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ORIGINAL ARTICLE

Prevalence of carbapenem-hydrolyzing OXA-type β-lactamases among *Acinetobacter baumannii* in patients with severe urinary tract infection

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ABSTRACT

Acinetobacter baumannii produces carbapenemase-hydrolyzing class D β-lactamases (CHDLs) as one of the major drug resistance mechanisms. This investigation is thus aimed to assess the prevalence and to characterize the CHDL-producing strains of *A. baumannii* by both phenotypic assays and genotypic characterization. A total of 73 isolates of *A. baumannii* were phenotypically and genotypically characterized from patients ($N = 1{,}000$) with severe urinary tract infection. Tested strains were subjected to double disk synergy testing by Kirby–Bauer disk diffusion method with modified Hodge test (MHT) for carbapenemase production. Plasmid DNA was molecularly screened for CHDL-encoding $bla_{\text{oxa-51}}$, $bla_{\text{oxa-23}}$, and $bla_{\text{oxa-143}}$ genes by polymerase chain reaction. Carbapenem-resistant profile showed 100%, 61.64%, and 67.12% resistance by Kirby–Bauer disk diffusion method that correlated with MHT positivity for 100% ($n = 73$), 80% ($n = 36$), and 78% ($n = 38$) of the isolates against imipenem, doripenem, and meropenem, respectively. The $bla_{\text{oxa-51}}$ and $bla_{\text{oxa-23}}$ were observed in 41.09% ($n = 30$) and 35.61% ($n = 26$) with co-occurrence in 4.10% ($n = 3$) of the isolates. MHT-positive isolates showed 100%, 91.66%, and 71.4% for $bla_{\text{oxa-51}}$ and 91.78%, 51.11%, and 34.69% for $bla_{\text{oxa-23}}$ with imipenem, doripenem, and meropenem resistance, respectively. None of the strains yielded $bla_{\text{oxa-143}}$ gene. The findings of this study showed prevalence of carbapenem resistance and high frequency of $bla_{\text{oxa-51}}$ and $bla_{\text{oxa-23}}$ among *A. baumannii*.

KEYWORDS

Acinetobacter baumannii, carbapenems, $bla_{\text{oxa-51}}$, $bla_{\text{oxa-23}}$, $bla_{\text{oxa-143}}$

INTRODUCTION

Acinetobacter baumannii is an important nosocomial pathogen associated with recalcitrant urinary tract infections, septicemia and pneumonia, and is considered as a frequent cause of infections among patients in intensive care units (ICUs) [1]. In recent years, it is of major concern that *A. baumannii* exhibits multidrug resistance against the routine drugs of choice [2, 3]. *A. baumannii* infections are alarming with greater concern due to their dramatic rise in the carbapenem resistance pattern and are considered as sentinels of drug resistance with the designation as carbapenem-resistant *A. baumannii* (CRAB) [4]. Resistance to carbapenems is mainly mediated by carbapenemases through different classes of genetic determinants [5]. Metallo-β-lactamases (MBLs) are rare among these species but prevalence of MBLs was reported as 53.4% in our earlier studies [6]. However, major contribution for carbapenem resistance was induced through the action of carbapenem-hydrolyzing class D β-lactamases

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(CHDLs), which are also referred as oxacillinases that can cause mild hydrolysis of the administered carbapenems in patients [7, 8] and are often overexpressed in association with insertion sequences [9].

At present, oxacillinases are encoded by five different subclasses of bla_{oxa} in *A. baumannii* strains. The $bla_{oxa\text{-}51}$ is documented to be associated with intrinsic resistant with 70 variants. Few acquired genes are also reported namely, $bla_{oxa\text{-}23\text{-}like}$, $bla_{oxa\text{-}24\text{-}like}$, $bla_{oxa\text{-}58\text{-}like}$, and $bla_{oxa\text{-}143\text{-}like}$ genetic determinants encoded by both chromosomes and plasmids [10]. Basically, oxacillinases are considered as unusual β-lactamases forming a heterogenous group based on structural and biochemical properties with a potent hydrolyzing effect on oxacillin than benzyl penicillin. They are also known to hydrolyze amoxicillin, methicillin, cephaloridine, and to some extent cephalothin. Hydrolytic efficiency of carbapenemase hydrolyzing class D β-lactamase (CHDL) is 100–1,000-fold lower compared to that of MBL; however, it plays a role in inducing carbapenem resistance and still is frequently reported in *A. baumannii* [11]. Although MBLs are considered to be more potent than CHDLs, oxacillinases are known to hydrolyze imipenem but not always meropenem [12].

