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Persistent Carriage and Infection by Multidrug-Resistant *Escherichia coli* ST405 Producing NDM-1 Carbapenemase: Report on the First Italian Cases▽#

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We report on the first detection of the NDM-1 carbapenemase in Italy, in *Escherichia coli* isolated in October 2009. Prolonged colonization and relapsing infection by NDM-1-positive *E. coli* were observed in a patient (index case) with an indirect epidemiological link with areas of endemicity. Transient colonization was apparently observed in another patient linked with the index case.

CASE REPORT

All patients admitted to the bone marrow transplantation unit (BMTU) of Modena University Hospital (northern Italy) are routinely screened for intestinal colonization by antibiotic-resistant enterics.

The first NDM-1-positive *Escherichia coli* isolate (CVB-1) was detected from the fecal swab of an inpatient (patient 1, index case) of the same unit in October 2009 (Fig. 1a). The isolate exhibited a multidrug-resistant (MDR) phenotype to aminoglycosides, fluoroquinolones, and β-lactams, including carbapenems (Table 1), and was found to produce metallo-β-lactamase (MBL) activity (specific imipenemase activity, 128 ± 1 nmol/min/mg protein, inhibited >90% by EDTA) and to carry the $bla_{\text{NDM-1}}$ gene along with several other resistance determinants (see below). The patient, with a history of acute myeloid leukemia since 2006, had been admitted for fever and pancytopenia (but with no obvious signs of infection) and empirically treated with piperacillin-tazobactam and teicoplanin; oral vancomycin and intravenous metronidazole were also administered due to a previous positivity for *Clostridium difficile*. Tigecycline was added following the detection of fecal carriage of the MDR *E. coli*, but subsequent fecal swabs collected during the admission period continued to yield *E. coli* showing the same MDR phenotype as CVB-1 (Fig. 1a; Table 1). Similar NDM-1-positive *E. coli* isolates were cultured again from this patient in February and June 2010, from the purulent exudate of a relapsing toe infection, while the patient was attending the day hospital clinic. In August 2010, a control fecal swab was obtained, and the patient was found to still be colonized by NDM-1-positive *E. coli* (Fig. 1a; Table 1).

An additional case of colonization by NDM-1-positive *E. coli* was detected in October 2009 in another inpatient (patient 2), with a history of amyloidosis and end-stage renal disease, who was admitted to the same BMTU during a period overlapping the admittance of the index case. The isolate from patient 2 (isolate CVV-1) was contemporary with the first isolate from patient 1 and exhibited a similar MDR phenotype. In this case, however, subsequent fecal swabs did not yield NDM-1-positive *E. coli* isolates (Fig. 1a), suggesting that colonization had been transient or that the resistant strain was subsequently present only in a small proportion.

Neither patient 1 nor patient 2 reported a history of hospitalization or recent visits to areas where NDM-1 is known or suspected to be endemic (the Indian subcontinent and the Balkan region) or where secondary spread has been documented (United Kingdom). However, a careful analysis of hospital records revealed an epidemiological link between the two cases and a third patient (patient 3) with a history of hospital admission in India (in 2008) and of fecal carriage of carbapenem-resistant *E. coli* during a stay in the same BMTU from May to July 2009 (Fig. 1a). In particular, during the period from March to October 2009 (i.e., before detection of the first NDM-1-producing isolate from patient 1), all three patients had attended the same day hospital clinic, sharing the same room, restroom, nurses, and physicians (Fig. 1a), with 18 simultaneous day hospital admissions for patients 1 and 3 and with 1 simultaneous day hospital admission for patients 2 and 3. Altogether, these findings suggested that patient 3 could have been the original source, while patients 1 and 2 were secondary cases acquired via cross-contamination likely occur-

