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Spread of NDM-1-Producing *Enterobacteriaceae* in a Neonatal Intensive Care Unit in Istanbul, Turkey

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Twenty-two consecutive carbapenem-resistant enterobacterial isolates were recovered from patients hospitalized between January and April 2013 in different units at a university hospital in Istanbul, Turkey. These were *Klebsiella pneumoniae* isolates producing the carbapenemases OXA-48, NDM-1, and KPC-2, *Enterobacter cloacae* isolates producing NDM-1, and *Escherichia coli* isolates producing OXA-48. Most of the OXA-48-producing *K. pneumoniae* and all the NDM-1-producing *E. cloacae* were clonally related. The NDM-1-producing *E. cloacae* isolates recovered from a single neonatal intensive care unit corresponded to a single cluster, highlighting the spread of that clone in that setting.

Carbapenem-hydrolyzing β-lactamases belonging to Ambler classes A, B, and D have been reported worldwide among *Enterobacteriaceae* (1, 2). In Turkey, the wide dissemination of OXA-48-producing isolates (from *Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii*, and *Enterobacter cloacae*) has been demonstrated, defining this country as being endemic for OXA-48 (3–5). Apart from OXA-48 producers, isolates producing other types of carbapenemases (NDM-1, IMP-1, and KPC-2) were recently identified in Turkey as well. The non-OXA-48-producing carbapenemase producers reported from Turkey are the following: six NDM-1-producing *K. pneumoniae* isolates (6, 7), four IMP-1-producing *K. pneumoniae* isolates (7, 8), and a single KPC-2-producing *K. pneumoniae* isolate (9).

The spread of NDM-1-producing *Enterobacteriaceae* is now considered to be almost global (10). However, only a few outbreaks of NDM-1 producers have been reported. So far, most of the reports from many countries have been scattered and corresponded mainly to importation cases (1).

Our study was designed to evaluate retrospectively the occurrence of carbapenemase-producing *Enterobacteriaceae* in a university hospital in Istanbul, Turkey. An increased number of carbapenem-resistant isolates recovered from patients hospitalized in the neonatal intensive care unit (NICU) during a short period prompted us to perform such a study.

All enterobacterial isolates presenting with reduced susceptibility to imipenem (MIC $\geq$ 0.5 μg/ml) were collected between 1 January and 1 May 2013 (a 4-month period), consisting of a total of 22 isolates. The MICs of the carbapenems were determined by the Etest (AB bioMérieux, La Balme-les-Grottes, France) on Mueller-Hinton agar plates at 37°C, and the results of susceptibility testing were interpreted according to CLSI guidelines (11). Those isolates were recovered mostly from urine samples, and also from rectal swabs (Table). All isolates were considered to be colonizers. These were *K. pneumoniae* ($n = 12$), *E. cloacae* ($n = 8$), and *E. coli* ($n = 2$) (Table 1). The isolates were resistant to all β-lactams, including carbapenems for most isolates (Table 2). However, some isolates were still categorized in the susceptible range for carbapenems (Table 2). In addition, they were mostly resistant to aminoglycosides, fluoroquinolones, chloramphenicol, sulfonamides, fosfomycin, and nitrofurantoin. All except two of them had low MIC values ($<$0.5 μg/ml) for colistin, as measured by the Etest.

Carbapenemase detection was performed using the Carba NP test (12), and positive results were obtained for the 22 isolates. Thus, PCR assays were carried out with a series of primers designed to detect Ambler class A, B, and D carbapenemase genes, i.e., bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{OXA-48} (13), followed by sequencing of the PCR amplicons. Two isolates were positive for bla_{KPC-2}, 12 for bla_{NDM-1} gene, and eight for bla_{OXA-48} (Table 1). None of the isolates coharbored two carbapenemase genes. Note that the two KPC-2-producing isolates were *K. pneumoniae*, the 12 NDM-1-producing isolates were *K. pneumoniae* ($n = 4$) and *E. cloacae* ($n = 8$), and the eight OXA-48-producing isolates were *K. pneumoniae* ($n = 6$) and *E. coli* ($n = 2$) (Table 1).

