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MECHANISMS OF RESISTANCE

High Rate of Association of 16S rRNA Methylases and Carbapenemases in *Enterobacteriaceae* Recovered from Hospitalized Children in Angola

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ABSTRACT Acquired 16S rRNA methylases (RMTases) conferring pan-drug resistance to aminoglycosides were searched among enterobacterial isolates recovered in Angola. A total of 36 hospitalized children were screened for rectal colonization using the Superaminoglycoside selective medium. Twenty-two pan-aminoglycoside-resistant enterobacterial isolates were recovered, all of which produced RMTases, i.e., RmtB, ArmA, and RmtC. Highly diverse genetic backgrounds were identified for *Escherichia coli* and *Klebsiella pneumoniae* isolates, most of which coproduced carbapenemases NDM-1 or NDM-5, respectively.

KEYWORDS 16S rRNA methylases, Angola, aminoglycosides, carbapenemases

Aminoglycosides (AGs) play an important role in antimicrobial therapy in severe infections, usually in combination with β-lactam agents. AG resistance usually arises from modification of the AG molecules by various enzymes, namely, phosphotransferases, adenylyltransferases, nucloetidyltransferases, and acetyltransferases, which affect some but not all AGs (1). Another mechanism corresponds with the production of 16S rRNA methylases (RMTases) that are mostly encoded by plasmids and found among Gram-negative bacteria (1). They methylate the AG binding site in the bacterial 16S RNA ribosomal subunit, leading to loss of interaction between the antibiotic and its target (1, 2). The recent emergence and spread of genes encoding these RMTases is worrisome because they are often identified in isolates that produce extended-spectrum β-lactamases (ESBLs) (3) and carbapenemases, particularly the class B NDM-like metalloenzymes in the latter case (2, 4). Among the 10 RMTases described so far, the most widespread are ArmA and RmtB (1). They have been described worldwide with a variable prevalence of ~1% in Europe and Japan (3, 5) and likely higher rates in Asia and the Middle East, with a rate of 37% estimated in a monocentric study in Saudi Arabia (6). However, few data exist for Africa. A rate of 18% of RMTases has been identified in Algeria among ESBL enterobacterial producers (7). In addition, RmtB was recently identified in sporadic enterobacterial isolates recovered in Angola and Kenya in association with the bla_{NDM-1} gene (8, 9).

In 2015, we conducted a study to evaluate the occurrence of carbapenemase genes among enterobacterial isolates recovered from hospitalized children in Angola, and we identified a series of isolates harboring $bla_{OXA-181}$ (carried by IncX3-type plasmids) or bla_{NDM-1} (carried by IncA/C- or IncL/M-type plasmids) (8) carbapenemase genes. In view of the very high rate of carbapenemase producers identified there, we wondered

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whether Angola might also be a reservoir of pan-AG resistance determinants, particularly RMTase producers.

We recently developed the first culture medium for selecting pan-AG-resistant bacteria, mainly corresponding to RMTase producers. This so-called Superaminoglycoside medium has been validated on a collection of AG-resistant and -susceptible reference strains (10). Our objective was therefore to perform a prospective evaluation for screening RMTase producers using this novel selective medium. Our study was performed using a pediatric population from a hospital in Angola, where the prevalence of carbapenemase producers is known to be high (8).

