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NDM-5 Carbapenemase-Encoding Gene in Multidrug-Resistant Clinical Isolates of *Escherichia coli* from Algeria

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Here, we report the first autochthonous cases of infections caused by bla_{NDM-5} New Delhi metallo-β-lactamase-producing *Escherichia coli* strains recovered from urine and blood specimens of three patients from Algeria between January 2012 and February 2013. The three isolates belong to sequence type 2659 and they coexpress $bla_{CTX-M-15}$ with the bla_{TEM-1} and bla_{aadA2} genes.

Escherichia coli is one of the most common causative agents of infection in humans, and the emergence of resistance to third-generation cephalosporins by extended-spectrum β-lactamases (ESBLs) has led to an increased use of carbapenem compounds (1). The growing incidence of resistance to carbapenems among *Enterobacteriaceae* is of major concern worldwide (1). Carbapenemase producers are mainly identified in *Klebsiella pneumoniae* and, to a lesser extent, in *E. coli* and other enterobacterial species. Carbapenemases are classified into three different classes (A, B, and D) and are now a serious problem due to their rapid spread in *Enterobacteriaceae* (1). Among the newly emerged β-lactamases in the world, New Delhi metallo-β-lactamase (NDM) represents the latest threat for public health (2). It was first reported from *K. pneumoniae* and *E. coli* isolates recovered from a Swedish patient previously hospitalized in India (2). Since then, seven additional NDM variants have been described worldwide (2) (Fig. 1). New Delhi metallo-β-lactamase 1 (NDM-1), which can be produced by different *Enterobacteriaceae*, has been reported worldwide, including recently in *Acinetobacter baumannii* clinical isolates in Algeria (3). In this report, we describe the first detection of bla_{NDM-5}-containing New Delhi metallo-β-lactamase-producing *E. coli* clinical isolates in Algeria.

A total of 105 consecutive and nonduplicate *E. coli* clinical isolates were recovered from hospitalized and nonhospitalized patients at the University Hospital of Annaba, Algeria, and were screened for carbapenem resistance between January 2012 and February 2013. During the study period, out of the 105 isolates, a total of 3 isolates harbored the bla_{NDM-5} gene. Out of these 3 bla_{NDM-5}-positive isolates, one was isolated from the blood of a 5-month-old child hospitalized in the pediatric ward, while two were isolated from urine samples from a 63-year-old man and a 75-year-old man. The isolates were identified using the Bruker Daltonics Microflex matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometer (Bremen, Germany), as previously described (3).

These three isolates were resistant to all β-lactams, with an imipenem MIC of >32 μg/ml and a high level of resistance to aminoglycosides and fluoroquinolones. They were susceptible only to tigecycline, fosfomycin, and colistin. Thus, NDM-5-harboring strains might be highly multidrug resistant, and a previous report on NDM-5-producing *E. coli* sequence type 648 (ST648) (GenBank accession no. JN104597) demonstrated that the strain was resistant to all available antimicrobials except tigecycline and colistin (4), and we observed the same phenotype for our three isolates. Carbapenemase activity was determined using the modified Hodge test and the disk approximation tests using EDTA (4) and was also confirmed using the recently described new MALDI-TOF MS carbapenemase assay, as published previously (5). Therefore, this method has emerged as a powerful and cost-effective tool for the rapid detection of carbapenem resistance (5). In our study, the presence of the bla_{NDM-5} gene in the three isolates was confirmed by real-time PCR and further verified by standard PCR and sequencing (5). In addition, the three isolates coexpressed the $bla_{CTX-M-15}$ gene with the bla_{TEM-1} and bla_{aadA2} genes. In order to study the transferability of the resistance phenotype, a conjugation experiment was performed between our clinical donor isolates and azide-resistant *E. coli* strain J53 as a recipient. The transconjugants were selected on MacConkey agar plates containing 2 μg/ml imipenem and 100 μg/ml sodium azide, as described previously (2). PCR amplification of the plasmid DNA and susceptibility profiling showed that all transconjugants became resistant to all tested antibiotics, except aztreonam and ciprofloxacin (Table 1), and they acquired the bla_{NDM-5}, bla_{TEM-1}, and bla_{aadA2} genes. The result clearly revealed that these resistance genes were transferred via a plasmid that also confers resistance to most β-lactams, including imipenem and all aminoglycosides. Multilocus sequence typing (MLST) was performed to characterize the genetic relationship of the *E. coli* strains; it was carried out on 30 *E. coli* strains, in addition to the three NDM-5-positive isolates, using seven housekeeping genes (*adk*, *fumC*, *icd*, *purA*, *gyrB*, *recA*, and *mdh*), as described at the *E. coli* MLST Database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) (Fig. 2). The results revealed that the *E. coli* carbapenemase-positive strains belong to sequence type 2659, which was different from the NDM-5-producing ST648 *E. coli* strain (GenBank accession no. JN104597) isolated from the United Kingdom (6). This is the first reported ST2659 *E. coli* strain producing the NDM-5 carbapenemase-encoding gene.

