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Short Communication

First report of NDM-1-producing *Klebsiella pneumoniae* imported from Africa to Italy: Evidence of the need for continuous surveillance

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ABSTRACT

Objectives: NDM-producing Enterobacteriaceae are considered emergent on the African continent and have been increasingly reported in recent years. In contrast, strains producing NDM-type enzymes have been rarely reported in Italy, usually associated with sporadic cases or small outbreaks. Here we report two cases of infection caused by NDM-1-producing *Klebsiella pneumoniae* (NDM-KP) in two unrelated patients returned from travel to Egypt.
Case reports: The two patients had been previously hospitalised for a short period in two different Egyptian hospitals. In our institution in Italy, NDM-KP isolates were detected from surgical wound drainage (patient #1) and respiratory secretions and blood cultures (patient #2). Rectal swabs of both patients were persistently positive for NDM-KP. In both cases, NDM-1-producing isolates exhibited a multidrug-resistant phenotype, being susceptible only to tigecycline and colistin. Analysis by multilocus sequence typing (MLST) revealed that the two *K. pneumoniae* isolates were not clonally related, belonging to different sequence types (STs), namely ST15 from patient #1 and ST11 from patient #2.
Conclusions: To the best of our knowledge, this is the first report of NDM-producing isolates imported from Africa to Italy, with no obvious link to the Indian subcontinent. Our experience confirms that Egypt is an emergent source of NDM-producing Enterobacteriaceae, thus representing a cause of concern for Mediterranean countries. Owing to its geographical position, Italy is a first-line European checkpoint with respect to African countries and plays a pivotal role in limiting the dissemination of high-risk clones, especially considering the latest strong migration flows.

1. Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) have become a worldwide health issue and pose a major threat due to the narrowing range of therapeutic options [1]. In most cases, resistance is due to carbapenemase production and affects *Klebsiella pneumoniae* strains. New Delhi metallo-β-lactamase-1 (NDM-1) was first described in 2008 in a *K. pneumoniae* isolate from a Swedish patient who had previously been hospitalised in New Delhi, India [2]. It is a class B carbapenemase that is able to confer resistance to almost all β-lactams, except the monobactam aztreonam. Of note, owing to the co-presence of other resistance mechanisms, NDM-producing isolates are usually also resistant to aminoglycosides, fluoroquinolones and sulphonamides, leaving few or no therapeutic options [1]. Following the first description, NDM-type enzymes have been detected in different strains from all continents, and several variants have been identified (NDM-1 to -14) [1,3]. The Indian subcontinent, the Balkan region and the Middle East are considered to be the main endemic areas for NDM-producers [4]. Focusing on the African continent, the first identification of NDM-1 occurred in 2007 in a *K. pneumoniae* strain obtained from the urinary tract of a patient hospitalised in Kenya [5]. NDM-producing Enterobacteriaceae have been subsequently reported from several African nations and have been

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increasingly described in recent years [6]. In Europe, a significant spread, although more limited than the Indian subcontinent, was also observed in the UK, which has close ties with India and Pakistan [7]. In Italy, the first detection of NDM-1 occurred in 2009, in two patients hospitalised in Modena (northern Italy) and colonised at the intestinal level by a NDM-1-producing *Escherichia coli*. Analysis of hospital records revealed an epidemiological link between these two cases and a third patient with a history of hospital admission in India (in 2008). The latter patient had faecal carriage of carbapenem-resistant *E. coli* during a stay in the same unit in the same period [8]. Two years later, in 2011, NDM-1 was detected in *K. pneumoniae* and *E. coli* isolates obtained from six patients hospitalised in four healthcare facilities in the Bologna area (northern Italy, close to Modena), with one patient having a history of hospitalisation in New Delhi, India [9]. More recently, in 2012, the NDM-4 variant was detected in *E. coli* from two patients hospitalised in a ward of a large tertiary healthcare facility in Genoa (northern Italy). Also in this case, one of the patients had been previously hospitalised in India [10]. This kind of epidemiology highlights the pivotal role of the Indian subcontinent in the worldwide dissemination of NDM-producing isolates. In particular, patients repatriated after hospitalisation abroad may be a risk for introducing multidrug-resistant microorganisms into hospitals in their home countries.

Here we report two cases of infection caused by NDM-1-producing *K. pneumoniae* in two patients returned from travel to Egypt, where they had also been hospitalised. To the best of our knowledge, this is the first report of infections caused by NDM-1-producing isolates imported from Africa to Italy.

