_{SPM} and bla_{VIM}) (Figure 1 and Table 1). The AID carbapenemase LPA was performed according to the manufacturer's instructions. Briefly, DNA was extracted from bacterial cultures or clinical urine samples followed by PCR amplification. DNA was

Table 1. Verification analysis of the AID carbapenemase LPA using carbapenemase-producing GNB or synthetically made carbapenemase genes

Bacterial strain/synthetic DNA	AID carbapenemase LPA containing probes for 13 carbapenemase genes													Seq./multiplex PCR confirmed[a]
	AIM	BIC	DIM	GIM	IMI	IMP	KPC	NDM	NMC-A	OXA-48	SIM	SPM	VIM	
P. aeruginosa bla_{AIM}	+	−	−	−	−	−	−	−	−	−	−	−	−	positive
Synthetic DNA bla_{BIC}	−	+	−	−	−	−	−	−	−	−	−	−	−	positive
Synthetic DNA bla_{DIM}	−	−	+	−	−	−	−	−	−	−	−	−	−	positive
E. cloacae bla_{GIM}	−	−	−	+	−	−	−	−	−	−	−	−	−	positive
Synthetic DNA bla_{IMI-1} and bla_{IMI-3}	−	−	−	−	+	−	−	−	−	−	−	−	−	positive
E. cloacae bla_{IMP}	−	−	−	−	−	+	−	−	−	−	−	−	−	positive
K. pneumoniae bla_{KPC}	−	−	−	−	−	−	+	−	−	−	−	−	−	positive
K. pneumoniae bla_{NDM}	−	−	−	−	−	−	−	+	−	−	−	−	−	positive
Synthetic DNA bla_{NMC-A}	−	−	−	−	−	−	−	−	+	−	−	−	−	positive
K. pneumoniae bla_{OXA-48}	−	−	−	−	−	−	−	−	−	+	−	−	−	positive
Synthetic DNA bla_{SIM}	−	−	−	−	−	−	−	−	−	−	+	−	−	positive
P. aeruginosa bla_{SPM}	−	−	−	−	−	−	−	−	−	−	−	+	−	positive
E. cloacae bla_{VIM}	−	−	−	−	−	−	−	−	−	−	−	−	+	positive

+, signal band on the LPA; −, no signal observed.
[a]The presence of the carbapenemase gene was confirmed by a carbapenemase multiplex PCR[15] or sequence analysis of the synthetic carbapenemase genes.

extracted from bacterial cultures using the InstaGene Matrix (Bio-Rad, Reinach, Switzerland). DNA from urine samples was extracted using the Abbott RealTime m2000sp instrument (Abbott, Baar, Switzerland). In the case of synthetically made carbapenemase genes, 200 ng of plasmid DNA was used for testing the specificity of the AID carbapenemase LPA. The PCR mixture was used for reverse hybridization with the carbapenemase probes present on the nitrocellulose strip followed by signal detection of hybridized biotinylated PCR amplicons. Five units of FastStart Taq Polymerase (Roche Diagnostics, Rotkreuz, Switzerland) were used per PCR.

Ethics

The research was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Results

Accuracy of the AID carbapenemase LPA

To verify the accuracy of the probes present in the AID carbapenemase LPA (Figure 1) a set of selected, phenotypically and genotypically well-characterized clinical carbapenemase-positive GNB strains ($n = 8$) and a set of cloned synthetic carbapenemase genes ($n = 5$) for which corresponding clinical strains were not available was used (Table 1).

The LPA detected PCR amplicons representing carbapenemase genes with 100% accuracy (Table 1).

Sensitivity and specificity of the AID carbapenemase LPA in clinical strains

The sensitivity and specificity of the AID carbapenemase LPA was studied using clinical GNB strains ($n = 151$; 56 *E. cloacae*, 29 *K. pneumoniae*, 21 *E. coli*, 11 *Enterobacter aerogenes*, 8 *Citrobacter freundii*, 6 *Hafnia alvei*, 5 *P. aeruginosa* and 15 isolates of other species; Table 2). Results showed an excellent performance of the AID carbapenemase LPA, with a sensitivity of 100% and a specificity of 100% for carbapenemase gene detection (Table 2).