ST2659 has been reported only once, from a domesticated cat

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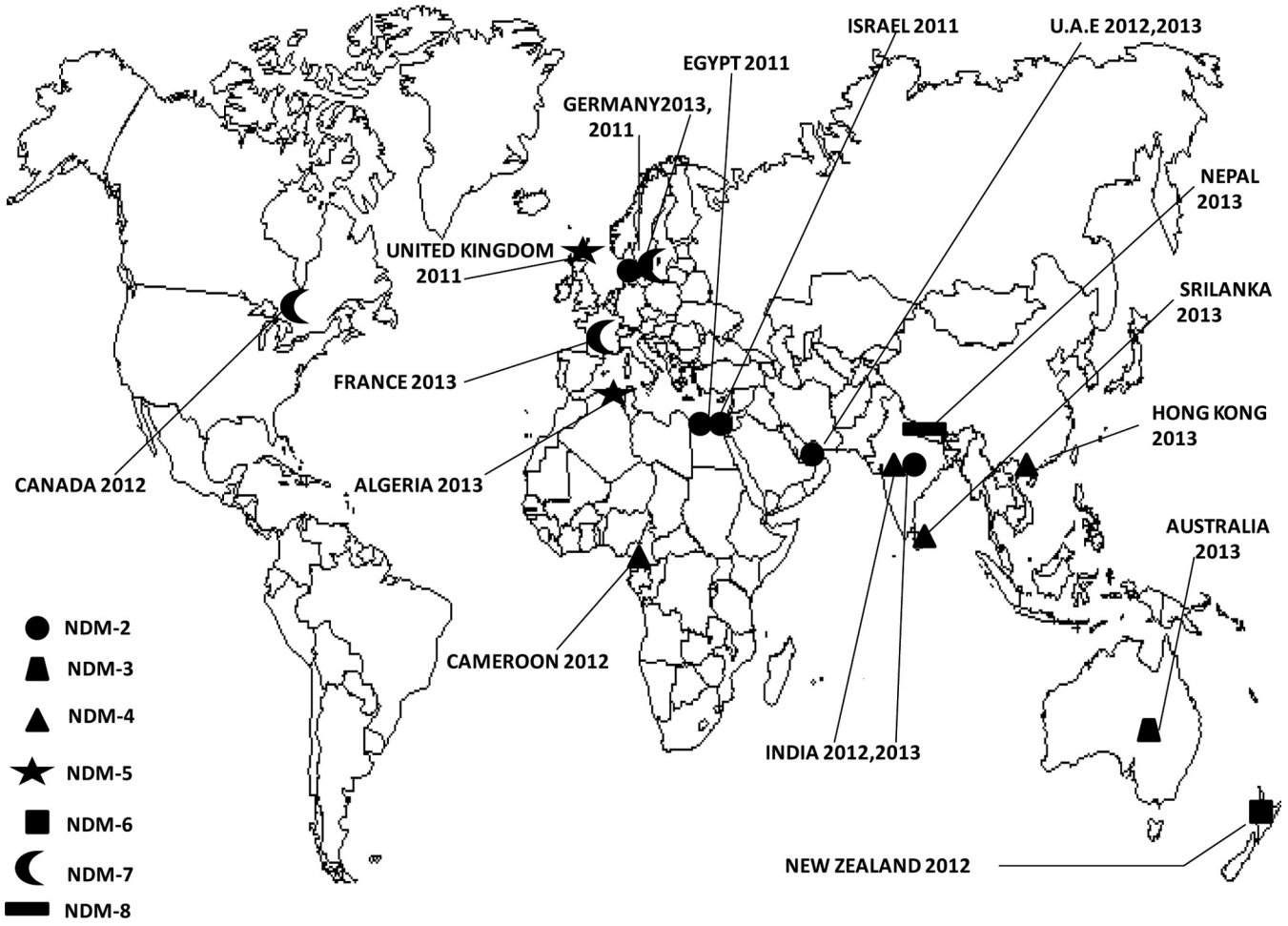

FIG 1 Geographic distribution of NDM variants detected worldwide.

in Germany (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). The reservoir of this gene in Algeria is unknown, but several contamination sources can be implicated. Various mobile genetic structures (insertion sequences, integrons, and transposons) can play an important role in the horizontal transfer of the bla_{NDM} gene between different species of bacteria, such as from *Acinetobacter* spp. to *E. coli* (6). Travelers contribute significantly to the global movement of microbes and resistance genes (6). Although nosocomial transmission of the bla_{NDM} gene has occurred in many countries (6), traveling to the Indian subcontinent is a significant risk factor for infection with an NDM-producing strain (6). The emergence of NDM-1-producing strains was linked to Asia and the Balkans (6). However, it has also been reported in autochthonous human cases worldwide (6). In contrast to other countries, where the bla_{NDM} gene has been identified mainly in *Enterobacteriaceae*, the bla_{NDM} gene has been reported only in *A. baumannii* clinical isolates from Algeria (3, 7). However, other types of carbapenemase-acquiring isolates have been reported in Algeria, such as bla_{VIM} in *Enterobacteriaceae* (8) and *P. aeruginosa* (9). The bla_{OXA-24}, bla_{OXA-23}, and bla_{OXA-58} genes have been also reported in Annaba and Tlemcen, Algeria (3, 10, 11). However, no reports are available on isolates of NDM-producing *E. coli* from Algeria and, to the best of our knowledge, we report here the first bla_{NDM-5} gene in *E. coli* from Algeria and on the African continent.