2. Case reports

2.1. Case #1

On 3 April 2014, a 2-year-old Italian child of African origin was involved in a traffic accident during a holiday in Egypt and was hospitalised in an intensive care unit (ICU) at a hospital in Cairo. Radiological examination evidenced abdominal crushing, pelvic fracture, hepatic laceration and spleen contusion. The child underwent abdominal surgery by laparotomy with partial hepatectomy and the placement of subhepatic drainage. No data on the antibiotic regimen during this hospitalisation period are available. On 8 April 2014, he returned to Italy with the family and on 11 April 2014 was admitted to our institution (A. Manzoni Hospital, Lecco, Italy) with an extended abdominal haematoma, diffuse bruises and a surgical abdominal wound with loss of peritoneal fluid as a consequence of the drainage. On admission, he had fever and abdominal pain. The drainage fluid and a rectal swab for screening of CRE were taken for laboratory analysis. Empirical antimicrobial therapy based on ampicillin (500 mg four times daily) plus netilmicin [50 mg once daily (o.d.)] was administered. Topical gentamicin was applied to the surgical wound. Cultures both from the rectalswab and the drainage fluid revealed the presence of a carbapenem-resistant *K. pneumoniae* isolate. On 16 April 2014, the child was transferred to the Paediatric Surgery Unit of another hospital for surgical examination, but no surgical operation was conducted. He was discharged on 29 April 2014, but 2 days later the child was re-admitted to our hospital for abdominal pain and vomiting syndrome, likely related to his surgical condition. During hospitalisation he underwent radiological and microbiological examination. Cultures were negative for common faecal pathogens (*Salmonella*, *Shigella* and *Campylobacter*), whilst the rectal swab was again positive for carbapenem-resistant *K. pneumoniae*. The child was discharged on 6 May 2014 in good condition.

Table 1
Clinical information of the two cases.

Patient	Age (years)	Sex	Previous hospitalisation			Admission to Italian hospital	Diagnosis at admission	NDM-producing strain (ST)	NDM variant	Source of collection	Other bacterial strains collected	Antimicrobial treatment in Italy	Outcome
			Date of admission	City, country	Antimicrobial treatment								
Patient #1	2	Male	3 April 2014	Cairo, Egypt	NA	11 April 2014	Polytrauma due to traffic accident	*Klebsiella pneumoniae* (ST15)	NDM-1	Abdominal drainage fluid, stool	–	AMC, AMP, GEN, NET, TZP	Discharged in good conditions after 26 days
Patient #2	63	Male	17 October 2014	Hurghada, Egypt	FLU, MTZ, MEM, VAN	20 October 2014	Pneumonia	*K. pneumoniae* (ST11)	NDM-1	Respiratory secretions, blood, urine, stool	*Acinetobacter baumannii*, *Stenotrophomonas maltophilia*	COL, CRO, IMI, LEV, LIN, MEM, RIF, SXT	Discharged in good conditions after 61 days

ST, sequence type; NA, not available; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; GEN, gentamicin; NET, netilmicin; TZP, piperacillin/tazobactam; FLU, fluconazole; MTZ, metronidazole; MEM, meropenem; VAN, vancomycin; COL, colistin; CRO, ceftriaxone; IMI, imipenem; LEV, levofloxacin; LIN, linezolid; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole.

Table 2
Antimicrobial resistance profile of NDM-1-producing *Klebsiella pneumoniae* isolates.

Antibiotic	MIC (mg/L) [susceptibility category[a]]	
	NDM-KP isolate from patient #1	NDM-KP isolate from patient #2
Amoxicillin/clavulanic acid	>16 [R]	>16 [R]
Piperacillin/tazobactam	>64 [R]	>64 [R]
Cefotaxime	>32 [R]	>32 [R]
Ceftazidime	>32 [R]	>32 [R]
Cefepime	>32 [R]	>32 [R]
Ertapenem	>4 [R]	>4 [R]
Imipenem	16 [R]	16 [R]
Meropenem	16 [R]	32 [R]
Amikacin	>32 [R]	>32 [R]
Gentamicin	>8 [R]	>8 [R]
Ciprofloxacin	>4 [R]	>4 [R]
Tigecycline	1 [S]	0.5 [S]
Trimethoprim/sulfamethoxazole	>8 [R]	>8 [R]
Colistin	≤0.5 [S]	≤0.5 [S]

MIC, minimum inhibitory concentration; R, resistant; S, susceptible.
[a] Results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [11].

