Screening for Carbapenemases in Ertapenem-Resistant *Enterobacteriaceae* Collected at a Tunisian Hospital Between 2014 and 2018

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Background: Carbapenem-resistance is frequently detected in *Enterobacteriaceae* isolated from patients in Tunisia. The study was performed to identify frequent carbapenemases in Tunisian isolates.
Methods: Between May 2014 and January 2018, 197 ertapenem-resistant *Enterobacteriaceae* were isolated at the microbiological department of the Military Hospital of Tunis. The strains were phenotypically characterized and then subjected to in-house polymerase chain reaction (PCR) targeting the carbapenemase genes bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{AIM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{BIC}, and bla_{OXA-48}.
Results: The assessed 197 ertapenem-resistant *Enterobacteriaceae* from Tunis comprised 170 *Klebsiella pneumoniae*, 19 *Enterobacter cloacae*, 6 *Escherichia coli*, 1 *Citrobacter sedlakii,* and 1 *Enterobacter asburiae*. Thereby, 55 out of 197 isolates (27.9%) were from blood cultures, suggesting a systemic disease. The carbapenemase gene bla_{OXA-48} quantitatively dominated by far with 153 detections, followed by bla_{NDM} with 14 detections, which were distributed about the whole study interval. In contrast, bla_{BIC} and bla_{VIM} were only infrequently identified in 5 and 3 cases, respectively, while the other carbapenamases were not observed.
Conclusions: The carbapenemase gene bla_{OXA-48} was identified in the vast majority of ertapenem-resistant Tunisian *Enterobacteriaceae* while all other assessed carbapenemases were much less abundant. In a quantitatively relevant minority of isolates, the applied PCR-based screening approach did not identify any carbapenemases.

Keywords: carbapenemase, *Enterobacteriaceae*, Mediterranean, Tunisia, epidemiology, military hospital, resistance

Introduction

The worldwide distribution of carbapenemases was recently summarized by the Center for Disease Dynamics, Economics & Policy (CCDEP) in a map based on a literature review (https://cddep.org/tool/global_distribution_carbapenemases_enterobacteriaceae_country_and_region/, last accessed 7th January 2019). According to this summary, endemic – i.e., nationwide distributed – carbapenemases comprise bla_{KPC} in America, bla_{IMP}, bla_{KPC}, bla_{OXA}, and bla_{VIM} in Europe, bla_{KPC}, and bla_{OXA} in Africa, and bla_{IMP}, bla_{KPC}, bla_{NDM}, and bla_{OXA} in Asia. Focusing on Northern Africa, endemicity was reported for bla_{OXA} in Morocco, and regional spread for bla_{NDM} in Egypt, while occasional occurrence or sporadic outbreaks were described for bla_{KPC} in Morocco, Algeria, Libya, and Egypt, for bla_{NDM} in Morocco, Algeria, Tunisia, and Libya, and for bla_{OXA} in Morocco, Algeria, Tunisia, and Libya, as well as for bla_{VIM} in Algeria, and Tunisia. In a recent review on carbapenemase-producing bacteria in Africa [1], prevalence in the hospital setting ranged from 2.3% to 67.7% in North Africa and from 9% to 60% in sub-Saharan Africa.

Focusing on Northern African Tunisia, resistant Gram-negative rod-shaped bacteria are frequently isolated in Tunisian hospitals. Knowledge on prevalent resistance mechanisms helps to mitigate the resistance problem by allowing a sound choice of first line antibiotic drugs as demonstrated on a Tunisian intensive care unit (ICU) during a study from 2006 [2]. Starting in the 1980s, the problem of multidrug-resistance in Gram-negative pathogens in Tunisia has emerged from resistance against penicillins and cephalosporins towards substantial carbapenem resistance in Gram-negative rod-shaped bacteria [3]. Although a lot has been published on multidrug-resistance in Gram-negative bacteria in patients from war- and crisis-haunted neighboring Libya [1, 4–18], the resistance problem in Tunisia started much earlier than the Libyan civil war. As early as in the late 90s of the last century, already 2% imipenem resistance was observed in Gram-negative pathogens in sepsis patients at a Tunisian Hospital [19]. In *Enterobacteriaceae*, which were isolated from blood cultures in Sfax between 1993 and 1998, 27.7% resistance against 3rd-generation-cephalosporins was observed [20]. In a study from 2006 on a pediatric and neonatal ICU, 85% *Klebsiella pneumoniae* isolates were reported to be multidrug-resistant [21]. In a similar study from 2007, 87% *Klebsiella pneumoniae* isolates were

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positive for an extended spectrum beta-lactamase (ESBL). In the same study, this species accounted for 19.5% nosocomial blood stream infections [22].

