New Microbiologica, 41, 3, 230-231, 2018, ISSN 1121-7138

SHORT COMMUNICATION

Detection of ST1702 *Escherichia coli* $bla_{\text{NDM-5}}$ and $bla_{\text{CMY-42}}$ genes positive isolates from a Northern Italian hospital

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SUMMARY

We describe two multi drug-resistant (MDR) carbapenemase-producing *Escherichia coli* clinical isolates from an acute hospital in Milan. Both strains, isolated from a surgical wound sample and a surveillance rectal swab respectively, were positive for a bla_{NDM}-type gene by Xpert Carba-R test. The whole-genome sequence characterization disclosed several resistance determinants: $bla_{\text{NDM-5}}$, $bla_{\text{CMY-42}}$, $bla_{\text{TEM-198}}$, *rmtB*, *mphA*. The two isolates belonged to phylogenetic group A, sequence type (ST) 1702 and serotype O89:H9. PCR-based replicon typing and conjugation assay demonstrated an IncI1 plasmid localization for both $bla_{\text{NDM-5}}$ and $bla_{\text{CMY-42}}$ genes. This is the first report of a ST1702 NDM-5 and CMY-42- producing *E. coli* clone in Italy.

Received March 22, 2018 *Accepted* June 16, 2018

Emergence of New Delhi metallo β-lactamase (NDM)-producing *Enterobacteriaceae* has become a crucial issue of global concern. The bla_{NDM} gene not only confers resistance to most β-lactams, but is often accompanied by several resistance determinants, thus making pathogens multidrug-resistant. NDM-producing bacteria have been implicated in both hospital - and community - acquired infections and have been recovered from several infection sites, including those associated with septicemia, urinary tract infections, and wound infections as well as from companion and livestock animals (Ranjan *et al.,* 2016). The evolution and spread of NDM are rapid, and to date, 21 variants of NDM enzyme have been reported (Naas *et al.,* 2017). $bla_{\text{NDM-5}}$ gene was first reported in an *Escherichia coli* strain (EC045) from a patient in the United Kingdom; the protein differed from NDM-1 by only two amino acid substitutions (Val88Leu and Met154Leu) and showed increased resistance to carbapenems and broad-spectrum cephalosporins (Hornsey *et al.,* 2011). Since then, NDM-5-producing strains have also been identified in Algeria, the United States, Australia, China, Denmark, India, Italy, Japan, Poland, Singapore, Spain, the South Korea, Egypt and the Netherlands (Zhu *et al.,* 2016).

Key words:
ST1702, *Escherichia coli,* NDM-5, CMY-42.

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Many reports have indicated a high sequence type (ST) diversity for $bla_{\text{NDM-5}}$-positive *E. coli* and there are several indications of isolates co-harbouring other resistance determinants like the plasmid-borne colistin resistance gene *mcr-1* (Chen *et al.,* 2017; Zhou *et al.,* 2017; Mediavilla *et al.,* 2016) or oxacillinase $bla_{\text{OXA-181}}$ (Rojas *et al.,* 2017; Gamal *et al.,* 2016). These data, together with the demonstrated horizontal transferability of the $bla_{\text{NDM-5}}$ gene either through plasmids or transposon-related mobile elements, highlighted the importance of continuous epidemiological investigation and surveillance of NDM isolates.

Here we report the detection of two multi drug-resistant (MDR) NDM-5 *E. coli* clinical isolates from the ASST Fatebenefratelli-Sacco hospital (Milan). The first isolate was collected in July 2015 from an Italian man in his 60s with a known history of several hospitalizations. The patient, HIV-positive and suffering from an HCV-related chronic hepatitis, was admitted to the surgical ward for a small intestine partial resection. After surgery, a carbapenemase-producing *E. coli* strain, Ec_S1_3/4, was isolated from a wound sample; it was confirmed as NDM-positive by Xpert Carba-R test (GeneExpert, Cepheid).

The second strain, Ec_S1_6, was isolated in August 2015 from a screening rectal swab of a 76-year-old female patient transferred to the Medicine ward from a long-term care facility. The woman, affected by a urinary tract infection, was empirically treated with a piperacillin/tazobactam, meropenem and fluconazole. The therapy was discontinued based on a reduction of the inflammation indices. The bla_{NDM}-type determinant was detected by Xpert Carba-R test.