2.2. Case #2

On 17 October 2014, a 63-year-old Italian citizen developed an acute respiratory syndrome during a cruise on the Red Sea and was hospitalised at the Red Sea Hospital (Hurghada, Egypt). On admission he was soporose, with fever (41 °C), dyspnoea and respiratory failure. An empirical antibiotic treatment was initiated using meropenem [1 g three times daily (t.i.d.)], vancomycin [1 g twice daily (b.i.d.)], metronidazole (500 mg t.i.d.) and fluconazole (400 mg o.d.). Cultures of respiratory secretions revealed the presence of *Streptococcus pneumoniae*. Because of this severe clinical condition, the patient was intubated for mechanical ventilation. On 20 October 2014, the patient was transferred to the ICU of our institution in a very critical condition, with fever, septic shock, respiratory failure, ischaemic cardiopathy and atrial fibrillation. Intubation for mechanical ventilation as well as a central venous catheter (CVC) were present at admission. His urine was positive for pneumococcal urinary antigen, and therapy was started with ceftriaxone (1 g b.i.d.), meropenem (1 g t.i.d.) and levofloxacin (500 mg b.i.d.). Cultures of purulent respiratory secretions (carried out on the first day of hospitalisation) were positive for carbapenem-resistant *K. pneumoniae*, carbapenem-resistant *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. In addition, the CVC (previously placed in Egypt) was positive for carbapenem-resistant *A. baumannii*. Based on these findings, colistin (2 MU t.i.d.) and trimethoprim/sulfamethoxazole (20 mg/kg o.d.) were added to therapy. On 12 November 2014, blood cultures resulted positive for carbapenem-resistant *K. pneumoniae*. Screening rectal swabs were positive for carbapenem-resistant *K. pneumoniae* and *A. baumannii* during the entire hospitalisation period. On 3 December 2014, he was transferred to the medical ward. Urine cultures were positive for carbapenem-resistant *K. pneumoniae* on 5 and 15 December 2014, but no antibiotic therapy was administered. The patient was discharged in good condition on 19 December 2014.

Clinical information about the two case reports is summarised in Table 1.

In both cases, bacterial identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (VITEK® MS; bioMérieux, Marcy-l'Étoile, France). Antimicrobial susceptibility testing against the carbapenem-resistant *K. pneumoniae* was performed by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method using lyophilised custom plates (Sensititre; ThermoFisher Scientific, Basingstoke, UK) and the results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [11]. Carbapenem-resistant *K. pneumoniae* isolates were susceptible only to tigecycline and colistin (Table 2). The presence of a metallo-β-lactamase (MBL) was phenotypically evaluated on the basis of synergistic activity between meropenem and dipicolinic acid using both the double disk approximation test (Rosco Diagnostica A/S, Taastrup, Denmark) and a commercially available combination method (KPC + MBL Confirm ID Kit; Rosco Diagnostica A/S).

The bla_{NDM} gene of both isolates was first detected by the GeneXpert® System using the Xpert Carba-R test (Cepheid, Sunnyvale, CA). Conventional PCR and sequencing for confirmation were performed as previously described [8,12] and revealed the bla_{NDM-1} allelic variant. Multilocus sequence typing (MLST) was carried out according to protocols and conditions as described on the *K. pneumoniae* MLST website (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). MLST analysis revealed that the two *K. pneumoniae* isolates were not clonally related, belonging to two different STs, namely ST15 in patient #1 and ST11 in patient #2. Carbapenem-resistant *A. baumannii* from patient #2 was negative for the bla_{NDM} gene.

3. Discussion and conclusions

The global spread of NDM-type carbapenemases is a serious threat in the field of carbapenem resistance because of rapid dissemination and association with multidrug resistance that greatly limits therapeutic options. To date, the reservoir of this carbapenemase was mostly related to the Indian subcontinent, which is inhabited by the second largest population in the world and where NDM-1-producers are reported also for community-acquired infections [4]. The size of that reservoir may explain the rapidity of the worldwide dissemination of NDM-producers [4]. According to this context, all previously described NDM-producing Enterobacteriaceae isolated in Italy were epidemiologically connected to a history of hospitalisation on the Indian subcontinent [8–10]. Interestingly, here we describe the first finding of NDM-producing isolates imported from Africa to Italy, with no obvious link to the Indian subcontinent. In these cases, in fact, NDM-1-producing *K. pneumoniae* were already present at admission and showed a strong correlation with recent previous hospitalisation in Egyptian hospitals. Overall, limited information is available for this geographic area, although NDM determinants have been previously described in *K. pneumoniae*, *Pseudomonas aeruginosa* and *A. baumannii* isolated in Egypt [6,12,13]. Our data confirm that this area is an emergent source of NDM-producing Enterobacteriaceae, thus representing a cause of concern for Mediterranean countries.