Detection of carbapenemase genes in urine samples using the AID carbapenemase LPA

A total of 299 consecutive clinical urine samples were directly tested for the presence of carbapenemase genes with the AID carbapenemase LPA. These urine samples had been submitted to the clinical laboratory for routine cultural testing as UTIs were suspected. Urine samples were scored clinically UTI-positive when $>10^4$ cfu/mL GNB were detected (standard cut-off for bacterial culture used in our clinical laboratory). Fifty-one of 299 samples (17.1%) were found positive for growth of GNB. The 54 GNB were identified as *E. coli* ($n = 42$), *K. pneumoniae* ($n = 10$), *E. cloacae* complex ($n = 1$) and *Citrobacter koseri* ($n = 1$). Three of the 51 positive samples showed growth of two GNB species, i.e. *E. coli* and *K. pneumoniae* ($n = 2$) and *E. coli* and *C. koseri* ($n = 1$). Standard disc diffusion antibiotic susceptibility testing using meropenem discs did not indicate carbapenemase activity in these 54 isolates.

The analytical sensitivity for detection of *K. pneumoniae* strains carrying bla_{KPC} or bla_{NDM} (two of the most prevalent carbapenemase genes) in urine was determined by spiking to be in the range of 10^1–10^2 cfu (data for *K. pneumoniae* bla_{KPC} shown in Figure 1). Testing the DNA extracts of the 299 urines with the carbapenemase LPA resulted in 20 carbapenemase-positive urines. Signals were found for bla_{KPC} (six), bla_{NMC-A} (five), bla_{AIM} (three), bla_{BIC} (three), bla_{GIM} (two), bla_{DIM} (one), bla_{SIM} (one) and bla_{VIM} (one) (Table 3). One urine was positive for three carbapenemase genes, i.e. bla_{BIC}, bla_{AIM} and bla_{NMC-A} (2013262916). Twelve of the 20 positive samples (60%) were confirmed by the carbapenemase multiplex PCR test,[15] which was used as an independent verification test (Table 3). The other eight samples could not be confirmed by this multiplex PCR although signals on the LPA were visible. For one LPA-positive and multiplex PCR-positive sample, a KPC-producing

Table 2. Testing of 151 clinical Enterobacteriaceae, *A. baumannii* and *P. aeruginosa* strains using the AID carbapenemase LPA

		Strains (*n*) detected with a carbapenemase gene as detected by a multiplex carbapenemase PCR[15] and the AID carbapenemase LPA[a]												
Bacterial strains[b]	*n*	bla_{AIM}	bla_{BIC}	bla_{DIM}	bla_{GIM}	bla_{IMI}	bla_{IMP}	bla_{KPC}	bla_{NDM}	bla_{NMC-A}	bla_{OXA-48}	bla_{SIM}	bla_{SPM}	bla_{VIM}
P. aeruginosa	5	1			1								3	
A. baumannii	2	1					1							
E. cloacae	56				1	1	3	1						3
Enterobacter sp.	1							1						
E. coli	21							1			3			
K. pneumoniae	29							6	1		5			1
E. aerogenes	11													1
C. freundii	8													2
Providencia rettgeri	1								1					
H. alvei	6													
Pantoea sp.	1													
Proteus mirabilis	2							1	1					
Serratia marcescens	4													
Citrobacter sp.	1													
Providencia stuartii	1								1					
Enterobacter asburiae	1													
Citrobacter amalonaticus	1													
Total	151	2	0	0	2	1	4	10	4	0	8	0	3	7

[a]Total DNA was extracted from cultured strains and used for PCR.
[b]Bacterial species: *P. aeruginosa*, *A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *E. aerogenes*, *C. freundii*, *P. rettgeri*, *H. alvei*, *P. mirabilis*, *S. marcescens*, *P. stuartii*, *E. asburiae* and *C. amalonaticus*.

K. pneumoniae was detected by culture in a parallel sample from the same patient (Table 3). For 3 of the 20 bacterial isolates with a positive LPA, but a negative culture (1 *K. pneumoniae* and 2 *E. coli*), meropenem disc diffusion showed a borderline susceptibility to meropenem (inhibition zone diameters of 25, 27 and 28 mm, respectively; EUCAST carbapenemase screening cut-off <25 mm) indicating the potential presence of a low-expressed carbapenemase gene (Table 3).