More up-to-date studies from Tunisian hospitals suggest resistance in Gram-negative isolates at an ongoing high level. In a collection of 113 *Escherichia coli* strains, which were isolated at two Tunisian hospitals between 2004 and 2012, 46 isolates (40.1%) were positive for the extended-spectrum beta-lactamase (ESBL) gene $bla_{CTX\text{-}M\text{-}15}$ [23]. Within a period of 16 months from 2015 till 2016, 18 carbapenem-resistant *Enterobacteriaceae* and Gram-negative nonfermentative rod-shaped bacteria were isolated at a hospital in South-East-Tunisia. Identified carbapenemase genes comprised $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}23}$, $bla_{OXA\text{-}48}$, and $bla_{VIM\text{-}2}$ [24]. During an assessment at a hospital in Tunis for about 3 months in 2014, 19 Gram-negative strains with resistance against 3rd generation cephalosporins were isolated from rectal swabs of 14 out of 31 patients (45.2%), one week after admission to hospital. Of note, rectal swabs of the same patients had been negative at the time of admission, indicating either nosocomial transmission as suggested by the authors of the study [25] or, alternatively, selection under the selective pressure of antibiotic treatment [26]. Identified ESBL genes comprised $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, and $bla_{GES\text{-}2}$, while also carbapenemase genes, namely, $bla_{GES\text{-}11}$, $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}23}$, and $bla_{OXA\text{-}48}$ were identified [25].

In the here described study, we used 3 previously described multiplex polymerase chain reactions (PCRs) to screen for 11 carbapenemase genes [27] in 197 ertapenem-resistant *Enterobacteriaceae,* which were isolated at the Military Hospital of Tunis between 2014 and 2018.

Methods

Assessed Strains. Assessed strains comprised 197 *Enterobacteriaceae,* which were isolated from inpatients and outpatients at the Military Hospital of Tunis between 28th of May 2014 and 17th of January 2018. Both screening isolates and strains, which were isolated from sample material in the case of clinical suspicion of an infection, were included in the study. Multiple isolates from the same patient and body site were excluded. Cultures were processed, applying standard laboratory practices, and, once pure culture was obtained on blood agar plates, strains were identified according to the operating procedures of the Military Hospital of Tunis. This included microscopy of Gram-stained preparations and biochemical analysis using the VITEK 2 system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. Initial resistance testing was performed using the VITEK 2 AST 235 cards. Interpretation of resistance testing was based upon the interpretation standards of EUCAST, version 2016. Bacteria from single colonies were stored in microbank cryogenic vials (Mast Diagnostica, Germany) at −70 °C. Prior to matrix-assisted laser desorption/ionization (MALDI–TOF) mass spectroscopy (MS) analysis in a Biotyper Microflex LT mass spectrometer (Bruker Daltonik, Bremen, Germany) for re-identification of the 197 *Enterobacteriaceae* at the Bundeswehr Institute of Microbiology in Munich, all strains were grown aerobically on blood agar plates at 37 °C for 24 h and checked for purity.

Screening PCRs for Carbapenemases. Culture material of the strains was boiled in 0.5-mL volumes of PCR-grade water at 95 °C for 10 min to inactivate the bacteria and release sufficient amounts of DNA. The resulting suspension was used for PCR without additional nucleic acid extraction.

