First report of NDM-5-producing *Escherichia coli* ST1284 isolated from dog in Bejaia, Algeria

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Carbapenemase-producing isolates have been isolated from humans, environmental samples and, more recently, companion animals [1]. Among the newly emerged carbapenemases, New Delhi metallo-β-lactamase (NDM) represents the latest threat for public health [2]. Sixteen variants of NDM-type carbapenemase (NDM-1 to NDM-16) have been reported (http://www.lahey.org/Studies).

Here we report what is to our knowledge the first case of *Escherichia coli* carrying the bla_{NDM-5} gene isolated from a dog in Algeria.

In March 2015 a rectal swab was collected from a 3-year-old German Shepherd dog with a tumor at its third finger. The sample was cultured in 10 mL nutrient broth containing half a disc of ertapenem. After incubation at 37°C for 24 hours, 200 μL was streaked on MacConkey agar plates containing imipenem at a concentration of 0.5 μg/mL. An *Escherichia coli* isolate was identified by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; Microflex, Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed by the disk diffusion method and interpreted according to the European Committee on Antimicrobial Susceptibility Testing guidelines (http://www.eucast.org/). The minimum inhibitory concentration (MIC) values of β-lactams were determined using Etest strips according to the manufacturer's recommendations (AB bioMérieux, Solna, Sweden).

The *E. coli* isolate was found resistant to β-lactams, including carbapenems (MICs >32 μg/mL) and extended-spectrum cephalosporins (MICs >256 μg/mL). The isolate was also resistant to tetracycline and fluoroquinolones but was susceptible to amikacin, cotrimoxazole, colistin and tigecycline.

The isolate was screened for carbapenemase production using the modified Carba NP test [3], and the production of metallo-β-lactamases was detected by the inhibition of the metallo-β-lactamase activity by ethylenediaminetetraacetic acid (EDTA) as previously described [4]. The *E. coli* isolate was positive for production of carbapenemase, which is inhibited by EDTA.

PCR was used to screen for the presence of genes encoding for class A carbapenemases (bla_{KPC} and bla_{GES}), class D carbapenemases (bla_{OXA-48}) and metallo-β-lactamases (bla_{NDM-1} and bla_{VIM}) as previously described [5], followed by sequencing, which led to the identification of bla_{NDM-5}. A review the literature at PubMed found 12 reports on NDM-5, which were all recovered from clinical specimens; no reports were from veterinary isolates, however. Among these 12 reports, one was from Algeria [6]. The authors of this study reported three isolates of *E. coli* sequence type (ST) 2659 recovered from urine and blood specimen isolated between January 2012 and February 2013 in the university hospital of Annaba (east Algeria). Thus, this is the first report of NDM-5-producing *E. coli* isolated from companion animals. Furthermore, other types of carbapenemases have been reported from domesticated animals, including NDM-1 in multidrug-resistant *E. coli* strains recovered from companion animals in the United States [7], and OXA-48 carbapenemase-producing *Klebsiella pneumoniae* and *E. coli* isolates obtained from a dog in Germany [8]. The *E. coli* isolate also carried the bla_{TEM-33} gene.

The transferability of the resistance phenotype was studied by a conjugation experiment using sodium azide–resistant *E. coli* strain J53 as a recipient. The transconjugants were selected on Luria agar plates containing ceftazidime (8 μg/mL) and sodium azide (200 μg/mL) [2]. Only transconjugants carrying the bla_{TEM-33} gene were obtained. To determine the localization of the bla_{NDM-5} gene the isolate was subjected to plasmid extraction using the High Purity Plasmid Miniprep Kit (Neobiotech, Los Angeles, CA, USA) and the QIAquick Gel extraction kit (Qiagen, Germantown, MD, USA), followed by PCR amplification targeting bla_{NDM-5} performed on the extracted plasmid. The result showed that the isolate harboured only one plasmid which did not contain the bla_{NDM-5} gene, indicating that this gene is probably integrated into the chromosome.

Genotyping of the isolate was performed by multilocus sequence typing (MLST) using seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA* and *recA*), as described at the *E. coli*

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MLST Database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). According to MLST analysis, the *E. coli* isolate was attributed to ST1284. This ST has been identified previously in *E. coli* isolates producing extended-spectrum β-lactamase recovered from animals [9].

In summary, this report documents the emergence of NDM-5 among *E. coli* ST1284 isolated for the first time from a companion animal in the North African countries.

Conflict of interest

None declared.

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