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Characteristics of NDM-1-Producing *Escherichia coli* Isolates That Belong to the Successful and Virulent Clone ST131▽

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An NDM-1 carbapenemase-producing *Escherichia coli* isolate of sequence type 131 (ST131) that belonged to phylogenetic group B2 was obtained from a patient with a urinary tract infection who returned to the United States after a recent hospitalization while visiting India. NDM-1-producing *E. coli* ST131 had significantly more virulence factors than NDM-1-producing *E. coli* ST101, previously isolated from a patient in Canada. The presence of NDM β-lactamases in a very successful and virulent *E. coli* sequence type is of concern.

The development of resistance to the carbapenems in the *Enterobacteriaceae* is a major issue, since these agents are often the last line of effective therapy available for the treatment of infections caused by these bacteria (13). There are 3 types of β-lactamases that inactivate the carbapenems (also called carbapenemases): the KPC types, the metallo-β-lactamases (MBLs), and the oxacillinases (13). The 2 most common types of MBL found in *Enterobacteriaceae* are the VIM and IMP types (7).

Recently, a new type of MBL, named NDM-1, has been described in bacteria (*Klebsiella pneumoniae* and *Escherichia coli*) recovered from a Swedish patient who was hospitalized in New Delhi, India (24). Kumarasamy and colleagues (11) provided evidence that NDM-producing *Enterobacteriaceae* (mostly *K. pneumoniae* and *E. coli*) are widespread in India, Pakistan, and the United Kingdom. NDM-producing *Enterobacteriaceae* have also recently been isolated from patients residing in the United States (1), the Netherlands (12), Australia (20), Canada (15), France (19), and the Sultanate of Oman (18). Most of the patients received medical care while visiting the subcontinent of Pakistan, India, and Bangladesh.

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A study was designed to characterize two carbapenem-resistant *E. coli* isolates, from Chicago, IL, and Alberta, Canada (14), obtained from patients with recent hospitalization while visiting India. Since very little information about the virulence factors (VFs) associated with *E. coli* that produces NDM-1 is available, we investigated the phylogenetic groups and virulence traits of these isolates.

Antimicrobial susceptibility was determined with a Microscan NEG MIC 30 panel (Siemens, Burlington, ON, Canada). The MICs of the following drugs were determined: amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefoxitin (FOX), ceftriaxone (CRO), ceftazidime (CAZ), aztreonam (ATM), meropenem (MER), ertapenem (ERT), amikacin (AMK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), nitrofurantoin (NIT), and trimethoprim-sulfamethoxazole (SXT). Additional susceptibilities for imipenem (IPM), MER, ERT, tigecycline (TIG), and colistin (CST) were performed using Etest methodology per the manufacturer's instructions (AB BioDisk, Solna, Sweden). Throughout this study, results were interpreted using CLSI criteria for broth dilution (6). Fosfomycin (FOF) susceptibility was determined using the CLSI disk methodology (6).

The presence of MBLs in *E. coli* was determined with an MBL Etest (AB BioDisk, Solna, Sweden). Isoelectric focusing (IEF) was performed as previously described (16). PCR amplification and sequencing of bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{CTX-M}, bla_{OXA}, bla_{TEM}, and bla_{SHV} were carried out as previously described (14, 16). Amplification of the *qnrA*, *qnrS*, and *qnrB* genes was undertaken with a multiplex PCR (22), while *aac(6′)-Ib* and *qepA* were amplified as previously described (21, 23). Multilocus sequence typing (MLST) was performed using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) (http://mlst.ucc.ie/mlst/dbs/Ecoli). Serotype O25 was confirmed using a PCR for the *pabB* allele (5). The *E. coli* isolates were assigned to one of the four main *E. coli* phylogenetic groups by the use of a multiplex PCR-based method (4). VFs for extraintestinal pathogenic *E. coli* (ExPEC)-associated genes were determined by established PCR-based assays (17). Plasmid sizes were determined as previously described (2), and plasmids were assigned to families by PCR-based replicon typing (3). A conjugation experiment was performed by mating-out assays with nutrient agar containing IPM, 2 μg/ml, and with *E. coli* J53 (azide concentration of 100 μg/ml) as the recipient.

The patient was a 40-year-old male paraplegic who traveled to New Delhi, India, for a surgical procedure in 2010. Three to 4 months after his return to the United States, he presented to an emergency department at a hospital in the Chicago metropolitan area with nausea, vomiting, dysuria, and frequent urination. The diagnosis of urinary tract infection (UTI) was made, and the patient was admitted. Due to the history of sepsis with multiresistant *E. coli*, the patient was placed in

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TABLE 1. Susceptibilities and molecular characteristics of NDM-1-producing *E. coli* CH01 and MH01