We can conclude that the epidemiology of carbapenemase-encoding genes has changed in the African continent, and NDM gene variants efficiently disseminate worldwide. These cases

TABLE 1 Antimicrobial susceptibility of the three clinical *E. coli* isolates producing the bla_{NDM-5} gene and their transconjugants

Antibiotic(s)	MIC (μg/ml) for:		
	E. coli J53	*E. coli* NDM-5	*E. coli* J53–NDM-5 transconjugants
Ampicillin	2	>256	>256
Amoxicillin-clavulanate	2	>256	>256
Piperacillin-tazobactam	1	>256	>256
Cefoxitin	4	>256	>256
Cefotaxime	2	>256	>256
Cefuroxime	4	>256	>256
Ceftazidime	0.064	>256	>256
Aztreonam	0.094	>256	0.094
Imipenem	0.25	>32	>32
Gentamicin	<1	>512	>512
Amikacin	<4	>512	>512
Tobramycin	<2	>512	>512
Ciprofloxacin	0.032	>32	0.032

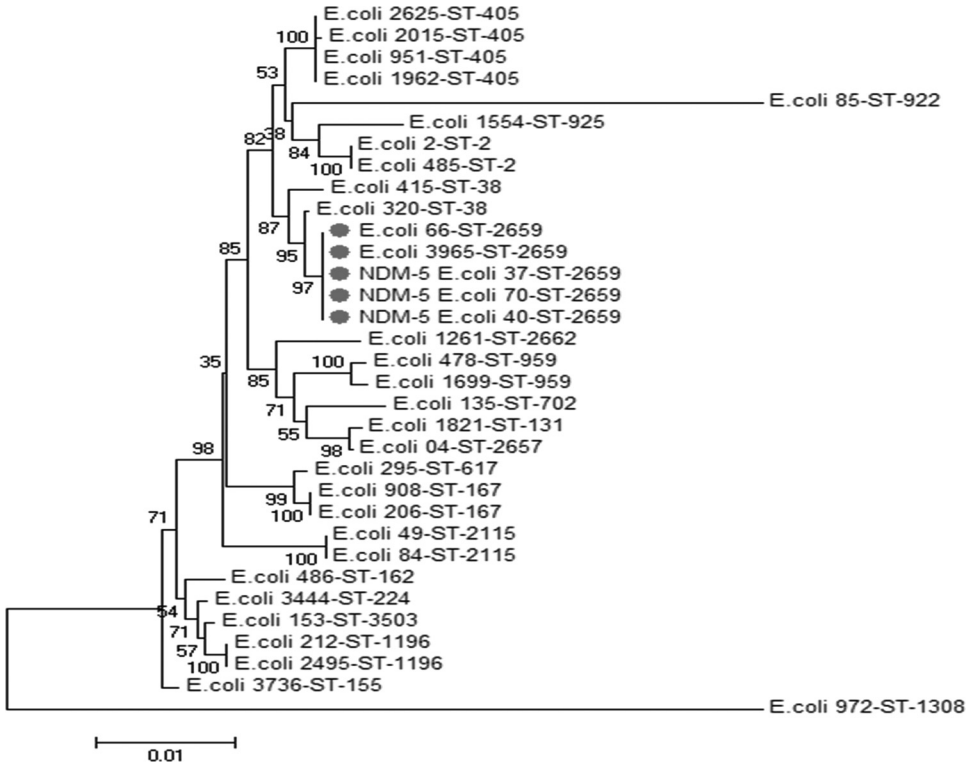

FIG 2 Concatenated phylogenetic tree showing the molecular relationships of the seven genes analyzed (*adk*, *fumC*, *icd*, *purA*, *gyrB*, *recA*, and *mdh*) for 33 clinical *E. coli* isolates, including the three NDM-5-positive isolates. Gray circles indicate *E. coli* strains belonging to sequence type 2659. The number shown at each node indicates the bootstrap level from 500 replicates.

should raise public concern once again over the increasing incidence of highly multidrug-resistant NDM-harboring strains.

Nucleotide sequence accession numbers. The nucleotide sequences of the three bla_{NDM-5}-containing *E. coli* strains have been deposited in the GenBank database under accession no. KF408072, KF408073, and KF408074.

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We declare no conflicts of interest.

All authors read and approved the manuscript.

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