Genotyping was performed to evaluate the clonal relationship of the *K. pneumoniae* and *E. cloacae* isolates by pulsed-field gel electrophoresis (PFGE) (14), and *K. pneumoniae* isolates were additionally genotyped by multilocus sequence typing (MLST), as described previously (15). The two KPC-2-producing *K. pneumoniae* isolates were clonally related and belonged to the sequence type 307 (ST307). The four NDM-1-producing *K. pneumoniae* isolates were clonally unrelated, belonging to ST15, ST45, ST278, and ST1059. The eight NDM-1-producing *E. cloacae* isolates were clonally undistinguishable by PFGE, thus corresponding to a single clone. Finally, the six OXA-48-positive *K. pneumoniae* isolates were all clonally related and belonged to ST101 (Table 1).

Interestingly, looking at the hospitalization wards from which the patients originated, it appeared that a cluster of colonizing NDM-1-producing *E. cloacae* isolates occurred in the NICU, with a total of eight neonates harboring this same resistant strain. Similarly, the two patients colonized with the KPC-2-producing *K.*

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TABLE 1 Features of the carbapenem-resistant isolates and patient characteristics

Patient/strain no.	Date of isolation (mo/day/yr)	Isolate	Site of isolation[a]	Carbapenemase produced	Associated β-lactamase(s)	16S rRNA methylase	Sequence type	Hospitalization unit[b]	Patient age
1	01/25/2013	*K. pneumoniae*	Urine	NDM-1	CTX-M-15 + CMY-6 + SHV-1	RmtC	ST15	Internal medicine	60 yr
2	02/13/2013	*K. pneumoniae*	ETA	OXA-48	CTX-M-15 + OXA-1 + TEM-1 + SHV-1	None	ST101	ICU	31 yr
3	02/25/2013	*K. pneumoniae*	ETA	OXA-48	CTX-M-15 + OXA-1 + TEM-1 + SHV-1	None	ST101	ICU	56 yr
4	03/09/2013	*K. pneumoniae*	Body fluid	OXA-48	CTX-M-15 + OXA-1 + TEM-1 + SHV-1	None	ST101	Orthopedics	73 yr
5	03/13/2013	*E. coli*	Body fluid	OXA-48	CTX-M-3 + OXA-1 + TEM-1	None	ND[c]	Gynecology	65 yr
6	03/30/2013	*E. cloacae*	ETA	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	Newborn
7	04/02/2013	*E. cloacae*	Urine	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	3 mo
8	04/03/2013	*E. cloacae*	Urine	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	4 mo
9	04/03/2013	*E. cloacae*	Rectal swab	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	Newborn
10	04/06/2013	*E. cloacae*	Urine	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	Newborn
11	04/09/2013	*E. cloacae*	Nasal swab	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	3 mo
12	04/10/2013	*E. coli*	Urine	OXA-48	CTX-M-15	None	ND	Pediatrics	7 yr
13	04/16/2013	*E. cloacae*	Rectal swab	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	1 mo
14	04/21/2013	*E. cloacae*	Rectal swab	NDM-1	CTX-M-15 + OXA-1	RmtC	ND	NICU	4 mo
15	04/24/2013	*K. pneumoniae*	Urine	OXA-48	CTX-M-15 + OXA-1 + TEM-1 + SHV-1	None	ST101	ICU	12 yr
16	04/04/2013	*K. pneumoniae*	Rectal swab	NDM-1	CTX-M-3 + SHV-27	RmtC	ST1059	NICU	Newborn
17	04/04/2013	*K. pneumoniae*	Rectal swab	NDM-1	OXA-1 + SHV-1	RmtC	ST45	NICU	Newborn
18	04/04/2013	*K. pneumoniae*	Rectal swab	NDM-1	OXA-1 + SHV-27	RmtC	ST278	NICU	Newborn
19	04/29/2013	*K. pneumoniae*	Urine	OXA-48	CTX-M-15 + OXA-1 + TEM-1 + SHV-1	None	ST101	Neurosurgery	12 yr
20	04/30/2013	*K. pneumoniae*	BAL fluid	OXA-48	CTX-M-15 + OXA-1 + TEM-1 + SHV-1	None	ST101	Pneumology	60 yr
21	04/30/2013	*K. pneumoniae*	Skin tissue	KPC-2	OXA-9 + SHV-1 + TEM-1	None	ST307	Plastic surgery	64 yr
22	04/30/2013	*K. pneumoniae*	Urine	KPC-2	OXA-9 + SHV-1 + TEM-1	None	ST307	Plastic surgery	12 yr

[a] ETA, endotracheal aspirate; BAL, bronchoalveolar lavage.
[b] ICU, intensive care unit; NICU, neonatal intensive care unit.
[c] ND, not determined.