In addition, CHDL-producing *A. baumannii* often exhibits resistance against clavulanate and tazobactam, with susceptibility to NaCl inhibition, which aids in the laboratory investigations. Among several phenotypic detections, Clinical Laboratory Standards Institute, CLSI guidelines, 2012, advocates the application of modified Hodge test (MHT), CarbaNP test, and/or a molecular based assay for the confirmation of the CHDL producers among *Enterobacteriaceae* and *A. baumannii* strains [13]. Genotypic characterization of CHDL-producing strains is based on the detection of genetic determinant bla_{oxa}, that is usually performed by polymerase chain reaction (PCR) and clonal relatedness can be analyzed by various molecular methods [14]. Periodic surveillance on the CHDL-producing *A. baumannii* would definitely aid in the eradication of the carbapenem-resistant strains in hospitalized patients.

With this background, the present investigation is aimed to phenotypically and genotypically characterize the CHDL producers among *A. baumannii* strains with the phylogenetic assessment of CHDL-based genetic determinants namely, $bla_{oxa\text{-}51}$, $bla_{oxa\text{-}23}$, and $bla_{oxa\text{-}143}$ screened from the patients with severe urinary tract infections from South India.

MATERIALS AND METHODS

Study design

A total of 73 consecutive, non-repetitive *A. baumannii* isolates that were isolated and identified for a period of 12 months (2014–2015) were phenotypically and genotypically characterized from urine samples of patients with severe urinary tract infections ($N = 1{,}000$). Severe urinary tract infection was defined in patients with one or more symptoms of frequency or urgency in urination, suprapubic pain, dysuria, and flank pain. Study cases included the outpatients (OP cases), inpatients (IP cases), and hospitalized patients in ICUs (ICU patients). Proper ethical guidelines and informed consents were obtained prior to beginning of the study. The strains were phenotypically and genotypically confirmed by conventional microbiological analytical tests and PCR, respectively. These characterized strains were subjected to antibiotic susceptibility test by standard Kirby–Bauer disk diffusion method using imipenem (10 μg), doripenem (10 μg), and meropenem (10 μg) for the carbapenem-resistant profile of the selected strains under study [15].

Phenotypic confirmatory test

Detection of CHDL-based oxacillinases or carbapenemases was carried out by MHT. Briefly, 0.5 McFarland standard turbid *Escherichia coli* ATCC 25922 broth suspensions was lawn cultured on a sterile Mueller–Hinton agar plate. Using a sterile forceps, imipenem (10 μg) disk (HiMedia laboratories, Mumbai, India) was placed at the center of the plate and the overnight fresh suspension of *A. baumannii* test strain was streaked from the center to the periphery of the plate. Based on the CLSI guidelines, a distorted zone after overnight incubation is interpreted as positive for carbapenemase production among members of *Enterobacteriaceae*. Although it is not recommended for non-fermenting Gram-negative bacilli, the test is conducted as many previous studies have suggested the test to detect CHDLs among *A. baumannii* strains [16, 17].

Molecular detection of $bla_{oxa\text{-}51}$, $bla_{oxa\text{-}23}$, and $bla_{oxa\text{-}143}$ genetic determinants in CHDL producers

Extraction of plasmid DNA and PCR amplification. All the strains were stored at −80 °C in 80%/20% (v/v) glycerol in Luria–Bertani medium for genetic stability of resistance upon storage [18]. Plasmid DNA was extracted from fresh cultures of *A. baumannii* using Qiagen extraction kit in accordance with the manufacturer's instructions and was stored in −20 °C until further use. An amount of 15 μl of amplification reaction mixtures was prepared by mixing 7.8 μl of 2× Master Mix (Takara, Japan) in 5.6 μl of double distilled water. Specific forward and reverse primers (Eurofins Genomic India Pvt. Ltd., Bangalore, India) of $bla_{oxa\text{-}51}$, $bla_{oxa\text{-}23}$, and $bla_{oxa\text{-}143}$ were added with the standard PCR conditions (Table I). PCR amplification was carried out and the resulting PCR amplicons were examined in 1% agarose gel electrophoresis containing ethidium bromide, which was visualized in a gel documentation system. The 100-bp DNA ladder was used to confirm the amplicon size.