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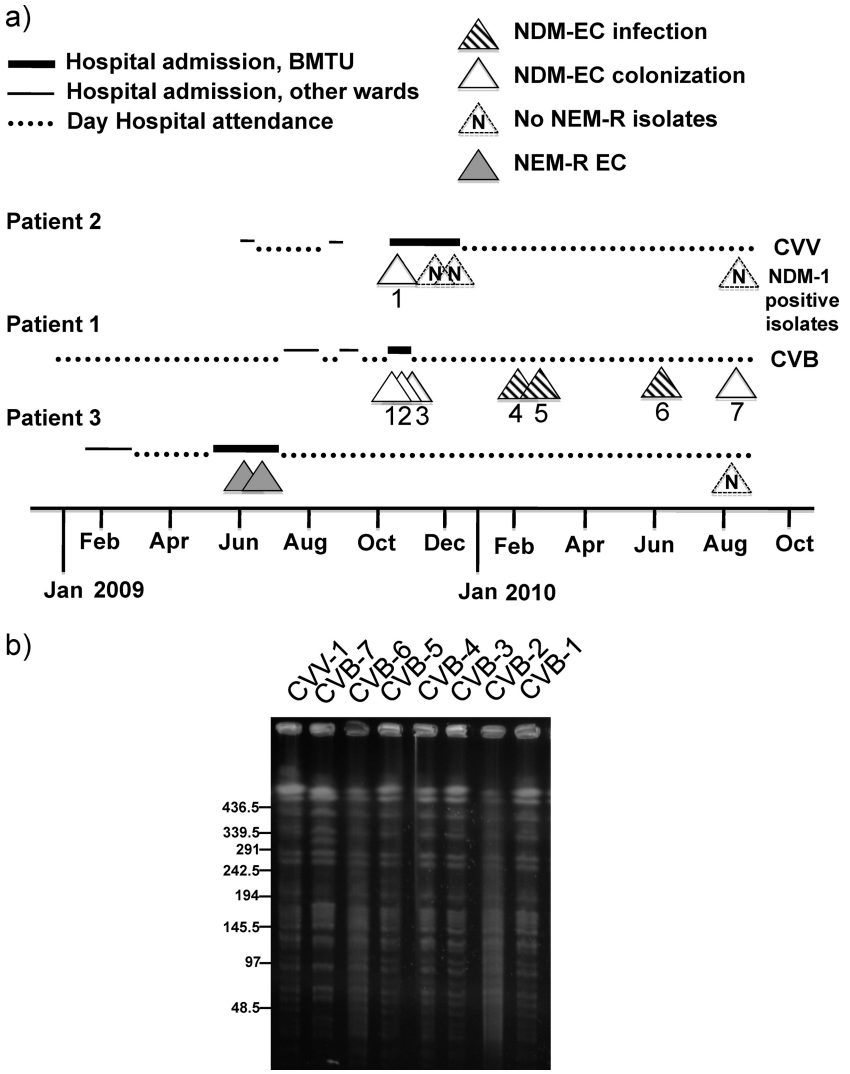

FIG. 1. (a) Diagram showing the times of isolation of NDM-1-positive *E. coli* (NDM-EC) from patients 1 and 2 and attendance at the BMTU (thick lines), other hospital wards (thin lines), and the day hospital clinic (dashed lines) by patients 1, 2, and 3. The lack of detection of NDM-1-positive isolates from fecal swabs is also indicated, as is the reported intestinal colonization by carbapenem-resistant *E. coli* (NEM-R EC), for which NDM-1-production could not be documented. (b) PFGE profiles of the $bla_{\text{NDM-1}}$-positive *E. coli* isolates following digestion with XbaI. Molecular size standards are indicated, in kb, on the left.

ring while attending the same health care setting. Unfortunately, evidence for NDM-1 positivity was not available for the carbapenem-resistant *E. coli* colonizers reported from patient 3, and a fecal swab obtained from the same subject on August 2010 did not yield any *E. coli* isolates with reduced carbapenem susceptibility (Fig. 1a).

Strain characterization. The NDM-1-positive *E. coli* isolates exhibited an MDR phenotype, being susceptible only to colistin and to tigecycline (Table 1).

In all isolates, microarray analysis combined with confirmatory PCR and sequencing detected the presence of $bla_{\text{NDM-1}}$ and of the following acquired resistance genes: $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-9}}$, $bla_{\text{CTX-M-15}}$, *tet*(B), *tet*(C), *armA*, *aac*(6′)-*Ib-cr*, *aac*(3)-II, *catB3*, and *arr3*. Five of these resistance determinants, namely, *aac*(6′)-*Ib-cr*, *aac*(3)-II, $bla_{\text{OXA-1}}$, *catB3*, and *arr3* were carried on gene cassettes inserted into a class 1 integron, resulting in a cassette array identical to that previously described in CTX-M-15-producing clinical isolates of *Klebsiella oxytoca* C994 from Spain (accession number GU189576). The $bla_{\text{NDM-1}}$ gene could not be transferred by electroporation or conjugation to *E. coli* recipients.