TABLE 2 MICs of carbapenems and coresistance to non-β-lactam antibiotics

Patient/strain no.	Isolate	Carbapenemase produced	MIC (μg/ml) of carbapenems[a] IMP	MER	ERT	Coresistances[b]
1	*K. pneumoniae*	NDM-1	2	4	16	TET SUL RIF AMK GEN TOB
2	*K. pneumoniae*	OXA-48	1	1	4	SUL AMK GEN TOB FQ FOS
3	*K. pneumoniae*	OXA-48	0.5	2	8	TET GEN TOB FQ CHL
4	*K. pneumoniae*	OXA-48	0.5	2	8	TET GEN TOB FQ CHL
5	*E. coli*	OXA-48	0.5	0.25	0.5	SUL RIF FOS
6	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
7	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
8	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
9	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
10	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
11	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
12	*E. coli*	OXA-48	0.5	0.25	0.5	SUL RIF FOS
13	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
14	*E. cloacae*	NDM-1	8	4	8	TET SUL RIF AMK GEN TOB
15	*K. pneumoniae*	OXA-48	1	4	8	TET SUL RIF GEN TOB FQ
16	*K. pneumoniae*	NDM-1	4	4	8	TET SUL RIF AMK GEN TOB COL
17	*K. pneumoniae*	NDM-1	4	4	4	TET SUL RIF AMK GEN TOB
18	*K. pneumoniae*	NDM-1	4	2	8	TET SUL RIF AMK GEN TOB
19	*K. pneumoniae*	OXA-48	1	4	16	TET RIF GEN TOB FOS COL
20	*K. pneumoniae*	OXA-48	8	8	16	TET SUL GEN TOB FQ CHL FOS
21	*K. pneumoniae*	KPC-2	2	4	8	RIF FQ FOS
22	*K. pneumoniae*	KPC-2	2	4	8	RIF FQ FOS

[a] IMP, imipenem; MER, meropenem; ERT, ertapenem.
[b] TET, tetracycline; SUL, sulfonamides; RIF, rifampin; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; FQ, fluoroquinolones (ciprofloxacin and ofloxacin); COL, colistin (indicated when the MIC was not <0.5 μg/ml); CHL, chloramphenicol; FOS, fosfomycin.

pneumoniae isolates had been hospitalized in the same ward (plastic surgery). Regarding the dissemination of the ST101 and OXA-48-producing *K. pneumoniae* isolates (five patients colonized in four distinct wards), a nosocomial route of dissemination might be also suspected. However, this hypothesis is not obvious considering that OXA-48-producing *K. pneumoniae* are very widespread in Turkey, and taking into account the heterogeneity observed in terms of antibiotic resistance for those isolates, the MICs of carbapenems, and coresistance markers (Table 2). Interestingly, ST101 and OXA-48-producing *K. pneumoniae* isolates have been reported in Libya (16) and were at the origin of an outbreak in Spain (17). In contrast, and even if OXA-48-producing *K. pneumoniae* isolates of many different STs have been identified in Turkey (18), that specific clone was never identified in that country. Considering the recent and frequent transfers of Libyan patients to Turkey, and in particular in the hospital where those samples have been recovered, this might explain the emergence of that specific clone.

Note that the majority of the NDM-1-positive isolates, regardless of their clonal lineage and of the species to which they belonged, were multidrug resistant. In particular, most of them coexpressed the extended-spectrum β-lactamase CTX-M-15 and the narrow-spectrum β-lactamase OXA-1, as identified by PCR and sequencing (19) (Table 1). In addition, since all NDM-1-positive isolates were resistant at a high level to all aminoglycosides, a search of 16S rRNA methylase-encoding genes was performed as described previously (20). The results showed that all those isolates (eight *E. cloacae* and the four *K. pneumoniae* isolates) were positive for the *rmtC* gene (21).

