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bla$_{NDM-5}$-carrying IncX3 plasmid in *Escherichia coli* ST1284 isolated from raw milk collected in a dairy farm in Algeria

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Sir,

Resistance to carbapenems in Enterobacteriaceae has become a major concern worldwide. New Delhi metallo-β-lactamases (NDMs) are carbapenem-hydrolysing enzymes first recognized in 2008 in a Swedish patient previously hospitalized in India.[1] To date, 16 NDM variants have been identified worldwide with NDM-1 being the most prevalent. The NDM-5 variant was reported for the first time in 2011 in the UK, also from a patient with a recent history of hospitalization in India, and was later identified in China, India, Japan, Australia and Algeria.[2–5] In Algeria, NDM-5-producing *Escherichia coli* isolates were reported in humans in 2014, and twice from companion animals in 2015 and 2016.[6–8] Here, we report four NDM-5-producing *E. coli* isolates from raw milk collected in a dairy farm in Algeria.

In March 2015, 34 healthy dairy cows from seven familial farms were randomly selected to ascertain the presence of carbapenemase producers in milk intended for local consumption in the area of Bejaia, Algeria. Milk samples were recovered from all animals and processed within an hour after collection. They were diluted 1:10 in nutrient broth supplemented with ertapenem (0.5 mg/L) and plated onto imipenem-supplemented (1 mg/L) MacConkey agar. For each cow, an additional swab from the mammal teat was also taken. One presumptive *E. coli* colony was selected per plate and identification was confirmed by MALDI-TOF (Vitek-MS, bioMérieux, France). All *E. coli* isolates were tested for antimicrobial susceptibility by disc diffusion according to the Antibiogram Committee of the French Society for Microbiology guidelines (www.sfm-microbiologie.org). MICs were determined by Etests (bioMérieux, France). Confirmation of carbapenemase production was achieved by the Modified Hodge test, Carba NP test, EDTA double-disc synergy test and PCR/sequencing for the *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM-like}$, *bla*$_{OXA-48-like}$, *bla*$_{KPC}$, *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{CMY}$ genes.[9]

The four *E. coli* isolates were recovered from two cows (milk and teat in both cases) from the same farm. Isolates were resistant to all β-lactams including carbapenems (ertapenem, meropenem and imipenem MICs of >32 mg/L), but susceptible to colistin and tigecycline (MICs of 0.5 mg/L and 0.25 mg/L, respectively). All four isolates had identical PFGE profiles and carried the genes *bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$ and *bla*$_{CMY-42}$, highlighting the spread of a unique *E. coli* clone, further identified to belong to ST1284. PCR-based replicon typing (Diatheva, Italy) and Southern blots on S1-PFGE gels with adequate probes demonstrated that *bla*$_{NDM-5}$ was located on an IncX3 plasmid whose size was ~50 kb. The IncX3 plasmid was conjugative but at a low frequency, since only one transconjugant presenting the *bla*$_{NDM-5}$ gene was obtained despite several conjugation experiments on MacConkey agar supplemented with imipenem (10 mg/L) using rifampicin-resistant *E. coli* as a recipient.

Our findings raise two main concerns. First, we highlight the probable direct transfer of NDM producers to humans through commonplace foodstuff intake in a country where NDM-producing Enterobacteriaceae are infrequent. NDM-1 was recovered once from cattle milk in India where these enzymes are highly endemic and the cow suffered from mastitis, rendering the milk unfit for human consumption.[10] In Algeria, cows were reared for local consumption and selling of crude milk, a common situation with >80% of the cattle milk production outside industrial processes. Thus, our data suggest previously unidentified sources and pathways for the spread of NDM producers in the community. Second, an NDM-5-producing ST1284 *E. coli* clone was recently reported from a diseased dog in Bejaia, Algeria.[8] Another NDM-5/CTX-M-15/CMY-42-producing *E. coli* isolate was also recovered from a healthy dog in the same city but the ST of the *E. coli* was not determined.[7] The occurrence of three similar NDM-5-producing *E. coli* isolates of animal origin in this small geographic area strongly suggests the spread of a unique clone. Since this ST1284 largely differs from the NDM-5-producing ST2659 *E. coli* reported in humans in Algeria or other countries,[6] plasmids may well be responsible for the *bla*$_{NDM-5}$ spread among different *E. coli*. Here, an IncX3-type plasmid carried the *bla*$_{NDM-5}$ gene, as also recently reported in India, Australia and China, but NDM-5-carrying plasmid types from clinical cases in Algeria were not determined.[3,5] Altogether, clonal spread and/or horizontal transfer of *bla*$_{NDM-5}$-bearing IncX3 plasmids may contribute to NDM-5 dissemination in Algeria. Neither the farmer nor the family or farm employees had a history of antibiotic treatment, hospitalization or travel in countries where NDM enzymes are endemic, and it is rather unlikely that the use of carbapenems in animals was the selective factor. Whatever the origin of these NDM-5 strains is, this study shows that raw milk consumption, at least in Algeria, is at risk to transfer and disseminate NDM-producing *E. coli* directly to the human population. Consequently, there is an urgent need for setting up global risk analysis approaches on carbapenem resistance, which include the animal/environmental reservoir, obviously in Algeria and surely on a more global scale.

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Transparency declaration

None to declare.

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