Three multiplex PCRs targeting the carbapenemase genes bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{AIM}, bla_{DIM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{BIC}, and $bla_{OXA\text{-}48}$ were performed on a Biometra T Professional cycler (analytik jena, Jena, Germany) as described before [27] with minor modifications. In detail, these modifications comprised the use of HotStar Taq Mastermix 2x (Qiagen, Hilden, Germany) and the use of concentrations of each primer of 0.4 µmol/L in 25-µL volumes of the reaction mix. Cycling conditions were 10 min at 95 °C followed by 36 cycles denaturation at 94 °C for 30 s, annealing at 50°C for 40 s, and amplification at 72 °C for 50 s, then followed by final elongation at 72 °C for 5 min with subsequent cooling to 4 °C. Visualization of the amplicons was performed using a FlashGel system (Lonza, Basel, Switzerland). Well-characterized positive control strains (bla_{IMP}, bla_{VIM}, bla_{DIM}, bla_{GIM}, bla_{KPC}, $bla_{BIC,}$ and $bla_{OXA\text{-}48}$) provided by the Robert-Koch-Institute, Berlin, Germany, or the Laboratory of Patrice Nordmann at the Unité de Microbiologie Médicale et Moléculaire, Université de Fribourg, Switzerland, or purchased plasmids (bla_{SPM}, bla_{AIM}, bla_{NDM}, and bla_{SIM}) were used as the positive controls for the PCRs. The sequence inserts of the plasmids, which were integrated into pEX-A2 vector backbones (Eurofins Genomics, Brussels), are provided in the supplementary material 1.

Ethics

All procedures performed in this study were in accordance with the national Tunisian and German ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The protocol for the retrospective and investigator-blinded study was considered and approved by the Ethics Committee of the Medical Association of Hamburg, Germany (registration number WF-023/18).

Results

Strain Characteristics. The *Enterobacteriaceae* with resistance against ertapenem, which were isolated at the Military Hospital of Tunis between May 2014 and January 2018, comprised *Citrobacter sedlakii* (n = 1), *Enterobacter asburiae* (n = 1), *Enterobacter cloacae* (n = 19), *Escherichia coli* (n = 6), and *Klebsiella pneumoniae* (n = 170). At the time of the isolation events, the patients were treated at different wards of the Military Hospital in Tunis (see Table 1). Sites of isolation comprised anal swabs (n = 35), axillary swabs (n = 2), a swab from a not-further defined location (n = 1), materials from broncho-alveolar lavages (n = 6), blood cultures (n = 55), cytobacteriological examinations of sputum (CBES) (n = 5), cerebrospinal fluid (n = 1), exudates from the ear (n = 2), biofilms at endotracheal tubes (n = 3), gastric samples (n = 1), catheters (KT) (n = 19), oral swabs (n = 1), protected tracheal sampling materials (PTS) (n = 13), pus (n = 18), a medical device (n = 1), and urine (n = 34).

Molecular Screening for Carbapenemases. The PCR-based screening identified the carbapenemase genes $bla_{OXA\text{-}48}$ in 153 stains (76.7%), bla_{NDM} in 14 strains (7.1%), bla_{BIC} in 5 strains (2.5%), and bla_{VIM} in 3 strains (1.5%). Thereby, all 3 strains with bla_{VIM}, 1 out of 5 strains (20%, *E. cloacae*) with bla_{BIC}, and 1 out of 14 strains (7.1%, *K. pneumoniae*) with bla_{NDM} were also positive for $bla_{OXA\text{-}48}$. In total, one or more carbapenemases could be identified in 170/197 strains (86.3%) using the applied molecular screening approach. Details on the characteristics of the strains with the carbapenemase genes $bla_{OXA\text{-}48}$, bla_{NDM}, bla_{BIC}, and bla_{VIM} are provided in Table 1.

Of note, 107 out of 197 assessed *Enterobacteriaceae* had been subjected to the "Hyplex® Superbug ID system" (amPLEX Diagnostics, Gars-Bahnhof, Germany), targeting the carbapemase genes bla_{VIM}, bla_{IMP}, $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}48}$, and bla_{KPC}, in a previous assessment [28]. From those

Table 1. Characteristics of isolates with the carbapenemase genes bla_{OXA-48}, bla_{NDM}, bla_{BIC}, and bla_{VIM}