RESULTS

Preliminary screening for the carbapenem resistance tested showed 100%, 61.64%, and 67.12% resistance against imipenem, doripenem, and meropenem, respectively, as per CLSI zone interpretative criteria. MHT was positive in 100% of imipenem-resistant isolates followed by 80% ($n = 36$) and

Table I. Primer sequence and PCR conditions to detect *bla*$_{OXA-51}$, *bla*$_{OXA-23}$, and *bla*$_{OXA-143}$ among CHDL producer *A. baumannii*

Target gene	Primers	Sequence (5′–3′)	Annealing temperature (°C)	Amplicon size (bp)
bla$_{OXA-23-like}$	OXA-23-F	GATCGGATTGGAGAACCAGA	52	501
	OXA-23-R	ATTTCTGACCGCATTTCCAT		
bla$_{OXA-51-like}$	OXA-51-F	TAATGCTTTGATCGGCCTTG	52	353
	OXA-51-R	TGGATTGCACTTCATCTTGG		
bla$_{OXA-143-like}$	OXA-143-F	TGGCACTTTCAGCAGTTCCT	52	149
	OXA-143-R	TAATCTTGAGGGGGCCAACC		

Note: PCR: polymerase chain reaction; CHDL: carbapenemase-hydrolyzing class D β-lactamase; F: forward; R: reverse.

78% ($n = 38$) among doripenem- and meropenem-resistant strains (Table II).

Genotypic characterization of the CHDL genetic determinants showed the presence of *bla*$_{oxa-51}$ and *bla*$_{oxa-23}$ in 41.09% ($n = 30$) and 35.61% ($n = 26$) of the isolates (Figures 1–3). Co-occurrence of *bla*$_{oxa-51}$ and *bla*$_{oxa-23}$ was observed in 4.10% ($n = 3$) of the isolates. MHT-positive isolates showed 100% positive for *bla*$_{oxa-51}$ with imipenem resistance, 91.66% ($n = 33$) with doripenem resistance, and 71.4% ($n = 35$) with meropenem resistance. Similarly, *bla*$_{oxa-23}$ was positive in 91.78% ($n = 67$) with imipenem resistance, 51.11% ($n = 23$) with doripenem resistance, and 34.69% ($n = 17$) with meropenem resistance among MHT-positive isolates. Among the three isolates with both *bla*$_{oxa-51}$ and *bla*$_{oxa-23}$ genes, only one strain was MHT-positive. However, none of the strains yielded *bla*$_{oxa-143}$ gene.

DISCUSSION

CRAB strains were declared as the priority number one pathogen by WHO in the year 2017 [19], due to a wide

Table II. Frequency of CHDL-producing *A. baumannii* based on phenotypic and genotypic characterization assays

Isolate under study	Kirby–Bauer method		MHT positivity (%)	Genes of target		
	Carbapenems tested	Resistance (%)		*bla*$_{OXA-51}$ (%)	*bla*$_{OXA-23}$ (%)	*bla*$_{OXA-143}$ (%)
A. baumannii ($N = 73$)	Imipenem	100	100	100	91.78	0
	Doripenem	61.64	80	91.66	51.11	0
	Meropenem	67.12	78	71.4	34.69	0

Note: MHT: modified Hodge test; CHDL: carbapenemase-hydrolyzing class D β-lactamase.

Figure 1. (a) Electrophoretogram of *bla*$_{oxa-51}$ gene run along with 100-bp DNA ladder. (b) Electrophoretogram of *bla*$_{oxa-23}$ amplicons run along with 100-bp DNA ladder