Pulsed-field gel electrophoresis (PFGE) genotyping revealed very similar (one band difference in one case) or identical profiles among all NDM-1-positive isolates (Fig. 1b).

Multilocus sequence typing (MLST) analysis revealed that all NDM-1-positive isolates belonged to sequence type 405 (ST405). This clonal complex has been detected worldwide and, together with ST131, has been suggested to be among the major drivers responsible for the worldwide spread of CTX-M-15 ESBL (2). Recently, ST405 *E. coli* isolates producing

CTX-M-3, CTX-M-14, or CTX-M-9 were also described, demonstrating the high propensity of members of this clonal lineage in acquiring different resistance genes (3, 9).

The NDM-1 metallo-β-lactamase (MBL) is the latest addition to the repertoire of acquired carbapenemases emerging in Gram-negative pathogens (13). This resistance determinant has been described in isolates of several Gram-negative species, including *Escherichia coli*, *Klebsiella pneumoniae*, and other *Enterobacteriaceae* (4).

The bla_{NDM-1} determinant appears to be endemic in the Indian subcontinent and possibly in the Balkans (4, 11). Intercontinental dissemination of NDM-1-producing strains has been documented, with imported cases reported in Europe, North America, Malaysia, Taiwan, Australia, and possibly Africa (references 4, 10, and 11 and references therein). In the United Kingdom, secondary dissemination has also been documented and the number of reported cases has rapidly increased during the past 3 years, suggesting that this resistance determinant could exhibit an unprecedented potential for spreading (4) and should be regarded as an emerging public health threat (8).

In this communication, we report on the first detection of NDM-1-producing bacteria in Italy, represented by *E. coli* of sequence type 405 (ST405), a well-known pandemic clonal lineage. We also show that prolonged intestinal carriage, as part of the dominant enterobacterial microbiota, is possible with similar strains.

Identification at the species level and antibiotic susceptibility testing were carried out using the Vitek-2 system (bioMérieux, Marcy-l'Etoile, France). MICs of amikacin, carbapenems, colistin, tigecycline, and piperacillin-tazobactam were confirmed using Etest strips (bioMérieux). For interpretation of the susceptibility data, the EUCAST breakpoints (document version 1.3, 5 January 2011, http://www.eucast.org/clinical_breakpoints/, accessed 23 March 2011) were considered.

Screening for intestinal carriage of antibiotic-resistant *Enterobacteriaceae* was carried out as follows: fecal swabs were plated onto MacConkey agar, and five colonies were randomly selected and subjected to identification and susceptibility testing. It should be noted that a similar approach is expected to detect with high likelihood strains that are representative of the predominant enterobacterial microbiome carried by the subject, while it could fail in detecting strains that are present in a small proportion (6).

Carbapenemase activity was measured in crude extracts by spectrophotometry, using imipenem (150 μM) as the substrate, either alone or in the presence of 5 mM EDTA as described previously (5).

Screening for resistance genes was carried out using a custom oligonucleotide microarray (1) modified to include probes for the most prevalent acquired resistance genes in Gram-negative pathogens (including β-lactamase genes, aminoglycoside resistance genes, tetracycline resistance genes, and quinolone resistance genes). A complete list of resistance genes detectable by the microarray is reported in Table S1 in the supplemental material. Positive hybridization signals were always confirmed by PCR and sequencing. Mapping of the vari-

