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SHORT COMMUNICATION

OXA-48 and NDM-1 *Klebsiella pneumoniae* of Sequence Type 101 from blood in a patient with travel history abroad, Italy

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SUMMARY

Klebsiella pneumoniae (KP) is an important pathogen involved in serious nosocomial infections all over the world. Here, we describe the first report on a blood-stream infection caused by an OXA-48/NDM-1 ST101-KP, in Italy. The patient was an Italian woman, transferred from Cairo Hospital to a Neurosurgery ward in Cuneo (IT). The detection described here enhances the need for an effective National infection control strategy in Italy.

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The global spread of carbapenem-resistant *Enterobacterales* (CRE), frequently due to class A (KPC, GES), class B (IMP, VIM, NDM), and class D (OXA-48) carbapenemases, is of special concern (Lee *et al.*; Grundmann *et al.*). NDM-producing *K. pneumoniae* strains (carrying *bla*NDM in IncA and IncC, N plasmids), were detected mainly in the Indian Subcontinent, in Balkan States (Pitout *et al.*), and recently in Italy (Principe *et al.*). The *bla*NDM-type genes circulation, reported sporadically since 2009 in *Escherichia coli* isolates, is now a growing problem in Italy (D'Andrea *et al.*, Bitar *et al.*). After its first detection in Turkey in 2003, *K. pneumoniae bla*OXA-48 positive has been described mainly in the Middle East, in Northern African countries (Morocco, Algeria, Tunisia, Libya, and Egypt) and in Saudi Arabia (Lomonaco *et al.*). The concurrent presence of *bla*NDM-1 and *bla*OXA-48 genes has been reported in ST11, ST14, ST101, ST147 and ST258 *K. pneumoniae* isolates from Morocco, Tunisia, United Arab Emirates, Turkey, Australia and Switzerland (Lomonaco *et al.*). High-risk clones have contributed to the spread of different plasmids, genetic platforms, and resistance genes among Gram-negative bacteria and have played a very important role in the global spread of antibiotic resistance (Pitout *et al.*). An NDM-1 and OXA-48-producing ST101 *K. pneumoniae* isolate was detected from screening rectal-swab of a patient transferred from the Intensive Care Unit (ICU) of a hospital located in Belgrade to Bern University Hospital, Switzerland (Seiffert *et al.*). A second case of rectal colonization by a NDM-1/OXA-48-producing MDR *K. pneumoniae* of a ST similar to ST101 was recently reported in a 7-week-old male baby admitted to the IRCCS-ISMETT Institute of Palermo; the infant was previously hospitalized in a Neonatal ICU, where an empirical antibiotic therapy was administrated (Monaco *et al.*). Previous colonization with MDR/XDR carbapenemase-producing *K. pneumoniae* strains represents an important risk factor for the development of invasive infections, particularly in immunocompromised patients in ICUs. Here we describe the detection in Italy of a ST101 *K. pneumoniae* strain, both NDM-1 and OXA-48 carbapenemases producer, from the bloodstream of a patient with a history of travel and hospitalization abroad.

On March 21, 2016 and during a holiday period, a 62-year-old woman without previous comorbidities underwent surgery for aneurysm rupture and cerebral haemorrhage at the Neurosurgery ward of Cairo Hospital, Egypt. On April 1, 2016, after discharge from the ICU of Cairo Hospital, the patient was transferred to the Neurosurgery ward of "S. Croce and Carle" Hospital in Cuneo, Italy. On admission, as a standard in-house surveillance procedure, the patient was checked for carbapenem-resistant Gram-negative microorganism colonization. A meropenem-resistant *Klebsiella pneumoniae* strain was detected by rectal swab screening on ChromID Carba agar plates (bioMeriéux, France). The *K. pneumoniae* isolate was identified as *bla*OXA-48 and *bla*NDM-type genes positive by GeneXpertCarbaR System (Cepheid, CA, USA). On the same day, the patient's blood samples resulted positive for the presence of both MDR *K. pneumoniae* (KP-1; April 2016) and *Acinetobacter baumannii* (AB-1; April 2016) strains; both isolates were stored. Routine identification and antibiotic susceptibility testing were carried out using the MALDI-TOF-MS and Vitek-2 automated System (bioMèrieux)

Key words:
Class D carbapenemase, class B carbapenemase, ST101 *Klebsiella pneumoniae*, blood.