Parameter	Value or characteristic for *E. coli* strain:	
	CH01	MH01
MIC[a] (μg/ml) of:		
AMC	>16/8	>16/8
TZP	>64/4	>64/4
FOX	>16	>16
CRO	>32	>32
CAZ	>16	>16
ATM	8	>16
MER	>8	>8
IPM	>8	>8
AMK	>32	>32
GEN	>8	>8
TOB	>8	>8
CIP	>2	>2
SXT	>1/19	>1/19
NIT	≤32	>64
β-Lactamase(s)	NDM-1	NDM-1, CTX-M-15
Plasmid-mediated quinolone resistance determinant	*aac(6′)-Ib-cr*	*aac(6′)-Ib*
MLST result	ST131	ST101
Plasmid sizes (kb)	120, 300	75, 165, 300, 400
Replicon typing results	IncA/C, IncFIA	IncA/C, IncFII
Phylogenetic group	B2	B1
Virulence characteristics[b]		
Adhesins		
F10 *papA*	Pos	Neg
papACEFG	Neg	Neg
sfa foc	Neg	Neg
focG	Neg	Neg
iha	Pos	Neg
fimH	Pos	Pos
tsh	Neg	Neg
hra	Neg	Pos
afa dra	Neg	Neg
Toxins		
hlyD	Neg	Neg
sat	Pos	Neg
pic	Neg	Neg
vat	Neg	Neg
astA	Neg	Neg
cnf1	Neg	Neg
Siderophores		
iroN	Neg	Neg
fyuA	Pos	Pos
ireA	Neg	Neg
iutA	Pos	Neg
Capsule		
kpsM II	Pos	Neg
K1	Neg	Neg
K2	Pos	Neg
K5	Neg	Neg
Miscellaneous		
usp	Pos	Pos
traT	Pos	Neg
ompT	Pos	Neg
iss	Neg	Neg
$fliC_{H7}$	Neg	Neg
malX	Pos	Neg

[a] MICs were determined using the Microscan NEG MIC 30 panel. MIC values for the respective drugs in a drug combination are separated by a slash.

[b] The presence (Pos) or absence (Neg) of each virulence characteristic is indicated. F10 *papA*, P fimbria subunit variant; *papACEFG*, genes of P fimbria operon; *sfa foc*, S or F1C fimbriae; *focG*, F1C fimbria adhesin; *iha*, adhesion siderophore; *fimH*, type 1 fimbriae; *tsh*, temperature-sensitive hemagglutinin; *hra*, heat-resistant agglutinin; *afa dra*, Dr-binding adhesins; *hlyD*, α-hemolysin; *sat*, secreted autotransporter toxin; *pic*, serine protease; *vat*, vacuolating toxin; *astA*, enteroaggregative *E. coli* toxin; *cnf1*, cytotoxic necrotizing factor; *iroN*, salmochelin (siderophore) receptor; *fyuA*, yersiniabactin (siderophore) receptor; *ireA*, siderophore receptor; *iutA*, aerobactin (siderophore) receptor; *kpsM* II, group 2 capsule; K1, K2, and K5, group 2 capsule variants; *usp*, uropathogen-specific protein; *traT*, serum resistance-associated protein; *ompT*, outer membrane protease T; *iss*, increased serum survival; $fliC_{H7}$, flagellin variant; *malX*, pathogenicity island marker.

isolation with contact precautions. A Foley catheter-collected urine culture yielded *E. coli* CH01 at $>10^5$ CFU per ml of urine. The patient was treated with oral nitrofurantoin at 100 mg twice a day for 7 days. His urinary tract infection improved clinically, and a follow-up urine culture taken 2 days after the completion of the nitrofurantoin treatment showed no growth. He was discharged after 7 days. Active surveillance was performed with rectal swabs in patients from the same ward and failed to identify additional cases.

The characteristics of *E. coli* CH01 (from Chicago) and *E. coli* MH01 (from Canada) are shown in Table 1, as are the MICs obtained with the Microscan NEG MIC 30 panels. Additional MICs were as follows; MER, 16 μg/ml; IPM, 16 μg/ml; ERT, >32 μg/ml; TIG, 0.25 μg/ml; and CST, 0.125 μg/ml. The zone size for FOF was 27 mm for CH01. CH01 was susceptible only to NIT, TIG, and FOF, while the CST MIC was 0.125 μg/ml. The transconjugant obtained with CH01 (i.e., CH01A) showed an MBL phenotype; plasmid analysis showed that CH01A harbored the 120-kb plasmid with markers for kanamycin, gentamicin, tobramycin, and trimethoprim-sulfamethoxazole. PCR confirmed that CH01A contained bla_{NDM}; CH01A belonged to the IncFIA replicon group. CH01 belonged to phylogenetic group B2 and sequence type 131 (ST131) and showed a virulence profile identical to those of a different ST131 strain isolated in Calgary, Alberta, Canada, during 2007 that produced CTX-M-15, OXA-1, and TEM-1 (data not shown). CH01 had significantly more virulence factors than MH01, previously isolated from a patient in Canada (Table 1).

ST131 is a very successful clone among CTX-M-producing and fluoroquinolone-resistant *E. coli* isolates (9). A study from Johnson and colleagues showed that that ST131 isolates have a fitness advantage because of their group B2 genomic backbone (8) and appear to combine resistance and virulence, which traditionally have been mutually exclusive in *E. coli* (10). *E. coli* ST131 that produces NDM-1 had previously been described from a patient that recently returned to France after living in India for several years (19).

The recent pandemic caused by *E. coli* ST131 with CTX-M β-lactamases, which began in 2003, highlights the ability of a successful clone to spread rapidly, and it is possible that *E. coli* ST131 isolates that produce NDM-1 could also cause a similar pandemic. If this emerging public health threat is ignored, it is possible that in the near future, the medical community will be confronted with carbapenem-resistant *E. coli* that causes common infections, such as UTIs. The identification of ST131 isolates that produce NDM-1 has grave implications for the future treatment of community-associated infections. The widespread and successful dissemination of ST131 and its distinctive combination of resistance and virulence need urgent investigation.

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