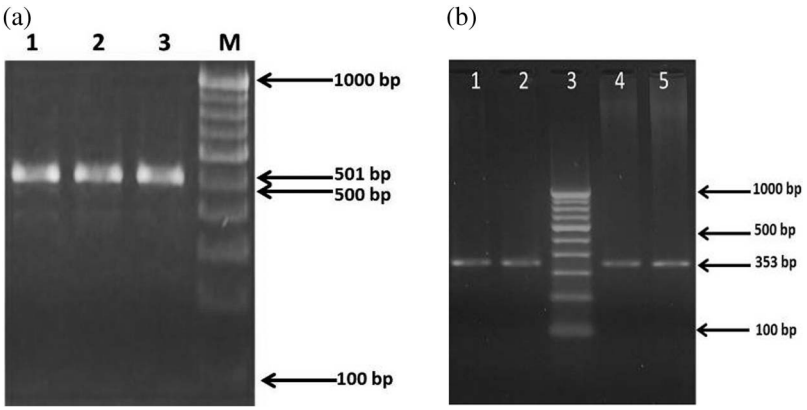
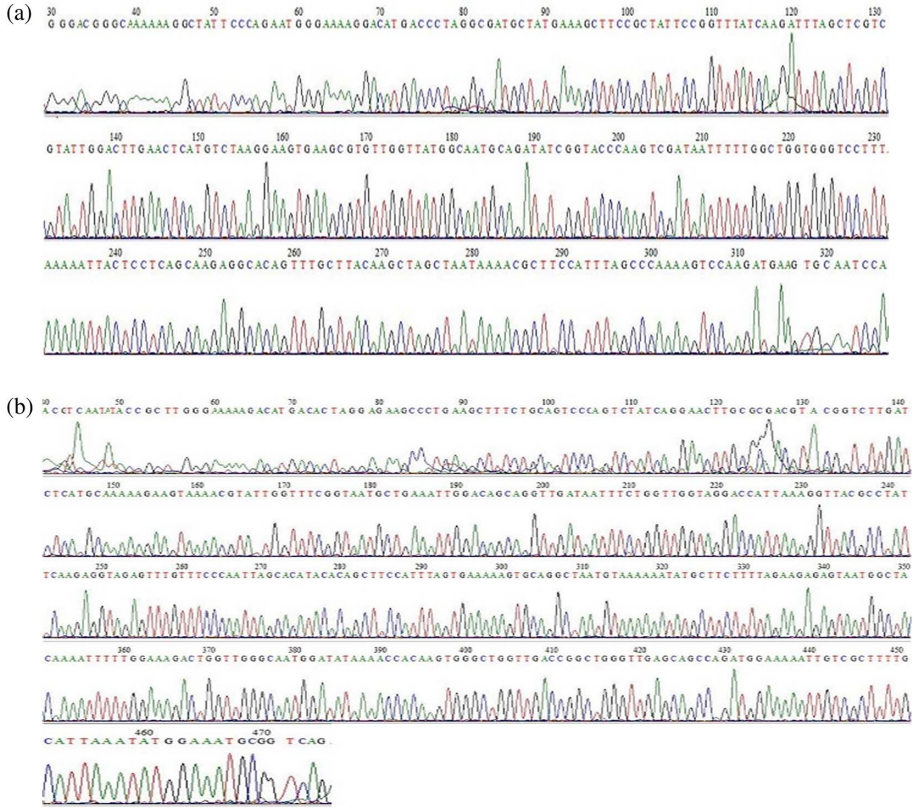