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at the Microbiology Laboratory, Cuneo. Antimicrobial susceptibility testing was interpreted by the 2016 European Committee on Antimicrobial Susceptibility Testing breakpoints (http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/). The KP-1 strain was MDR, being susceptible only to chloramphenicol (MIC≤8 mg/L), colistin (MIC≤2 mg/L), gentamicin (MIC≤2 mg/L) and tigecycline (MIC≤1 mg/L). The *A. baumannii* isolate resulted XDR, showing susceptibility only to colistin (MIC<2 mg/L). All the results agreed with those obtained by MicroScan AutoScan4 System (Beckman Coulter) at the Clinical Microbiology Unit, University of Pavia, where colistin MICs were confirmed by broth microdilution (MicronautS plates, Merlin) (*Table 1*). A colistin plus meropenem combination therapy was then administered. After her condition improved, on May 17, 2016, the patient was transferred to the Rehabilitation Unit III of "S.S. Trinità" Hospital of Fossano ASL CN1, Cuneo. At admission, a new rectal swab was collected and cultured on ChromID agar plates (bioMérieux); GeneXpertCarbaR System confirmed the persistence of a *bla*OXA-48 and *bla*NDM-type positive *K. pneumoniae* (KP-2; May 2016); *A. baumannii* resulted negative for the above resistance determinants. Whole Genome Sequencing (WGS, by Illumina system, MiSeq) was performed on:

1) KP-2 parental strain collected by rectal swab;
2) KP-1 and KP-2 transconjugants obtained using *E. coli*Az^r (azide-resistant) as recipient. KP-2 resistance and replicon content, determined by using default threshold parameters in ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/), allowed the identification of *bla*TEM1a, *bla*CTX-M-14b, *bla*CTX-M-15, *bla*OXA-9, *bla*OXA-48 and *bla*NDM-1 beta lactamase genes in this donor strain. The resistance to aminoglycosides, tetracycline, fluoroquinolones, macrolides and trimethoprim/sulphonamides was due to the presence of *aadA1*, *aac* (6')-Ib, *strA/B*, *aph* (3')-VIa/b, *tet(D), qnrS1, dfrA5, sul1*, *erm(B)*, *mph(A)* genes, respectively.

IncFII(K), IncL and IncM (pOXA-48), IncR, IncFIB (K) and small ColE (ColpVC) plasmid types were all detected in the KP-2 using PlasmidFinder (http://www.genomicepidemiology.org/).

E. coli J53Az^{r}RKP-1 transconjugant resulted *blaOXA-48*, *blaNDM-1*, *blaCTX-M-15*, *blaCTX-M-14b*, *blaOXA-9*, *blaTEM-1A*, *aph(3')-VIb*, *aac(6')-Ib*, *aadA1*, *strA*, *strB*, *qnrS1*, *sul1*, *erm(B)*, *tet(D)*, *mph(A)*, genes by ResFinder and IncL, IncR, ColpVC, positive by PlasmidFinder (Carattoli *et al.*); *E. coli* J53Az^{r}RKP-2 was *bla*OXA-48, *bla*CTX-M-14b, *aph(3')-VIb*, *strA*, *strB*, *erm(B)* genes and IncL positive. The molecular typing of both KP-1 and KP-2 isolates was carried out by XbaI digestion and Pulsed-Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) according to the scheme of the Pasteur Institute (https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef). The *K. pneumoniae* pulsotypes resulted identical (*Figure 1*) and the strains were finally assigned to the ST101. On May 18, due to the patient's worsening conditions, both urine and a blood-culture set were collected; bloodstream resulted positive for a *bla*OXA-48 gene harbouring *Enterobacter cloacae* strain by GeneXpert CarbaR System. Based on susceptibility testing data (imipenem MIC=2 mg/L and colistin MIC ≤0.5 mg/L), the above combination therapy was continued. Susceptibility profiles results for the MDR Gram-negative microorganisms and KP-1/KP-2 transconjugants are showed in *Table 1*. On November 3 the patient was discharged and transferred to a Long-Term Facility in Cuneo.