Children included in this study were hospitalized in different departments in the main pediatric hospital of Luanda, Angola, in January 2017. Rectal swabs were collected from 36 patients. Each sample was cultured overnight in Luria-Bertani (LB) broth. One calibrated inoculated loop (10 μl) of each sample was plated onto the Superaminoglycoside medium consisting of eosin-methylene blue agar (EMB) 3.75%, gentamicin 30 μg/ml, amikacin 30 μg/ml, vancomycin 10 μg/ml, and amphotericin B 5 μg/ml to select for AG-resistant Gram-negative isolates (10). In addition, selection of carbapenem-resistant isolates was performed using the Supercarba selective medium to select for carbapenem-resistant isolates (11). Once selected, the carbapenem-resistant isolates were tested by Carba NP to detect for carbapenemase producers (12). Finally, we included in our screening a search for colistin-resistant *Enterobacteriaceae* by using the Superpolymyxin selective medium (13). For all selected isolates, identification at the species level was performed with the API20E system (bioMérieux, La Balme-les-Grottes, France). Antimicrobial susceptibility testing was performed and interpreted according to the disk diffusion method following CLSI recommendations (14). Polymyxin susceptibility was performed by the broth microdilution method as recommended by EUCAST (www.eucast.org). Identification of the ESBL and carbapenemase genes, the aminoglycoside resistance 16S rRNA methylase genes, the *qnr*-type quinolone resistance genes, and the polymyxin resistance *mcr*-like genes was performed by PCR as described previously (15–19), followed by sequencing of the amplicons.

The main procedure and the main results obtained from our prospective screening are summarized in Fig. 1.

Out of the 36 patients from whom rectal swabs were taken, 22 distinct isolates were recovered from 20 different patients onto the Superaminoglycoside medium. In accordance with the high specificity of this selective medium, they all displayed a high level of resistance to the four AGs tested by the disk diffusion method (i.e., amikacin, tobramycin, kanamycin, and gentamicin) and were found to be positive by the rapid aminoglycoside NP test (20).

PCR and sequencing were performed to identify RMTase-encoding genes (Table 1). Surprisingly, all 22 isolates tested positive for an RMTase gene. A total of 16 isolates harbored the *rmtB* gene (9 *Escherichia coli*, 5 *Klebsiella pneumoniae*, 1 *Enterobacter cloacae*, and 1 *Enterobacter aerogenes*), 5 harbored the *armA* gene (2 *K. pneumoniae*, 1 *E. aerogenes*, 1 *E. cloacae*, and 1 *Citrobacter freundii*), and a single *K. pneumoniae* harbored the *rmtC* gene (Table 1). None of the strains carried two different RMTase-encoding genes.

All of the pan-AG isolates were resistant to broad-spectrum cephalosporins, and 10 of the 22 isolates were resistant to carbapenems. In addition, all of these isolates were resistant to fluoroquinolones. PCR amplification followed by sequencing was used to identify coresistance determinants and showed that 15 isolates produced the ESBL CTX-M-15, 2 isolates produced the ESBL CTX-M-55, and 10 isolates produced the NDM-5 carbapenemase (Table 1). In addition, the plasmid-mediated quinolone resistance gene *qnrB* was identified in 16 isolates (Table 1).

To characterize the plasmids bearing the RMTase genes, conjugation assays were performed, followed by PCR-based replicon typing (PBRT), Kieser extraction, and gel electrophoresis to evaluate the plasmid sizes (21, 22). In addition, IncX3-type replicons were searched by using specific primers as described previously (8). Azide-resistant *E. coli* J53 was used as a recipient for mating-out assays. Transconjugants were selected