Identified carbapenemase	bla_{OXA-48}	bla_{NDM}	bla_{BIC}	bla_{VIM}
Total number (percentage)	153 (76.5%)	14 (7.0%)	5 (2.5%)	3 (1.5%)
Years of isolation	2014 (n = 44), 2015 (n = 38), 2016 (n = 56), 2017 (n = 13), 2018 (n = 2)	2014 (n = 2), 2015 (n = 1), 2016 (n = 5), 2017 (n = 5), 2018 (n = 1)	2014 (n = 3), 2016 (n = 2)	2014 (n = 3)
Species[a]	*C. sedlakii* (1/1), *E. asburiae* (1/1), *E. cloacae* (14/19), *E. coli* (4/6), *K. pneumoniae* (133/170)	*K. pneumoniae* (14/170)	*E. cloacae* (5/19)	*K. pneumoniae* (3/170)
Departments/ units[b]	Cardiologic ward (1/1), cardio-thoracic-surgical ward (2/2), emergency department (5/6), outpatient departments (3/6), general surgical ward (2/4), intensive care unit (109/130), internal medicine department (5/6), neonatological ward (17/22), neurosurgical ward (3/3), orthopedic ward (1/2), pediatric ward (1/3), pneumological ward (1/1), pneumophtisiology unit (1/1), urological ward (1/3), vascular surgical ward (1/5)	Emergency department (1/6), intensive care unit (9/130), neonatological ward (2/22), vascular surgical ward (2/5)	Outpatient departments (1/6), intensive care unit (2/130), pediatric ward (1/3), neonatological ward (1/22)	Intensive care unit (3/130)
Sample materials[c]	Anal swabs (25/36), axillary swabs (2/2), swab from a not-further defined location (1/1), materials from broncho-alveolar lavages (5/6), blood cultures (49/55), CBES (5/5), cerebrospinal fluid (1/1), exudates from the ear (1/2), biofilms at endotracheal tubes (3/3), gastric samples (1/1), KT (16/21), PTS (12/13), pus (7/18), medical device (1/1), urine (24/34)	Anal swabs (4/36), blood cultures (3/55), KT (3/21), pus (2/18), urine (2/34)	Anal swabs (1/36), blood cultures (2/55), urine (2/34)	Anal swabs (2/36), blood cultures (1/55)

[a]Denominators show total numbers of isolates of the respective species.
[b]Denominators show total numbers of isolates from the respective wards.
[c]Denominators show total numbers of isolates from the respective sample materials.

107 strains, the ampPLEX assay had detected bla_{OXA-48} in 76 strains, bla_{NDM-1} in 7 strains, bla_{OXA-48} and bla_{NDM-1} in 4 strains, and bla_{OXA-48} and bla_{VIM} in 4 strains, while no carbapenemase had been detected in 16 strains. Matches and mismatches of the results of the in-house PCRs [27] as applied in the present study and the results of the commercial assay from the previous assessment [28] are shown in Table 2 for the carbapenemase genes bla_{VIM}, bla_{NDM}, and bla_{OXA-48} which were included in both assays (Table 2).

Altogether, the combination of the results led to additional detections of carbapenemase genes in 15 strains (4x bla_{NDM-1}, 11x bla_{OXA-48}), for which no carbapenemase was detected with the in-house PCRs [27] in the present study. Accordingly, the number of strains without any detection of carbapenemase genes could be reduced to 12 out of 197 strains (6.1%).

Discussion

The applied panel of multiplex PCRs identified carbapenemases in altogether 170/197 (86.3%) assessed *Enterobacteriaceae* from the Military Hospital of Tunis. Similar to previous analyses [24, 25], the genes bla_{OXA-48} and bla_{NDM} were most frequently detected with an overwhelming quantitative dominance of bla_{OXA-48}. In contrast, bla_{BIC} and bla_{VIM} were only sporadically observed, and the remaining 7 carbapenemase genes which were covered by the in-house PCR approaches were not detected at all. Of note, bla_{OXA-48} was described as a frequent carbapenemase in multidrug-resistant isolates from neighboring Libya as well [4, 6, 8, 10, 14, 18]. The carbapenemase gene bla_{BIC}, which has been rarely included in surveillance assessments, has also been recently detected in an *E. cloacae* complex isolate from a patient from Syria applying the same screening PCRs [18].