Discussion

Rapid detection and accurate identification of carbapenemase genes is crucial for proper antibiotic therapy, to monitor resistance epidemiology and to promptly initiate hospital hygiene procedures. Although carbapenemase strains can be detected by phenotypic screening, integration of genotypic methods is often faster and thus facilitates cost-effective and accurate carbapenemase detection.

Several advantages of commercial tests as compared with laboratory 'in-house' developed tests are observed, i.e. their manufacturing process is highly standardized and quality controlled and they provide lot number tracking with a guaranteed shelf-life for laboratory accreditation purposes and protocols optimized for practical use. Several commercial test assays for the genetic detection of carbapenemase genes were recently introduced, e.g. Check-Direct CPE kit (Check-Points, Wageningen, The Netherlands), the eazyplex® Superbug CRE test (Amplex, Giessen, Germany), the Xpert MDRO test (Cepheid, Sunnyvale, CA, USA) and the LightMix modular carbapenemase kit (Roche, Rotkreuz, Switzerland). Although these assays are relatively easy to perform and macroarrays can be used for testing many probes in parallel, all four test systems require a dedicated RT–PCR, individual readers and special software for detection and interpretation of the results. In addition, the costs per test are relatively high for the above-mentioned assays particularly when used for screening.

In this study we evaluated the performance of a new commercially available carbapenemase LPA developed by AID, which contains probes for 13 carbapenemase genes, i.e. bla_{AIM}, bla_{BIC}, bla_{DIM}, bla_{GIM}, bla_{IMI}, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{NMC-A}, bla_{OXA-48}, bla_{SIM}, bla_{SPM} and bla_{VIM} genes.

The advantages of LPAs are that: (i) they are easy to perform and faster than conventional diagnostic tests; (ii) they are already commonly used in the molecular diagnostic laboratory; (iii) apart from a 'simple' PCR machine for performance of endpoint PCRs they do not require additional sophisticated equipment; and (iv) they provide specific genetic detection, even directly from clinical samples (without culturing the pathogen). A disadvantage of LPAs is that the amount of probes is restricted to ~15–20 probes, whereas an array can contain more probes. As with every LPA, the AID carbapenemase LPA is limited to the detection of genes, for which primers and probes are present in the assay.

Our results show that the LPA accurately detected all genes for which probes were present in clinical isolates or which were synthesized on plasmids and transformed into *E. coli* (Table 1). No false-negative or false-positive result was observed for any of the carbapenemase probes.

Table 3. Detection of carbapenemase genes in urine samples prospectively collected in the clinical laboratory

Sample number	AID carbapenemase LPA result	Carbapenemase multiplex PCR result[15]	Culture result	Meropenem zone diameter (EUCAST screening cut-off <25 mm)	Culture from parallel clinical sample results
2013262714	KPC	KPC	*K. pneumoniae*	25 mm, imipenem, ertapenem, second- and third-generation cephalosporin susceptible	negative
2013261833	KPC	KPC	*E. coli*	27 mm, imipenem and ertapenem susceptible, ESBL producer	negative
2013261183	KPC	KPC	negative	NA	*K. pneumoniae* KPC-positive (meropenem diameter 14 mm)
2013263519	NMC-A	NMC-A	negative	NA	negative
2013263533	NMC-A	NMC-A	negative	NA	negative
2013262920	NMC-A	negative	negative	NA	negative
2013262916	AIM, BIC, NMC-A	BIC	negative	NA	negative
2013262938	NMC-A	negative	negative	NA	negative
2013262732	BIC	negative	negative	NA	negative
2013262765	KPC	negative	negative	NA	negative
2013262761	AIM	AIM	negative	NA	negative
2013262763	AIM	AIM	negative	NA	negative
2013263280	BIC	negative	negative	NA	*P. aeruginosa* (meropenem diameter 26 mm)
2013263245	DIM	negative	negative	NA	negative
2013262426	KPC	negative	negative	NA	negative
2013262262	GIM	GIM	negative	NA	negative
2013262510	SIM	SIM	negative	NA	negative
2013262436	GIM	GIM	negative	NA	*Klebsiella oxytoca* (meropenem diameter 34 mm)
2013263249	VIM	VIM	negative	NA	negative
2013260983	KPC	negative	*E. coli*	28 mm, imipenem and ertapenem susceptible, ESBL producer	negative

NA, not applicable (as no GNB were cultured).