In order to evaluate whether the cooccurrence of the $bla_{\text{NDM-1}}$ and the *rmtC* genes might be related to the spread of a specific plasmid, mating-out assays were performed using all those isolates as donors and with *E. coli* J53 as the recipient, as described previously (22). Interestingly, *E. coli* transconjugants coproducing NDM-1 and RmtC were obtained for all the isolates, and a single plasmid of ca. 150 kb was identified in all transconjugants. That plasmid additionally conferred resistance to rifampin to all transconjugants. However, it did not harbor the $bla_{\text{CTX-M-15}}$ gene, in accordance with the susceptibility to aztreonam (a substrate spared by NDM-1) (23) observed for all *E. coli* transconjugants. Attempts to type this plasmid using the PCR-based replicon typing (24) remained unsuccessful. PCR mapping was performed to identify the genetic sequences surrounding the $bla_{\text{NDM-1}}$ gene in all positive isolates. The same structure was found, with the $bla_{\text{NDM-1}}$ gene preceded by a truncated version of IS*Aba125*, followed by the ble_{MBL} gene encoding resistance to bleomycin (25). Similar structures were identified previously in different species from different countries (19).

Our study reports an outbreak of NDM-1-producing *E. cloacae* in a NICU setting and describes the emergence of plasmid-mediated 16S rRNA methylases in Turkey. Here, the RmtC enzyme was involved and was encoded by a gene located on the same plasmid carrying the $bla_{\text{NDM-1}}$ gene. Such an association between those two genes was previously reported in a single *K. pneumoniae* isolate from India (19) and in a series of *K. pneumoniae* isolates from Kenya (26). A 150-kb and untypeable plasmid coharboring the two genes was similarly identified here and in the Indian isolate. The wide spread of multidrug-resistant NDM-1 producers in a NICU setting is extremely worrisome, since infections developed by those immunocompromised patients are very difficult to treat. While this work was in progress, an outbreak of NDM-1-producing *K. pneumoniae* was also reported in a neonatal unit located in Bogotá, Colombia (27). In that study, two out of the six infected newborns died. The strain involved corresponded to ST1043, which is unrelated to the STs identified in our study.

In many countries, the occurrence of NDM-1 producers in hospitalized patients is mostly related to previous patient hospitalization on the Indian subcontinent. Nevertheless, the first case of NDM-1-producing *K. pneumoniae* in Turkey was related to a transfer from Iraq (8). While this work was in progress, another study showed the emergence of NDM-1-producing *K. pneumoniae* isolates in a Turkish hospital located in Kayseri, the center of Turkey, which is 800 km from Istanbul (7), further emphasizing the actual NDM-1 producer problem in Turkey and therefore identifying a possible hidden reservoir for those multidrug-resistant isolates. In that study, as in ours, the origin of the NDM-1-producing isolates is unknown. Altogether, those studies indicate that the spread of NDM-1 producers in the Middle East may be more important than was suspected and is now likely out of control.

This clinical experience was interesting since an outbreak was suspected as soon as two similar carbapenem-resistant *E. cloacae* isolates were recovered from two different patients in the NICU, which is a 30-bed unit. Nasal and rectal swabs were obtained from all 30 patients, and 10 more swabs were obtained from the incubators and environment (a total of 70 surveillance cultures). None of the swabs from the incubators and environment grew carbapenem-resistant *Enterobacteriaceae* (CRE). In order to detect the flaws in work practices causing this colonization, a hospital video surveillance system was used, and significant compromises in the infection control procedures were identified, especially during night shifts. A detailed and rigorous infection control training program was therefore performed, in which all staff participated. Contact isolation precautions were strictly implemented with all the patients who were positive for CRE, and after 30 to 45 days, there were no patients in the NICU who were infected or colonized with any CRE organism, and there has not been a single case since then (8 months). This further shows that complementary works between the clinical and microbiological units may contribute significantly to preventing the spread of CRE.

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