For 13.7% of the isolates, however, the applied in-house carbapenemase PCRs failed to identify the mechanism of carbapenem resistance. The multiplicity of potential mechanisms makes a PCR panel for a comprehensive screening for all likely reasons of carbapenem resistance virtually impossible. Next generation sequencing (NGS) with subsequent analysis of the reads for genetic resistance determinants as described elsewhere [29, 30] represents a much more comprehensive approach than PCR but is usually not applied in the diagnostic routine due to high costs and debatable clinical relevance of the results.

The study has a number of limitations. The used PCR panel allowed a screening for a limited number of carbapenemases only and did not discriminate variants of the enzymes. Further, available patient data were limited due to the blinded assessment as demanded by the ethics committee. Accordingly, the assessment just provides an idea on the distribution of some frequently identified carbapenemases within the setting of the Military Hospital of Tunis. However, it neither provides data on the affected population nor on potential routes of nosocomial transmission within the hospital.

Table 2. Matches and mismatches of commercial amPLEX PCR as applied in a previous study [28] and in-house PCRs as applied in the present study with 107 out of 197 strains for the carbapenemase genes bla_{OXA-48}, bla_{VIM} and bla_{NDM}

		Detected with in-house PCR [27]	
		Positive for bla_{OXA-48}	Negative for bla_{OXA-48}
Detected with the "Hyplex® Superbug ID system" (amPLEX Diagnostics, Gars-Bahnhof, Germany)	Positive for bla_{OXA-48}	70	14
	Negative for bla_{OXA-48}	11	12
		Positive for bla_{VIM}	Negative for bla_{VIM}
	Positive for bla_{VIM}	2	2
	Negative for bla_{VIM}	1	102
		Positive for bla_{NDM}	Negative for bla_{NDM}
	Positive for bla_{NDM}	2	9
	Negative for bla_{NDM}	2	94

Of note, there were a number of mismatches between the results of a previous screening for carbapenemase genes using a commercial PCR assay [28] and the present screening using in-house multiplex PCRs [27]. For the genes bla_{OXA-48} and bla_{VIM}, the numbers of missed samples and additional detections were in a similar range for both platforms, while considerably more detections of bla_{NDM} were observed with the commercial system. This likely sensitivity problem of the in-house platform [27] is in line with the results of a previous study [18], in the course of which the in-house PCR had failed to identify bla_{NDM} in an *A. baumannii* complex strain from a Libyan patient. Although factors like loss of plasmids during frozen storage might theoretically have played a role in the observed discrepant results, differing sensitivity and specificity of the applied tests are the more likely reason. The in-house PCR approach, which was applied for the present study, was directly adapted from the literature [27] for research purposes without the possibility of a thorough in-house validation due to a lack of well-characterized positive and negative control strains, which is an admitted limitation of the study.

Next to this, the applied PCR panel from the present study was designed for the analysis of culture isolates only [27], not for clinical samples. If direct assessment of clinical samples is desired, for example in the case of suspected nosocomial transmission of a strain with a known carbapenemase during an outbreak, real-time PCR is an alternative. In-house multiplex real-time PCR approaches for the detection of the carbapenemase genes bla_{OXA-48} and bla_{NDM}, which were most frequently identified in this study in the Tunisian isolates, as well as also for bla_{VIM}, were described [31–33] and even evaluated in multi-center studies [31, 33]. In addition, well evaluated commercial systems for the molecular rapid screening for such carbapenemase genes like the real-time PCR-based Xpert Carba-R system (Cepheid, Sunnyvale, CA, USA) [34-42] and the loop-mediated isothermal amplification-(LAMP-)based eazyplex SuperBug system (amPLEX, Gars-Bahnhof, Germany) [43] are available on the market. Although commercial tests do not necessarily provide better diagnostic performance than in-house approaches as shown in a recent assessment [44], they are usually more convenient to use in the diagnostic routine setting.

Similar to that shown for the molecular in-house approach, which was used in our study, also commercial PCR-based screening tools detect only a subset of carbapenem-resistant bacteria [41]. The proportion of detections depends on the choice of target genes of the PCR approach and on the local distribution of those target genes. Accordingly, a negative screening PCR cannot exclude the presence of carbapenem-resistant bacteria. Therefore, PCR for carbapenemases can only be an element of a more comprehensive screening procedure for carbapenem-resistant bacteria and cannot replace the culture-based diagnostic approach. More than this, most evaluation studies with carbapenemase PCRs have focused either on culture isolates or on screening swabs, while only few studies have been performed directly with clinical sample materials [41].