In the second part of this study, we analysed the specificity and sensitivity of the carbapenemase LPA by testing a collection of 151 clinical strains. All carbapenemase genes present in the set of 151 clinical GNB strains were correctly identified, resulting in 100% sensitivity and specificity of the LPA for the detection of carbapenemase genes in culture isolates (Table 2).

In the third part, we extracted DNA from 299 urine samples initially submitted to our clinical laboratory for the standard bacteriological analysis of UTIs for the direct detection of carbapenemase genes in clinical specimens. As most UTIs originate from the local bacterial flora, and as urogenital–gastrointestinal carriers of carbapenemase-producing Enterobacteriaceae can thus be detected by testing urine specimens,[30] we questioned if direct molecular testing of urine samples would be more rapid, accurate and sensitive than classical urine culture regarding the detection of carbapenemase producers. In addition, low cfu counts ($<10^4$/mL) in urine samples are usually classified as clinically irrelevant and such isolates are not further tested for antibiotic susceptibility thereby missing potential carbapenemase carriers. Our results show that molecular testing is probably more sensitive than standard urine culture for the detection of carbapenemase-harbouring GNB (Table 3). The lower sensitivity of culture can partly be attributed to non-growth or dismissal of low cfu counts, which are classified as clinically non-relevant in checking for UTIs, but even in samples with growth of GNB (Table 3) that were considered clinically important ($\geq 10^4$ cfu/mL) the LPA seemed to display superior sensitivity. A limitation of this study is that we could not completely verify carriers of carbapenemase-producing GNB that were positive in the LPA only, but negative in culture. Except for one positive LPA, we did not detect carbapenemase-producing GNB in parallel routine samples of these putative carbapenemase carriers (e.g. urines, wound swabs, respiratory specimens). However,

routine urine samples to test for UTIs were used in this study, and a specific carbapenemase-carrier screening taking parallel samples from other body sites had not been performed. Nevertheless, 12 of 20 samples were confirmed by an independent multiplex PCR pointing to a true-positive result (bla_{KPC} $n = 3$, $bla_{NMC\text{-}A}$ $n = 2$, bla_{BIC} $n = 1$, bla_{AIM} $n = 2$, bla_{GIM} $n = 2$, bla_{SIM} $n = 1$ and bla_{VIM} $n = 1$) and, for 1 of these 12 samples, the patient was found to carry bla_{KPC} in a parallel sample by culture and phenotypic and genotypic confirmation testing. Our results indicate that direct molecular LPA testing is very sensitive, but, to assure adequate specificity/positive predictive value, solely LPA-positive results should be followed by collection of other specimens to confirm, phenotypically and genotypically, the presence of a carbapenemase. The confirmed detection of more rarely encountered carbapenemase types such as $bla_{NMC\text{-}A}$, bla_{BIC} and bla_{AIM} with the LPA only may indicate that those carbapenemase types are so far underdetected by standard culture methods.

Another limitation of this study was the relatively small number of carbapenemase types that are rarely encountered in our epidemiological setting, e.g. AIM, GIM, IMP and SPM, or that are generally encountered very rarely, and for which we had to synthesize plasmids containing the corresponding genes (BIC, DIM, IMI, NMC-A, SIM).

In conclusion, the AID carbapenemase LPA is a rapid tool for the accurate detection of the most commonly occurring carbapenemase genes in Enterobacteriaceae, *P. aeruginosa* and *A. baumannii* isolates. It can be used either as a screening tool or for confirmation in the case of inconclusive phenotypic test results. This assay is easy to use and can readily be implemented in any diagnostic laboratory possessing a PCR machine for performance of endpoint PCRs and a water bath for reverse hybridization of PCR amplicons. In addition, direct testing of urine specimens may enhance sensitivity of carrier screening.

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Transparency declarations

None to declare.

Author contributions

G. V. B. and M. H. designed the study. A. B.-K., J. T., C. M., E. D. and C. R. were responsible for the microbiological work-up of the samples. G. V. B., A. B.-K. and M. H. did the data mining. G. V. B., A. B.-K. and M. H. analysed the data. G. V. B., A. B.-K., P. M. K. and M. H. wrote and revised the manuscript.

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