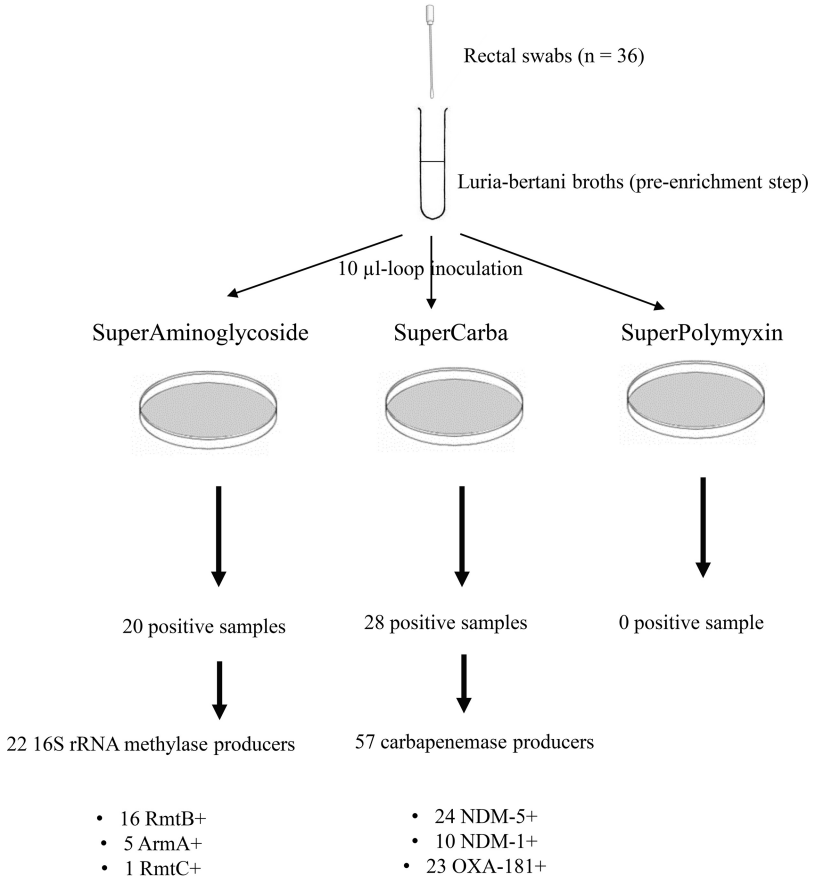

FIG 1 Flow diagram illustrating the procedure and results for the prospective screening of 16S rRNA methylase producers, carbapenemase producers, and colistin-resistant isolates using the Superaminoglycoside, Supercarba, and Superpolymyxin selective media, respectively.

on LB agar plates supplemented with sodium azide 100 μg/ml, gentamicin 50 μg/ml, and amikacin 50 μg/ml. The *rmtB* gene was carried, in most cases, by a conjugative 125-kb IncFIB plasmid that also carried the $bla_{\text{TEM-1}}$ penicillinase gene (10/16 isolates). The other identified *rmtB*-positive plasmids belonged to IncL/M, IncN, and IncY incompatibility groups, which carried $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$, and $bla_{\text{NDM-5}}$, respectively (Table 1). The *armA* gene was carried by a conjugative IncA/C plasmid, associated with the $bla_{\text{NDM-5}}$ carbapenemase gene for three of the five isolates harboring this gene. Even though transconjugants could be obtained, the incompatibility group of the plasmid bearing the *rmtC* gene could not be determined by PBRT, suggesting that the genetic background of this plasmid corresponded to one that was uncommon.

Clonality of the different RMTase-producing *E. coli* and *K. pneumoniae* isolates was investigated by multilocus sequence typing (MLST), which showed highly diverse genetic backgrounds for *E. coli* and *K. pneumoniae* isolates (Table 1). Noticeably, ST448 complex was the most frequently identified RmtB-producing *E. coli* clone (5/9).

By using the carbapenem-resistant selective medium followed by testing with Carba NP, 57 carbapenemase-producing enterobacterial isolates were recovered from 28 patients, leaving only 8 patients free of carbapenemase producers. This rate of colonization (78%) by carbapenemase-producing *Enterobacteriaceae* was much higher than the rate found in a study we conducted in the same hospital 2 years earlier that revealed a colonization rate of 36% (8).

The $bla_{\text{NDM-5}}$ gene was identified in 24 isolates, the $bla_{\text{NDM-1}}$ gene in 10 isolates, and the $bla_{\text{OXA-181}}$ gene in 23 isolates (Table 2). Four patients were colonized by the three different carbapenemase producers. The emergence of NDM-5-producing isolates was