Conclusions

The study confirmed bla_{OXA-48} as by far the most frequent carbapenemase gene in ertapenem-resistant *Enterobacteriaceae*, which were isolated at the Military Hospital of Tunis between 2014 and 2018, followed by bla_{NDM}. The genes bla_{BIC} and bla_{VIM} were only infrequently detected. Only bla_{OXA-48} and bla_{NDM} were identified in isolates during the whole period of the assessment. Due to its frequent and continuous occurrence, the bla_{OXA-48} gene should be considered as endemic in Tunisia, while occasional detections of bla_{NDM} and bla_{VIM} could be confirmed in this study. The bla_{BIC} gene was first described in *Enterobacteriaceae* from Tunisia. Of note, the bla_{KPC} gene was not observed, although it has been occasionally described in neighboring Algeria and Libya as detailed in the introduction.

The applied in-house PCR approach detected carbapenemases in 170/197 (86.3%) strains, while the resistance mechanism against carbapenems remained unresolved for the remaining 13.7% of cases. The addition of results from a previous study with a commercial carbapenemase PCR [28] reduced the number of strains without detected carbapenemase genes to 12/197 (6.1%).

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Authors' Contributions

HF, DFW, SH, RW, and MBM planned the study. HK, HF, RBH, HN, MSEA, ME, JJB, and FB performed the experiments and analyzed the results. HK and HF wrote the paper. All authors have read, amended and corrected the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary material 1: Sequence inserts for the positive control plasmids.