Resistance to broad-spectrum antibiotics such as third-generation cephalosporins in Gram-negative species of *E. coli* and *K. pneumoniae* is becoming more widespread. The subsequent increased use of carbapenems has promoted the emergence and dissemination of carbapenem-resistant *Enterobacterales,* with invasive infections from these organisms resulting in high mortality rates. Significant progress has been made in the development of new beta-lactamase inhibitors (i.e., avibactam, relebactam, vaborbactam) active against Ambler class A and C β-lactamases, including activity against *K. pneumoniae* carbapenemase (KPC)-producing organisms; however, their usefulness could be threatened by the co-presence of different carbapenemase resistance determinants (e.g., *bla*NDM plus *bla*OXA-48 and/or *bla*KPC type genes in the same pathogen or in mixed infections)

Table 1 - *Susceptibility profiles of clinical strains and their J53R E. coli transconjugants.*

Antibacterial drugs	*MICs (mg/L)/[S, I, R]*					
	KP-1	*KP-2*	*AB-1*	*OXA-48 E. cloacae*	*E. coli J53Az^R R KP-1*	*E. coli J53Az^R R KP-2*
Amoxicillin/clavulanic acid	>32 / [R]	>32 / [R]	>32 / [R]	>32 / [R]	>8/4 / [R]	>8/4 / [R]
Piperacillicn/tazobactam	>128 / [R]	>128 / [R]	-	-	>16 / [R]	>16 / [R]
Cefotaxime	>64 / [R]	>64 / [R]	>64 / [R]	>64 / [R]	>16 / [R]	>16 / [R]
Ceftazidime	>64 / [R]	>64 / [R]	-	>64 / [R]	>8 / [R]	>8 / [R]
Cefepime	>64 / [R]	>64 / [R]	-	>64 / [R]	>8 / [R]	>8 / [R]
Ertapenem	>1 / [R]	>1 / [R]	-	>8 / [R]	>1 / [R]	1/ [I]
Imipenem	>16 / [R]	>16 / [R]	>16 / [R]	2 / [S]	8 / [I]	<=2 / [S]
Meropenem	>8 / [R]	>8 / [R]	>16 / [R]	4 / [I]	8 / [I]	<= 2 / [S]
Amikacin	>64 / [R]	>64 / [R]	-	>64 / [R]	>16 / [R]	>16 / [R]
Gentamicin	4 / [I]	4 / [I]	>16 / [R]	<=1 / [S]	<=2 / [S]	<=2 / [S]
Ciprofloxacin	>4 / [R]	>4 / [R]	>4 / [R]	>4 / [R]	<=0.5 / [S]	<=0.5 / [S]
Tigecycline	1 / [S]	1 / [S]	2 / [S]	2 / [I]	<=1 / [S]	<=1 / [S]
Trimethoprim/sulfamethoxazole	>320 / [R]	>320 / [R]	>320 / [R]	320 / [R]	>4/76 / [R]	<=2/38 / [R]
**Colistin*	<=0.5 / [S]	<=0.5 / [S]	<=0.5 / [S]	<=0.5 / [S]	<=2 / [S]	<=2 / [S]

*Colistin MICs have been confirmed by broth microdilution (MicronautS plates, Merlin).
S, I, R = Susceptible, Intermediate, Resistant on the basis of EUCAST 2016 clinical breakpoints.

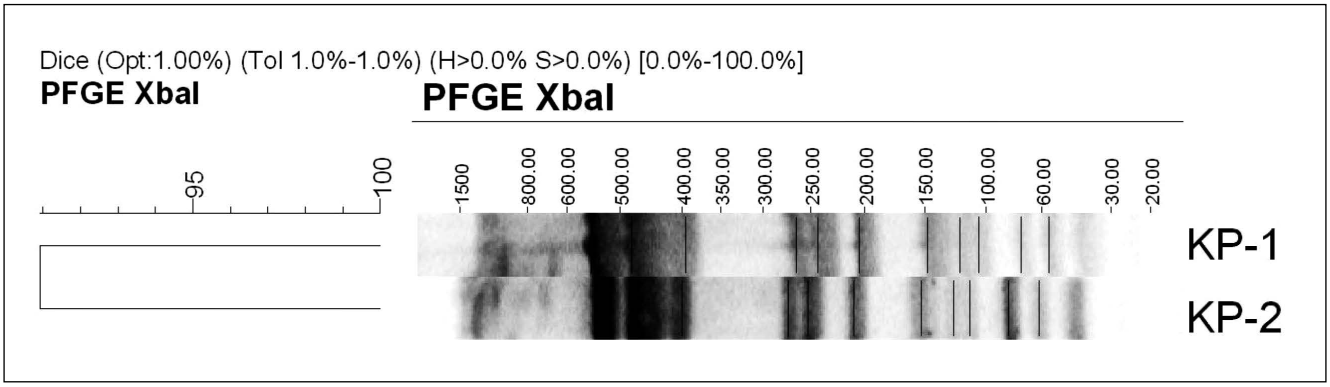

Figure 1 - *PFGE profiles of KP-1 and KP-2* Klebsiella pneumoniae.

or, merely, by their excessive and routine use. The results of a recent nationwide survey in Italy (2014-2017) showed a high and growing incidence of carbapenemase-producing *Enterobacterales* causing bloodstream infection (CPE-BSI). CPE-BSI cases more often occurred in ICU or general medicine ward patients and were mainly associated with the presence of invasive devices (Iacchini *et al.*). The microbiological results confirmed predominance of KPC-producing *K. pneumoniae* (95.2%); on the other hand, associations between MBL and KPC (0.9%) or MBL and OXA-48 (0.3%) enzymes were rarely identified in the same species (Iacchini *et al.*).