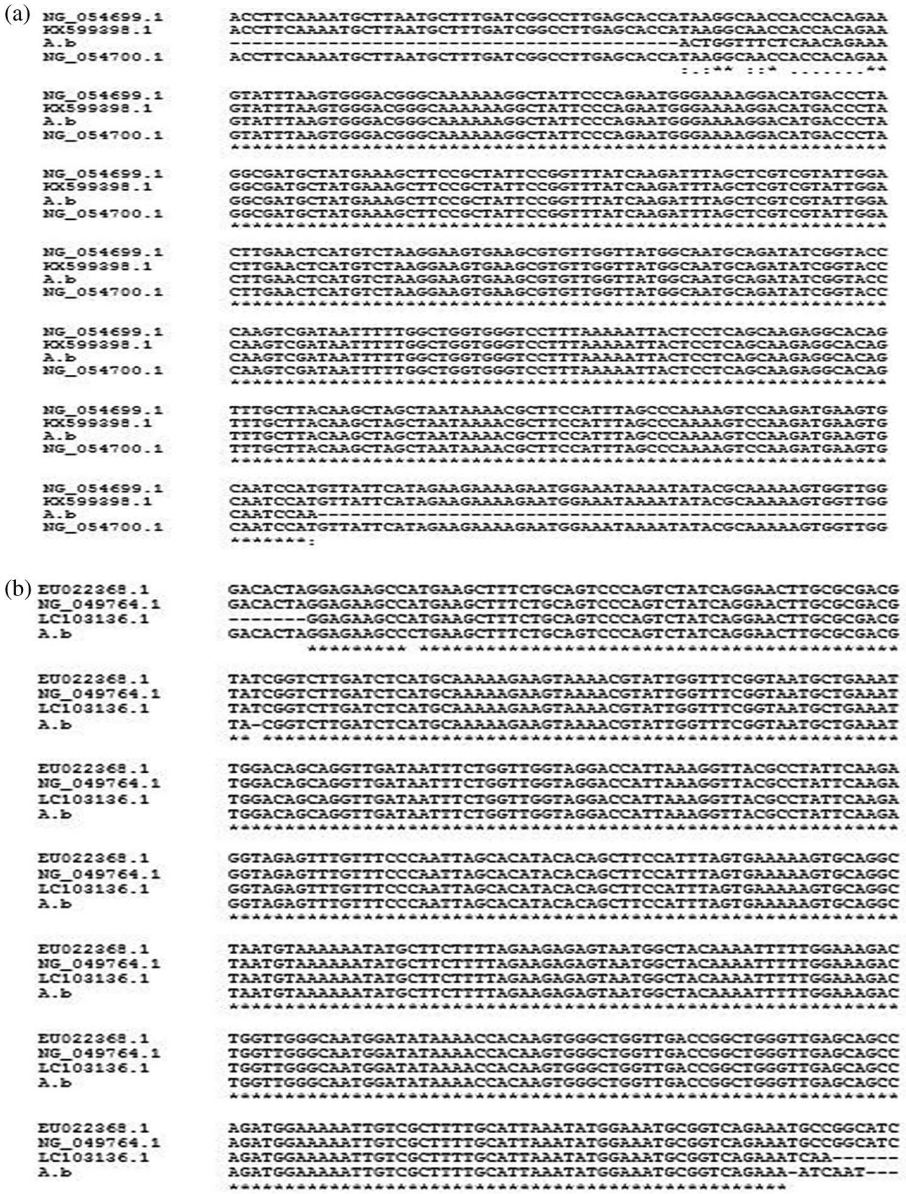
Figure 2. (a) The partial sequence chromatogram of *bla*$_{oxa-51}$ gene. (b) The partial sequence chromatogram of *bla*$_{oxa-23}$ gene



range of nosocomial infections resulted from the strains, encompassing meningitis, septicemia, pneumonia, skin, and wound infections with a major challenge in the patient health care [20]. In addition, severe and complicated infections of *A. baumannii* are treated with the last resort of carbapenems, such as imipenem, doripenem, meropenem, and ertapenem. High incidences of carbapenem-resistant strains in both community- and hospital-acquired infections have been documented [21]. The present investigation has also recorded 50.68% ($n = 37$) as carbapenem-resistant strains showing resistance against all the three drugs tested under the study. Hundred percent of the strains showing imipenem resistance in this study correlate with an earlier study from South India [22]. Resistance to imipenem in *A. baumannii* is reported [23] and in many earlier studies the isolates of *A. baumannii* for carbapenemase and MBL production were categorized based on imipenem susceptibility and resistance patterns [24]. Higher incidences of imipenem resistance are also documented in various studies globally [6, 25]. Our clinical strains had previously recorded 60%–65% of non-susceptibility against doripenem and meropenem with only 15.06% and 13.69% susceptibility, respectively, against the same [20] that had correlated with similar observations from Turkey with 66.6% resistance against meropenem and 49.9% against doripenem mediated by OXA-type carbapenemases [21]. Similar correlations were also observed from a study in the USA that showed 68% and 80% non-susceptibility to meropenem and doripenem, respectively [26]. On the contrary, a study from Punjab, India, has recorded only 6% of the isolates to exhibit non-susceptibility against doripenem and meropenem [22]. Among the routine carbapenems, it is stated that there is no impact in the susceptibility patterns of imipenem, which aids in the reduced administration of imipenem and ciprofloxacin [27]. However, this study has its own limitation where ertapenem is thus omitted under carbapenem-resistant profile for the test organisms under the study.

