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Genetic characterisation of NDM-1 and NDM-5 -producing Enterobacterales from retail chicken meat in Egypt

Sir,

Among the acquired carbapenemases that have been identified worldwide, NDM types are one of the most clinically significant since they hydrolyze almost all β-lactams at a high level. They have been found in all continents and almost all clinically-relevant Gram-negative pathogens. NDM producers are often multidrug-resistant, having accumulated a significant number of acquired resistance genes. The bla_{NDM-1} gene was first detected in *Klebsiella pneumoniae* from a patient who previously traveled in New Delhi, India, and the bla_{NDM-5} gene was identified from a multidrug-resistant *Escherichia coli* in the UK. NDM-5 differs from NDM-1 by two amino acid substitutions at positions 88 (Val3Leu) and 154 (Met3Leu) involved in increased carbapenemase activity. Although NDM-producing Enterobacterales are known to be widely disseminated among humans, animals, and the environment, limited studies are available regarding their occurrence and dissemination in the food chain.

In this study, a total of 145 retail chicken carcasses were collected between June and December 2017 from different supermarkets, poultry slaughterhouses, and butcher shops in Egypt. The samples (neck skin) were homogenized and enriched in a sterile buffer peptone water for 24 h at 37° C with shaking, followed by direct plating on a selective plate (MacConkey agar supplemented with 2 μg/mL of meropenem (Sigma-Aldrich, Tokyo, Japan). Colonies of different morphology, size, and color from each plate were selected and purified for DNA extraction and PCR screening. A total of 155 meropenem-resistant isolates were obtained and screened by PCR for the carbapenemase-encoding genes bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{BIC}, bla_{AIM}, bla_{SPM}, bla_{DIM}, bla_{GIM}, and bla_{SIM}. A single *K. pneumoniae* Kp38 and a single *E. coli* Ec34 isolate were found to produce the NDM-1 and NDM-5 enzymes, respectively. Both isolates possessed a multidrug resistance phenotype. MICs were determined using the broth microdilution technique (Table 1). Results showed that *E. coli* EC34 was resistant to ampicillin, amoxicillin-clavulanic acid, aztreonam, cefotaxime, ceftazidime, ceftriaxone, doripenem, imipenem, meropenem, nalidixic acid, ciprofloxacin, kanamycin, gentamicin, chloramphenicol, and tetracycline, and remained susceptible only to amikacin, colistin, tigecycline, and fosfomycin. *K. pneumoniae* isolate Kp38 was resistant to all antimicrobials tested, except to gentamicin, tigecycline, and colistin. Phenotypic carbapenemase production was confirmed by using the Carba NP test and the recently developed NitroSpeed Carba NP test [1,8]. PCR and DNA sequencing were used to detect ESBLs, plasmid-mediated quinolone-resistance determinants, and 16S rRNA methylases conferring resistance to all aminoglycosides. The *E. coli* Ec34 isolate carried bla_{OXA-1}, bla_{TEM-1}, $bla_{CTX-M-3}$, and *aac(6′)-Ib-cr*, while the *K. pneumoniae* Kp38 isolate harbored the bla_{SHV-1}, $bla_{CTX-M-15}$, and *aac(6′)-Ib-cr* genes. In both isolates, the IS*Aba*125 insertion sequence was identified immediately upstream of the bla_{NDM-1} and bla_{NDM-5} genes, while the bleomycin resistance gene ble_{MBL} was identified downstream of it. Those genetic structures corresponded to those usually found in the close vicinity of most of the bla_{NDM-1} genes in Enterobacterales [2].