The identification of the two *E. coli* strains was obtained by Maldi-TOF MS (BioMèrieux), while the susceptibility profiles were determined by Vitek2 System (BioMèrieux) and in-

Table 1 - *Genetic features of the Ec_S1_3/4 and Ec_S1_6 isolates.*

Carbapenemase/AmpC enzyme	*MLST 1/2**	*Serotype*	*Resistance profile*
NDM-5, CMY-42	1702/2	O89:H9	*bla*$_{TEM-198}$, *aad*A, *aad*A5, *sul1*, *tet*(A), *tet*(R), *dfr*A17, *dfr*A12, *mph*A, *rmt*B

*MLST 1: seven genes Achtman scheme, MLST 2: eight genes Pasteur scheme.

terpreted according to the EUCAST 2016 breakpoints. Both isolates (Ec_S1_3/4 and Ec_S1_6) resulted MDR, showing a high resistance level to all carbapenems, cephalosporins, fluoroquinolones and trimethoprim-sulfametoxazole by Vitek2 System. The carbapenem MIC values, determined using the broth microdilution Sensititre system (Termoscientific, Italy), of the two isolates were 8 and ≥16 mg/L for imipenem, ≥16 mg/L for ertapenem and ≥8 mg/L for meropenem.

The genomic DNA was extracted with the QIAamp DNA minikit (Qiagen) following the manufacturer's instructions, and sequenced using Illumina Miseq (2 x 250 paired-end run) after Nextera XT library preparation. Ec_S1_3/4 and Ec_S1_6 reads were assembled using the SPAdes (Nurk et al., 2013) program in 225 and 257 contigs, respectively (accession number ERS2527069 and ERS2527070, available in the project PRJEB27128).

The Enterobase (https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search), ResFinder, and SeroFinder (http://www.genomicepidemiology.org/) databases were used to characterize the STs, the antibiotic resistance mechanisms, and the serotypes of the *E. coli* isolates. The phylogenetic group analysis was also accomplished according to Clermont's scheme (Clermont *et al.,* 2000).

Ec_S1_3/4 and Ec_S1_6 belonged to phylogenetic group A, serotype O89:H9, and ST1702 (*Table 1*). The above ST was reported from an animal sample in China in 2015, and from human specimens in Yemen, Oman (2015) and Ireland in 2016. The strains from Yemen and Oman were NDM-producers but no information was available on the gene variant (http://enterobase.warwick.ac.uk/species/ecoli/search_strains?query=st_search). The presence of *bla*$_{NDM-5}$ and *bla*$_{NCMY-42}$ gene variants was ascertained in both isolates. Other resistance determinants detected were *bla*$_{TEM-1B}$, *rmtB, dfrA17, aadA5* and *mphA* (*Table 1*). The two isolates were identified as a single clone according to resistance phenotype results, coupled with the genotyping data. The co-existence of *bla*$_{NDM-5}$ and *bla*$_{CMY-42}$ genes in *E. coli* isolates was recently reported in Italy in a ST405 lineage, phylogroup D (Bitar *et al.,* 2017).

In order to assess the transferability of *bla*$_{NDM-5}$ gene, conjugation assay was performed using the *E. coli* J53 Azide^{R} as recipient strain at both 25°C and 37°C temperature. Transconjugants were obtained for both strains under the two thermal conditions. The identification and susceptibility profiles were obtained with Microscan Autoscan4 System (Beckman Coulter), and the presence of *bla*$_{NDM-5}$ and *bla*$_{CMY-42}$ was confirmed by PCR and sequencing. Plasmid analysis with PBRT kit (Diatheva) was accomplished for both isolates and transconjugants strains. The transconjugants resulted IncI1 replicon positive only, while IncFII and IncI1 were observed in the donor strains. This result differed from the previous Italian data, where a *bla*$_{NDM-5}$ determinant was detected in an IncFII harbouring plasmid, while the *bla*$_{CMY-42}$ unique resistance gene was present in an IncI1 plasmid (Bitar *et al.,* 2017).

To the best of our knowledge, this is the first description of ST1702 NDM-5 and CMY-42 co-producing *E. coli* clinical isolates in Northern Italy. In this study, no travel history was ascertained for the two patients and no further NDM-5-producing isolates have since been reported from the hospital. Ec_S1_3/4 and Ec_S1_6 isolates could represent autochthonous strains, with the community setting playing a role as reservoir. However, the patient's several previous hospitalizations may have constituted a risk factor for intra-hospital acquisition of these pathogens.

In summary, our study provides new evidence of persistence and dissemination of MDR NDM-5-producing isolates and the emergence of a new lineage in Italian clinical settings.

Competing Interests: None declared

Acknowledgments: Thanks to the Romeo ed Enrica Invernizzi Foundation.

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