Phenotypic detection of CHDL production was observed using MHT in this study. Among the tested isolates, with 100% resistance against imipenem and nearly 63% resistance against doripenem and meropenem, phenotypic confirmation was achieved in all the imipenem-resistant isolates but only in 36 and 38 isolates of doripenem- and meropenem-resistant isolates. Among the 73 imipenem-resistant isolates, all were positive for MHT, which might be due to the *bla*$_{oxa-51}$ intrinsic gene cassettes associated with integrons [28]. It might also be an additional fact for the 91.78% and 71.4% of the isolates showing MHT-positive *A. baumannii*, together with the expression of *bla*$_{oxa-23}$, suggesting the role of *bla*$_{oxa-51}$- and *bla*$_{oxa-23}$-type CHDL's in inducing carbapenem resistance. Isolates with positive MHT but showing negative genotypic results may be related to the variants exhibited among class I integron structures, which are detected frequently among *A. baumannii* [29, 30]. Comparative analysis between phenotypic and genotypic data observed in the present investigation suggests MHT to be highly reliable and easy to perform for the preliminary screening of CHDL production in accordance with earlier reports [31].

Figure 3. (a) Multiple sequence alignment of *bla*$_{\text{oxa-51}}$ gene using plasmid DNA as the template isolated from *A. baumannii*. (b) Multiple sequence alignment of *bla*$_{\text{oxa-23}}$ gene using plasmid DNA as the template isolated from *A. baumannii*



Molecular detection of the genetic determinants of CHDL production namely, *bla*$_{\text{oxa-23}}$, *bla*$_{\text{oxa-51}}$, and *bla*$_{\text{oxa-143}}$, was observed using PCR. All the resistant isolates ($n = 73$) of *A. baumannii* showed *bla*$_{\text{oxa-143}}$ negativity. In comparison with the carbapenem-resistant profile (IMP – 100%, Dor – 61.64%, Mero – 67.12%) and MHT-positive isolates, only 23 and 17 showed the presence of *bla*$_{\text{oxa-23}}$. This variation might be due to the other non-enzymatic mechanisms, such as presence of efflux pumps, role of outer membrane proteins, etc., exhibiting the carbapenem-resistance property among *A. baumannii* [32], which is the vital fact for the widespread distribution of CHDL producers among *A. baumannii* observed worldwide [33, 34].

Among the CHDL genetic determinants, co-occurrences of the genes are also not uncommon. Studies record the different patterns of co-occurring CHDL genes from different countries including India [35]. In view with this, this study also records the co-occurrence of *bla*$_{\text{oxa-23}}$ and *bla*$_{\text{oxa-51}}$, in three isolates. Comparative analysis between phenotypic and genotypic detection also shows a significant report. The study also records isolates with MHT + *bla*$_{\text{oxa-23}}$ and MHT + *bla*$_{\text{oxa-51}}$ positivity, respectively, with isolates showing MHT + *bla*$_{\text{oxa-23}}$ + *bla*$_{\text{oxa-51}}$ positivity. In an earlier study from Nepal, the coexistence of *bla*$_{\text{oxa-23}}$ and *bla*$_{\text{NDM-1}}$ was detected [36] with the presence of other class B MBLs, such as *bla*$_{\text{VIM}}$ and *bla*$_{\text{GIM}}$. These reports suggest that the variations exhibited by the test isolates in both phenotypic and genotypic characterizations are mainly due to the frequency of different genetic determinants prevailing among the *A. baumannii* species existing in different geographical location against the carbapenems.

Complications induced by *A. baumannii* traits that are acquired through different patterns of antimicrobial

resistance transform them as dreadful nosocomial pathogen posing serious impediments in infection control. Frequency of CHDLs and the distribution of their genetic determinants restrict the administration of carbapenems against *A. baumannii*. The present investigation thus concludes by stating the need for the proper and periodical antimicrobial surveillance programs for the use of carbapenems against *A. baumannii* due to the high prevalence of varying resistance pattern in association with the $bla_{\text{oxa-23}}$, $bla_{\text{oxa-51}}$, and $bla_{\text{oxa-143}}$ in inducing the carbapenemase resistance.

Conflict of Interest: The authors declare no conflict of interest.

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