Here we describe the occurrence of a bloodstream infection due to an OXA-48 and NDM-1-producing MDR ST101 *K. pneumoniae*, in a patient with a recent history of foreign hospitalization.

Egypt is a CPE emergent geographical area, thus representing a cause of concern for Mediterranean countries. The limited antimicrobial stewardship and infection control practices adopted could be the causes of CPE endemicity in Africa. As Italy represents a first-line European checkpoint with respect to African countries and their migration flows, it plays a pivotal role in limiting the dissemination of high-risk clones (Principe *et al.*). Recognition of carbapenemases *K. pneumoniae* hyper-epidemic clones of ST101 by molecular tools represents an important step toward tracing transmission routes, developing targeted control and prevention strategies, and monitoring their effectiveness (Roe *et al.*). Finally, our findings emphasise that unusual associations of carbapenemases are emerging in invasive *K. pneumoniae* strains in Italy. This poses a serious threat to detection, if phenotypic synergy tests are only ceftazidime/avibactam based or targeted to *bla*KPC detection.

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References

Bitar I, Piazza A, Gaiarsa S, Villa L, Pedroni P, et al. (2017). ST405 NDM-5 producing *Escherichia coli* in Northern Italy: the first two clinical cases. *Clin Microbiol Infect.* **23**, 489-490.

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, et al. (2005). Identification of plasmids by PCR-based replicon typing. *Journal of Microbiological Methods.* **63**, 219-228.

D'Andrea MM, Venturelli C, Giani T, Arena F, Conte V, et al. (2011). Persistent carriage and infection by multidrug-resistant *Escherichia coli* ST405 producing NDM-1 carbapenemase: report on the first Italian cases. *J Clin Microbiol.* **49**, 2755-2758.

Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, et al. (2017). European Survey of Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) Working Group. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE): a prospective,multinational study. *Lancet Infect Dis.* **17**, 153-163.

Iacchini S, Sabbatucci M, Gagliotti C, Rossolini GM, Moro ML, et al. (2019). Bloodstream infections due to carbapenemase producing *Enterobacteriaceae* in Italy: results from nationwide surveillance, 2014 to 2017. *Euro Surveill.* 24.

Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, et al. (2016). Global Dissemination of Carbapenemase-Producing *Klebsiella pneumoniae*: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. *Front Microbiol.* **13**, 895.

Lomonaco S, Crawford MA, Lascols C, Timme RE, Anderson K, et al. (2018). Resistome of carbapenem- and colistin-resistant *Klebsiella pneumoniae* clinical isolates. *PLoS One.* **8**, e 0198526.

Monaco F, Mento GD, Cuscino N, Conaldi PG, Douradinha B. (2018). Infant colonisation with *Escherichia coli* and *Klebsiella pneumoniae* strains co-harbouring *bla*(OXA-48) and *bla*(NDM-1) carbapenemases genes: a case report. *Int J Antimicrob Agents.* **52**, 121-122.

Pitout JD, Nordmann P, Poirel L. (2015). Carbapenemase-Producing *Klebsiella pneumoniae*, a Key pathogen Set for Global Nosocomial Dominance. *Antimicrob Agents Chemother.* **59**, 5873-5884.

Principe L, Mauri C, Conte V, Pini B, Giani T, et al. (2017). First report of NDM-1-producing *Klebsiella pneumoniae* imported from Africa to Italy: Evidence of the need for continuous surveillance. *J Glob Antimicrob Resist.* **8**, 23-27.

Roe CC, Vazquez AJ, Esposito EP, Zarrilli R, Sahl JW. (2019). Diversity, Virulence, and Antimicrobial Resistance in Isolates From the Newly Emerging *Klebsiella pneumoniae* ST101 Lineage. *Front Microbiol.* **10**, 542.

Seiffert SN, Marschall J, Perreten V, Carattoli A, Furrer H, et al. (2014). Emergence of *Klebsiella pneumoniae* co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA and ArmA in Switzerland. *Int J Antimicrob Agents.* **44**, 260-262.