Sequence insert for the positive control plasmid of *bla*$_{SPM}$ (NCBI reference number: NG_050140.1).
5‘-CGT-TTG-AAA-ATC-TGG-GTA-CGC-AAA-CGC-TTA-TGG-ATT-GGG-TGG-CTA-AGA-CTA-TGA-AGC-CGA-AGA-AAG-TAG-TAG-CCA-TCA-ATA-CGC-ACT-TTC-ATT-TGG-ACG-GCA-CGG-GTG-GAA-ATG-AAA-TTT-ACA-AGA-AGA-TGG-GCG-CGG-AGA-CGT-GGT-CGA-GCG-ATC-TGA-CAA-AGC-AGT-TGC-GAC-TTG-AGG-AAA-ACA-AGA-AAG-ACC-GGA-TAA-AAG-CAG-CTG-AGT-TCT-ATA-AAA-ACG-AGG-ATC-TGA-AGC-GAA-GGA-TTC-TGA-GTT-CCC-ATC-CTG-TTC-CAG-CGG-ATA-ATG-TTT-TTG-A-3’
Sequence insert for the positive control plasmid of *bla*$_{AIM}$ (NCBI reference number: NG_048689.1).
5‘-ATG-CCC-CTG-AAG-GTG-TAC-GGA-AAC-ACC-TGG-TAC-GTT-GGC-ACC-TGC-GGC-ATC-AGT-GCG-CTG-CTG-GTC-ACT-TCC-GAC-GCG-GGC-CAT-ATC-CTG-GTC-GAT-GCC-GCC-ACG-CCG-CAG-GCG-GGC-CCA-CAG-ATC-CTG-GCC-AAC-ATC-CGC-GCA-CTC-GGT-TTC-AGG-CCG-GAG-GAC-GTG-CGC-GCC-ATC-GTG-TTC-TCG-CAC-GAG-CAT-TTC-GAC-CAT-GCC-GGC-AGC-CTC-GCC-GAA-CTG-CAG-AAG-GCC-ACG-GGT-GCA-CCG-GTG-TAC-GCG-CGC-GCG-CCC-GCG-ATC-GAC-ACG-CTG-AAG-CGC-GGC-CTG-CCG-GAC-CGC-ACC-GAC-CCG-CAA-TTC-GAG-GTG-GCC-GAA-CCC-GTT-G-3’
Sequence insert for the positive control plasmid of *bla*$_{NDM}$ (NCBI reference number: LC381306.1).
5‘-TGG-CGA-CCA-ACG-GTT-TGG-CGA-TCT-GGT-TTT-CCG-CCA-GCT-CGC-ACC-GAA-TGT-CTG-GCA-GCA-CAC-TTC-CTA-TCT-CGA-CAT-GCC-GGG-TTT-CGG-GGC-AGT-CGC-TTC-CAA-CGG-TTT-GAT-CGT-CAG-GGA-TGG-CGG-CCG-CGT-GCT-GTT-GGT-CGA-TAC-CGC-CTG-GAC-CGA-TGA-CCA-GAC-CGC-CCA-GAT-CCT-CAA-CTG-GAT-CAA-GCA-GGA-GAT-CAA-CCT-GCC-GGT-CGC-GCT-GGC-GGT-GGT-GAC-TCA-CGC-GCA-TCA-GGA-CAA-GAT-GGG-CGG-TAT-GGA-CGC-GCT-GCA-TGC-GGC-GGG-GAT-TGC-GAC-TTA-TGC-CAA-TGC-GTT-GTC-GAA-CCA-GCT-TGC-CCC-GCA-AGA-GGG-GCT-GGT-TGC-GGC-GCA-ACA-CAG-CCT-GAC-TTT-CGC-CGC-CAA-TGG-CTG-GGT-CGA-ACC-AGC-AAC-CGC-GCC-CAA-CTT-TGG-CCC-GCT-CAA-GGT-ATT-TTA-CCC-CGG-CCC-CGG-CCA-CAC-CAG-TGA-CAA-TAT-CAC-CGT-TGG-GAT-CGA-CGG-CAC-CGA-CAT-CGC-TTT-TGG-TGG-CTG-CCT-GAT-CAA-GGA-CAG-CAA-GGC-CAA-GTC-GCT-CGG-CAA-TCT-CGG-TGA-TGC-CGA-CAC-TGA-GCA-CTA-CGC-CGC-GTC-AGC-GCG-CGC-GTT-TGG-TGC-GGC-GTT-CCC-CAA-GGC-CAG-CAT-GAT-CGT-GAT-GAG-CCA-TTC-CGC-CCC-CGA-TAG-3’
Sequence insert for the positive control plasmid of *bla*$_{SIM}$ (NCBI reference number: AY887066.1).
5’-CAA-GAG-TAC-AAG-GGA-TTC-GGC-ATC-GTT-AAA-AAA-CAA-GGC-TTA-GTA-GTT-CTT-GAC-AAT-CAC-AAG-GCA-TAT-CTC-ATC-GAC-ACT-CCA-GCT-TCC-GCA-GGA-GAT-ACT-GAA-AAG-CTA-GTA-AAC-TGG-CTC-GAA-AAA-AAT-GAT-TTC-ACT-GTC-AAT-GGA-AGC-ATT-TCA-ACA-CAT-TTC-CAC-GAC-GAC-AGT-ACT-GCT-GGG-ATA-GAG-TGG-CTT-AAT-ACA-AAG-TCC-ATC-CCC-ACA-TAT-GCA-TCT-AAA-TTG-ACA-AAT-GAA-TTG-CTA-AAT-AAA-AAT-GGC-AAA-ACT-CAA-GCC-AAG-CAC-TCT-TTT-GAT-AAA-GAG-AGC-TTT-TGG-TTG-GTC-AAA-AAT-AAA-ATT-GAA-ATT-TTT-TAT-CCA-GGC-CCA-GGA-CAC-ACT-CAA-GAT-AAC-GAA-GTT-GTC-TGG-ATA-CCT-AAT-AAA-AAA-ATC-CTA-TTC-GGG-GGC-TGT-TTT-ATA-AAA-CCG-AAT-GGC-CTT-GGC-AAT-CTA-AGT-GAC-GCA-AAT-TTG-GAA-GCT-TGG-CCA-GGC-TCC-GCA-AAA-AAA-ATG-ATA-TCA-AAA-TAC-AGT-AAG-GCA-AAA-CTT-GTT-ATC-CCA-AGC-CAC-AGT-GAA-ATC-GGA-GAC-GCA-TCA-CTA-TTG-AAA-CTC-ACA-TGG-GAA-CAG-GCC-ATT-AAA-GGT-T-3’