TABLE 1 Genetic features associated with the 16S RMTase-producing isolates

Strain	Species	ST	Resistance determinants[a]	Plasmid incompatibility[b]	Plasmid size (kb)[c]	Resistance phenotype[d]
R21	*E. coli*	ST448	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	95	CAZ, SXT, TET, NAL, GEN, AMK
R23	*E. coli*	ST10	*rmtB*, bla_{TEM-1}, bla_{NDM-5}, $bla_{CTX-M-15}$	IncFIB	95	CAZ, IMP, SXT, TET, NAL, GEN, AMK
R24	*E. coli*	ST10	*rmtB*, bla_{TEM-1}, bla_{NDM-5}	IncFIB	ND	CAZ, IMP, SXT, TET, NAL, GEN, AMK
R31r	*E. coli*	ST448	*rmtB*, bla_{TEM-1}, bla_{NDM-5}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	125	CAZ, IMP, SXT, TET, NAL, GEN, AMK
R46	*E. coli*	New ST	*rmtB*, bla_{TEM-1}, bla_{NDM-5}, $bla_{CTX-M-15}$	IncY	125	CAZ, IMP, SXT, NAL, GEN, AMK
R49	*E. coli*	ST448	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	125	CAZ, SXT, TET, NAL, GEN, AMK
R52	*E. coli*	ST448	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	125	CAZ, SXT, TET, NAL, GEN, AMK
R57r	*E. coli*	ST448	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	125	CAZ, SXT, TET, NAL, GEN, AMK
R62	*E. coli*	New ST	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-55}$	IncN	54	CAZ, SXT, TET, NAL, GEN, AMK
R28	*K. pneumoniae*	ST273	*rmtB*, bla_{TEM-1}, bla_{NDM-5}, $bla_{CTX-M-15}$, *qnrB*	ND	ND	CAZ, IMP, SXT, TET, NAL, GEN, AMK
R30	*K. pneumoniae*	ST147	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	125	CAZ, SXT, TET, NAL, GEN, AMK
R39	*K. pneumoniae*	ST307	*armA*, bla_{NDM-5}, $bla_{CTX-M-15}$, *qnrB*	IncA/C	154	CAZ, IMP, SXT, TET, NAL, GEN, AMK
R40	*K. pneumoniae*	ST307	*armA*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncA/C	ND	CAZ, SXT, TET, NAL, GEN, AMK
R45	*K. pneumoniae*	ST147	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-55}$, *qnrB*	IncFIB	125	CAZ, SXT, TET, NAL, GEN, AMK
R48	*K. pneumoniae*	ST273	*rmtC*, bla_{NDM-5}, $bla_{CTX-M-15}$, *qnrB*	ND	125	CAZ, IMP, SXT, TET, NAL, GEN, AMK
R57b	*K. pneumoniae*	ST101	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncL/M	66	CAZ, SXT, GEN, AMK
R69	*K. pneumoniae*	ST36	*rmtB*, bla_{TEM-1}, *qnrB*	ND	190	CAZ, SXT, TET, GEN, AMK
R31b	*E. aerogenes*	ND	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$, *qnrB*	IncFIB	125	CAZ, SXT, TET, NAL, GEN, AMK
R50	*E. aerogenes*	ND	*armA*, bla_{NDM-5}, *qnrB*	IncA/C	154	CAZ, IMP, SXT, TET, GEN, AMK
R72	*E. cloacae*	ND	*armA*, bla_{NDM-5}	IncA/C	135	CAZ, IMP, SXT, GEN, AMK
R79	*E. cloacae*	ND	*rmtB*, bla_{TEM-1}, $bla_{CTX-M-15}$	IncFIB	110	CAZ, SXT, GEN, AMK, TOB
R8	*C. freundii*	ND	*armA*, bla_{TEM-1}, bla_{NDM-5}, $bla_{CTX-M-15}$, *qnrB*	IncA/C	ND	CAZ, IMP, SXT, TET, NAL, GEN, AMK

[a]Underlined resistance genes are colocated with the RMTase-encoding gene.
[b]ND, not determinable by the PBRT method.
[c]Plasmid size of the plasmid carrying the RMTase-encoding gene. ND, not determined because of plasmid degradation on electrophoresis gel.
[d]CAZ, ceftazidime; IMP, imipenem; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; NAL, nalidixic acid; GEN, gentamicin; AMK, amikacin; TOB, tobramycin.

noticed, whereas only a few NDM-1 and most OXA-181 producers were recovered in our previous study (8).