Conjugation and transformation experiments were performed to evaluate the transferability of the bla_{NDM-1} and bla_{NDM-5} genes [3,9]. The azide-resistant *E. coli* strain J53 was used as the recipient and transconjugants were selected on LB agar plates supplemented with ampicillin (100 μg/mL) and sodium azide (150 μg/mL). The bla_{NDM-5} gene was successfully transferred into the *E. coli* strain J53, indicating that it was located on a transferable plasmid. On the opposite, and despite repeated attempts, conjugation failed to transfer the bla_{NDM-1} gene from *K. pneumoniae* Kp38 to *E. coli* J53. Consequently, the plasmid extraction was performed from *K. pneumoniae* Kp38 and electroporated into *E. coli* DH10B. Transformants were successfully selected on LB agar plates containing ampicillin (100 μg/mL) [9]. The *E. coli* transconjugant producing NDM-5 showed resistance to carbapenems and broad-spectrum cephalosporins but remained susceptible to aminoglycosides and aztreonam, indicating that the corresponding plasmid did neither harbor an ESBL nor an aminoglycoside resistance determinant. The *E. coli* transformant producing NDM-1 showed resistance to broad-spectrum cephalosporins, carbapenems, and aztreonam, but remained susceptible to aminoglycosides, showing that an ESBL encoding gene was co-located onto the same plasmid as the bla_{NDM-1} gene was. PCR and sequencing of the amplicon confirmed that the bla_{NDM-1}-positive plasmid additionally carried the $bla_{CTX-M-15}$ gene.

MICs of imipenem and meropenem for the NDM-5- and NDM-1-producing *E. coli* transconjugants and transformants were 4–8 and 4 μg/mL, respectively, similarly to what is commonly observed [4,5]. Those MIC values were much lower than those found for the respective parental strains (Table 1), showing that a series of additional resistance mechanisms (permeability defects, efflux overexpression) also occurred in those latter clinical isolates. PCR-based replicon typing (PBRT) method was used to identify the incompatibility group of those NDM-encoding plasmids. The bla_{NDM-5}-positive plasmid that was ca. 48 kb in-size belonged to the IncX3 incompatibility group, whereas the bla_{NDM-1}-positive plasmid that was ca. 100 kb in-size belonged to the IncR group. Multilocus sequence typing (MLST) analysis revealed that *E. coli* EC34 belonged to the ST648 sequence type, while *K. pneumoniae* Kp38 belonged to ST147. NDM-5-producing ST648 *E. coli* has been reported in India, UK, and Australia [6]. NDM-1-producing ST147 *K. pneumoniae* clone has been reported in several Mediterranean countries and beyond, including in Iraq, Oman, Tunisia, and Egypt. In Egypt, NDM-1 was first reported in 2013 from one ST11 *K. pneumoniae* isolate recovered from a hospitalized patient [7]. The first case of *E. coli* producing NDM-5 has been identified from the ascitic fluid of a 52-years-old female patient with post-hepatitis cirrhosis [4]. To the best of our knowledge, this is the first report of

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Table 1
Minimum inhibitory concentrations (MICs) of different antibiotics for NDM-5-producing *Escherichia coli* Ec34, NDM-1-producing *Klebsiella pneumoniae* Kp38 and derived *E. coli* transconjugants (Tc) and transformants (Tf).

Strain	MIC (μg/mL)											
	CTX	CRO	MEM	IPM	KAN	GEN	NAL	CIP	NOR	TET	CHL	COL
E. coli Ec34	256	256	128	64	256	256	256	256	256	256	256	0.5
E. coli Tc (NDM-5)	256	32	8	4	16	8	64	0.5	0.5	0.5	8	0.5
K. pneumoniae Kp38	256	256	128	64	256	8	256	256	256	16	256	0.5
E. coli Tf (NDM-1)	256	256	4	4	256	8	8	0.5	0.5	4	4	0.5

CRO, ceftriaxone; NOR, norfloxacin; CHL, chloramphenicol; CIP, ciprofloxacin; TET, tetracycline; IPM, imipenem; MEM, meropenem; COL, colistin; KAN, kanamycin; GEN, gentamicin; NAL, nalidixic acid; CTX, cefotaxime.

NDM-5 from chicken meat in Egypt and the Middle East. It further indicates that NDM producers may be a true One-Health issue disseminating in the environment and then in humans through the food chain.

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Ethical approval

Not required

Competing interests

None declared

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