TABLE 1. Antimicrobial susceptibilities of the NDM-1-positive *E. coli* isolates

Isolate	Patient no.	Isolation date (day/mo/yr)	Ward[a]	Specimen type	PFGE type	MIC (mg/liter)[b]															
						AMK[c]	GEN	AMC	AMP	FEP	CTX	CAZ	PIP	TZP[c]	IPM[c]	MEM[c]	ERT[c]	DOR[c]	LVX	SXT	CST[c]	TGC[c]
CVV-1	2	23/10/2009	BMTU	Fecal swab	A	>256	≤1	>32	>32	>64	>64	>64	>128	>128	8	>32	>32	12	>8	>320	0.75	0.5
CVB-1	1	23/10/2009	BMTU	Fecal swab	A	>256	>16	>32	>32	>64	>64	>64	>128	>128	8	32	>32	16	>8	>320	0.5	0.75
CVB-2	1	27/10/2009	BMTU	Fecal swab	A	>256	>16	>32	>32	>64	>64	>64	>128	>128	4	8	>32	2	>8	>320	0.38	0.5
CVB-3	1	02/11/2009	BMTU	Fecal swab	A	>256	>16	>32	>32	>64	>64	>64	>128	>128	4	8	>32	2	>8	>320	0.5	1.5
CVB-4	1	03/02/2010	DH	Wound exudate	A	>256	>16	>32	>32	>64	>64	>64	>128	>128	8	16	>32	8	>8	>320	0.5	0.5
CVB-5	1	26/02/2010	DH	Wound exudate	A	>256	>16	>32	>32	>64	>64	>64	>128	>128	4	16	>32	4	>8	>320	0.5	0.5
CVB-6	1	25/06/2010	DH	Wound exudate	A	>256	>16	>32	>32	>64	>64	>64	>128	>128	4	8	>32	1.5	>8	>320	0.5	0.5
CVB-7	1	30/08/2010	DH	Fecal swab	A1	>256	>16	>32	>32	>64	>64	>64	>128	>128	8	8	>32	4	>8	>320	0.5	0.5

[a] BMTU, bone marrow transplantation unit; DH, day hospital.
[b] AMK, amikacin; GEN, gentamicin; AMC, amoxicillin-clavulanate (1:2); AMP, ampicillin; FEP, cefepime; CTX, cefotaxime, CAZ, ceftazidime; PIP, piperacillin; TZP, piperacillin-tazobactam; IPM, imipenem; MEM, meropenem; ERT, ertapenem; DOR, doripenem; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole; CST, colistin; TGC, tigecycline.
[c] MIC determined by Etest.

able region of class 1 integrons was performed by PCR as described previously (7).

PFGE typing of bacterial isolates was carried out as described previously (14), using the XbaI restriction endonuclease. PFGE profiles were interpreted according to the method of Tenover et al. (12). Multilocus sequence typing (MLST) was carried out using protocols and conditions described on the *E. coli* MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli/documents/primersColi_html). Sequence types were assigned using the website interface.

Plasmid transfer experiments were performed by either electroporation or conjugation, using *E. coli* DH5α or J53 as the recipient, respectively, as described previously (5).

Concluding remarks. The NDM-1 carbapenemase is a rapidly spreading resistance determinant of major clinical concern (8). To our best knowledge, this is the first detection of NDM-1-positive isolates in Italy. The two cases exhibited a clear epidemiological link with each other but no direct epidemiological link with areas of known endemicity. The source of the NDM-1-positive strain was likely represented by cross-transmission from a third patient that had previously been hospitalized in India and had shared the health care setting with the other two patients. In particular, prolonged attendance at the day hospital clinic, where the patients shared the same room, restroom, nurses, and physicians, could have provided the most likely opportunity for cross-transmission of the NDM-1-positive strain from patient 3 to patient 1 (and possibly also to patient 2) (Fig. 1a).

The prolonged intestinal colonization by an *E. coli* strain belonging to ST405 and carrying a plethora of clinically relevant resistance determinants including $bla_{\text{NDM-1}}$, observed with patient 1, is an alarming finding, since similar carriers could play a relevant role in the dissemination of $bla_{\text{NDM-1}}$ and other resistance genes, especially if the colonizer is present as part of the dominant microbiota and if the hygienic conditions are suboptimal, as in low-resource settings. The reason for this prolonged intestinal colonization at a high level remains unknown. However, the long-lasting fluoroquinolone-based prophylaxis used with hematologic patients, received by patient 1 after discharge, could have played a role in this phenomenon.

Notably, according to the Hospital Microbiology Laboratory records, no further isolation of NDM-1-positive bacteria was reported during the study period either from infections or from the fecal screening of patients admitted to the BMTU. These findings suggest that the infection control procedures employed whenever MDR isolates are detected by surveillance cultures in BMTU patients [including (i) patient isolation in a single room, with dedicated bathroom, (ii) donning of disposable gowns and gloves and changing them for each patient, and (iii) deep cleaning of the rooms after patient discharge] could be effective overall at controlling the dissemination of strains carrying these resistance determinants.

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