Among the NDM-producing isolates were 14 *K. pneumoniae* isolates producing NDM-1 or NDM-5 (8 and 6 isolates, respectively) and 15 *E. coli* isolates producing NDM-5, among which 4 isolates coproduced RmtB (Table 2). The other species identified were *E. cloacae* (2 NDM-1 and 2 NDM-5 positive) and a single NDM-5-producing *C. freundii*. The 24 OXA-181 producers included 10 *K. pneumoniae*, 8 *E. coli*, 4 *E. cloacae*, and 1 *E. aerogenes*; and the $bla_{OXA-181}$ gene was located on an ~30-kb IncX3 plasmid in 20 isolates, as found previously (8). Transformation experiments using *E. coli* TOP10 as the recipient were performed for the 4 IncX3-negative OXA-181 producers, identifying the $bla_{OXA-181}$ gene on a 60-kb untypeable plasmid that did not carry any other resistance determinant. Two NDM-5 and 3 NDM-1 producers were also transformed. Most often, the bla_{NDM-1} gene was carried by an ~60-kb untypeable plasmid coharboring *qnrS* and $bla_{CTX-M-15}$, whereas the bla_{NDM-5} gene was located on an ~50-kb IncX3 plasmid (Table 2).

MLST analysis showed a predominance of the ST147 clone among the NDM-1-positive *K. pneumoniae* isolates (7/8) and the ST10 clone among the NDM-1-positive *E. coli* isolates (9/15). Six different *E. coli* and 8 different *K. pneumoniae* clones were identified among the OXA-181 producers.

Note that no enterobacterial isolate exhibiting acquired resistance to colistin was detected.

We report here a high rate of recovery of 16S RMTase and carbapenemase producers in this population of hospitalized children in Angola, colonizing 56% and 78% of the patients, respectively. The high rate of identification of 16S RMTase producers is of concern in the context of endemicity of carbapenemases.

TABLE 2 Genetic features associated with carbapenemase-producing isolates

Strain	Species	ST[a]	Resistance determinants[b]	Plasmid incompatibility	Plasmid size (kb)[c]	Resistance phenotype[d]
CR23r	*E. coli*	ST10	bla_{NDM-5}, bla_{CTX-M}, *rmtB*	IncX3	55	CAZ, ETP, IMP, NAL, TET, GEN, AMK, TOB
OR24	*E. coli*	ST10	bla_{NDM-5}, bla_{TEM-1}, *rmtB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, AMK, TOB
CR28	*E. coli*	New ST	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN
CR31r	*E. coli*	ST10	bla_{NDM-5}, bla_{CTX-M}, *rmtB*	IncX3	60	CAZ, ETP, IMP, NAL, TET, GEN, AMK, TOB
CR39r	*E. coli*	ST10	bla_{NDM-5}, *qnrB*	IncX3	60	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR40	*E. coli*	ST10	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR44	*E. coli*	ST10	bla_{NDM-5}, *qnrB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, TOB
CR47r	*E. coli*	ND	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, TET, TOB
CR48r	*E. coli*	ST2083	bla_{NDM-5}, bla_{CTX-M}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR50r	*E. coli*	ST448	bla_{NDM-5}, *qnrS*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR52r	*E. coli*	ST10	bla_{NDM-5}, *rmtB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, AMK, TOB
CR57r	*E. coli*	New ST	bla_{NDM-5}, bla_{CTX-M}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
OR62	*E. coli*	ND	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, GEN, AKN, TOB
CR74	*E. coli*	ST10	bla_{NDM-5}, bla_{CTX-M}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR79	*E. coli*	ST10	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
OR9r	*E. coli*	New ST	$bla_{OXA-181}$, *qnrS*, *qnrB*	IncX3	60	ETP, NAL, TET, GEN, TOB
OR26r	*E. coli*	New ST	$bla_{OXA-181}$, *qnrS*, bla_{CTX-M}	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR39r	*E. coli*	New ST	$bla_{OXA-181}$, *qnrS*, *qnrB*	IncX3	60	ETP, NAL, GEN, TOB
OR45r	*E. coli*	ST448	$bla_{OXA-181}$, bla_{CTX-M}	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR46r	*E. coli*	ST448	$bla_{OXA-181}$, bla_{CTX-M}	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR47r	*E. coli*	ST448	$bla_{OXA-181}$, bla_{CTX-M}	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR78	*E. coli*	New ST	$bla_{OXA-181}$, *qnrS*	IncX3	60	ETP, TET
OR79	*E. coli*	New ST	$bla_{OXA-181}$, *qnrS*	IncX3	60	ETP, NAL
CR2	*K. pneumoniae*	ST1031	bla_{NDM-5}, $bla_{CTX-M-like}$, *qnrB*	IncX3	50	CAZ, ETP, IMP, TET
CR9	*K. pneumoniae*	ST414	bla_{NDM-5}, bla_{CTX-M}, *qnrB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR21	*K. pneumoniae*	ST2602	bla_{NDM-5}, bla_{CTX-M}, *qnrB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR23b	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	100	CAZ, ETP, IMP, NAL, AMK, TOB
CR25	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	100	CAZ, ETP, IMP, NAL, AMK, TOB
CR30b	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	60	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR31b	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	60	CAZ, ETP, IMP, NAL, AMK, TOB
CR39b	*K. pneumoniae*	ST2602	bla_{NDM-5}, bla_{CTX-M}	IncX3	60	CAZ, ETP, IMP, NAL, TET, GEN I, TOB
CR47b	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	100	CAZ, ETP, IMP, NAL, AMK, TOB
CR48b	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	100	CAZ, ETP, IMP, NAL, AMK, TOB
CR50b	*K. pneumoniae*	ST414	bla_{NDM-5}, *qnrB*	IncX3	50	CAZ, ETP, IMP, GEN, TOB
Cr60	*K. pneumoniae*	ST11	bla_{NDM-1}, *qnrS*	IncFIA	100	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR69	*K. pneumoniae*	ST11	bla_{NDM-5}, bla_{CTX-M}, *qnrB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR79b	*K. pneumoniae*	ST147	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	60	CAZ, ETP, IMP, NAL, AMK, TOB
OR3	*K. pneumoniae*	ST11	$bla_{OXA-181}$, *qnrB*, bla_{CTX-M}	IncX3	60	CAZ, ETP, TET, GEN, TOB
OR9b	*K. pneumoniae*	ST1214	$bla_{OXA-181}$	IncX3	60	ETP
OR26b	*K. pneumoniae*	ST2092	$bla_{OXA-181}$, *qnrS*, *qnrB*, bla_{CTX-M}	IncX3	60	CAZ, ETP, NAL, TOB
OR30	*K. pneumoniae*	ST466	$bla_{OXA-181}$, *qnrS*, bla_{CTX-M}, *qnrB*	IncX3	60	CAZ, ETP, TET, GEN, TOB
OR31	*K. pneumoniae*	ST976	$bla_{OXA-181}$, *qnrS*, bla_{CTX-M}	IncX3	60	CAZ, ETP, TET
OR39b	*K. pneumoniae*	ST11	$bla_{OXA-181}$, *qnrS*, *qnrB*, bla_{CTX-M}	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR47b	*K. pneumoniae*	New ST	$bla_{OXA-181}$, *qnrS*, *qnrB*, bla_{CTX-M}	IncX3	60	CAZ, ETP, TET, GEN, TOB
OR50	*K. pneumoniae*	ST26	$bla_{OXA-181}$, *qnrS*, *qnrB*, bla_{CTX-M}	IncX3	60	CAZ, ETP, GEN, TOB
OR52b	*K. pneumoniae*	New ST	$bla_{OXA-181}$, *qnrS*, bla_{CTX-M}	IncX3	60	CAZ, ETP, GEN, TOB
OR57	*K. pneumoniae*	ST2092	$bla_{OXA-181}$, *qnrS*, *qnrB*, bla_{CTX-M}	IncX3	60	CAZ, ETP, GEN, TOB
CR3	*E. cloacae*	ND	bla_{NDM-1}, *qnrB*	IncFIA	100	CAZ, ETP, IMP, NAL, TOB
CR45	*E. cloacae*	ND	bla_{NDM-1}, bla_{CTX-M}, *qnrS*	IncFIA	100	CAZ, ETP, IMP, NAL, AMK, TOB
CR52b	*E. cloacae*	ND	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
CR57b	*E. cloacae*	ND	bla_{NDM-5}	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB
OR2	*E. cloacae*	ND	$bla_{OXA-181}$, bla_{CTX-M}, *qnrS*, *qnrB*	IncX3	60	CAZ, ETP, GEN, AMK, TOB
OR45b	*E. cloacae*	ND	$bla_{OXA-181}$, *qnrS*, *qnrB*	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR48	*E. cloacae*	ND	$bla_{OXA-181}$, *qnrS*	IncX3	60	CAZ, ETP, NAL, TET, GEN, TOB
OR60	*E. cloacae*	ND	$bla_{OXA-181}$	IncX3	60	CAZ, ETP, GEN, TOB
OR72	*E. aerogenes*	ND	$bla_{OXA-181}$, *qnrB*	IncX3	60	CAZ, ETP, TET, GEN, TOB
CR52v	*C. freundii*	ND	bla_{NDM-5}, bla_{CTX-M}, *qnrB*	IncX3	50	CAZ, ETP, IMP, NAL, TET, GEN, TOB

[a]ND, not determinable.

[b]Underlined resistance genes are colocated with the carbapenemase-encoding gene.

[c]Size of the plasmid carrying the carbapenemase-encoding gene.

[d]CAZ, ceftazidime; ETP, ertapenem; IMP, imipenem; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; NAL, nalidixic acid; GEN, gentamicin; AMK, amikacin; TOB, tobramycin.

It is worth highlighting that our methodological approach may explain this high detection rate. Our study was prospective, using the first screening medium developed to select for pan-AG-resistant *Enterobacteriaceae*, previously shown to exhibit excellent sensitivity. Therefore, the high rate in this study compared with those found in the literature might be the result of a very sensitive screening methodology for 16S RMTase producers. We may speculate that the global prevalence of 16S RMTase producers is largely underestimated worldwide and that the Superaminoglycoside selective medium will be an excellent tool for better assessment of the real extent of the problem.

Another concern that came out of the present study is the occurrence of NDM-5 producers, which were not identified in our previous study performed a year earlier in the same hospital (8). It has been shown that NDM-5 possesses a higher hydrolytic capacity than NDM-1 toward carbapenems and confers higher resistance levels to those molecules (23). Therefore, we can speculate that the carbapenem selective pressure may be responsible for a switch from NDM-1 producers to NDM-5 producers in that geographic area. Interestingly, the $bla_{\text{NDM-5}}$ gene was associated with an IncX3 plasmid, as previously reported in different European countries, Australia, Algeria, India, and China (23, 24). This plasmid type seems to play an important role in the horizontal transfer of the carbapenemase gene among enterobacterial isolates and has been found to be associated with the spread of both bla_{NDM} and $bla_{\text{OXA-181}}$ genes in our study. Note that our study identified NDM-1-producing *K. pneumoniae* ST147 as a source of nosocomial dissemination, as previously reported in China and recently in Tunisia and Algeria (25, 26). Therefore, this clone appears to be widespread on the African continent and elsewhere, and implementation of surveillance and control studies should be reinforced to prevent its massive dissemination.

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