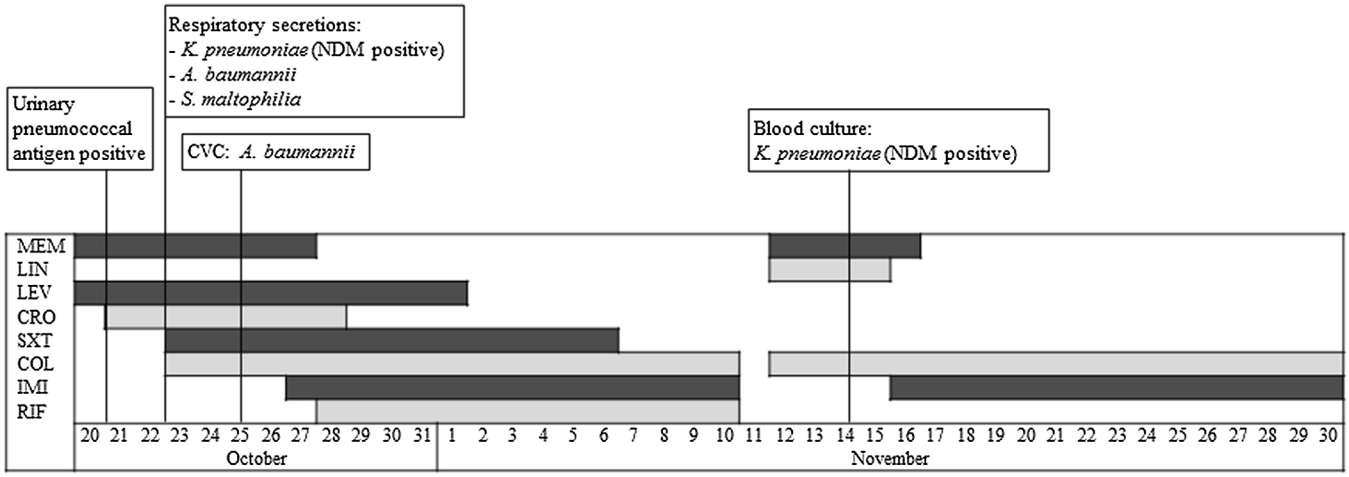

Fig. 1. Antimicrobial treatment during the hospitalisation period in the intensive care unit (ICU) for patient #2. Boxes indicate clinical isolates and the date of isolation. Rectal swabs were positive for *Acinetobacter baumannii* and NDM-producing *Klebsiella pneumoniae* during the entire hospitalisation period in the ICU (data not shown). CVC, central venous catheter; MEM, meropenem; LIN, linezolid; LEV, levofloxacin; CRO, ceftriaxone; SXT, trimethoprim/sulfamethoxazole; COL, colistin; IMI, imipenem; RIF, rifampicin.

The uncontained spread of carbapenemase-producing bacteria may occur in Africa for several reasons, primarily owing to the limited attention to antimicrobial stewardship and the suboptimal level of infection control practices, with limited opportunity for patient isolation or screening of high-risk individuals [6]. Of concern, there is an increase in over-the-counter use of carbapenems in Africa, especially in Egypt, owing to the quasi-absence of regulations governing antibiotic use [14].

In Italy, a recent countrywide surveillance showed that KPC-producing enterobacteria are now endemic in this country, whereas other carbapenemases (e.g. MBLs and OXA-type enzymes) are sporadic and are responsible for a small percentage of cases [15]. In our experience, early detection of NDM-producing isolates was performed by rectal swab screening on admission based on phenotypic and molecular methods. Further analysis by MLST evidenced the presence of two different STs, of which ST15 has been previously reported in Africa (Morocco) and belongs to CC14. It is considered to be endemic both in Hungary and Denmark and has been associated with a high content of virulence factors [16]. ST11 is the major ST among *K. pneumoniae* strains harbouring bla_{KPC} from Asia, but it has been also associated with NDM determinants [17]. Of note, the first NDM-1-producing *K. pneumoniae* reported from Egypt belonged to ST11, and isolates of this type have recently been found in the same country [18,19]. Both NDM-1-producing isolates exhibited a multidrug-resistant phenotype, being susceptible only to tigecycline and colistin. Of note, patient #1 was not appropriately treated for NDM-producing *K. pneumoniae*. However, the patient's conditions improved, thus suggesting probable colonisation by NDM-producing *K. pneumoniae* rather than infection. In contrast, patient #2 received an appropriate antimicrobial regimen based on several extended-spectrum antibiotics given during the hospitalisation period in the ICU. As shown in Fig. 1, colistin was administered for a long period of time (38 days), mostly in combination with carbapenems (imipenem for 30 days and meropenem for 10 days). Following a 3-week antimicrobial regimen, a 1-day treatment washout was applied thus allowing the emergence of pathogens causing bloodstream infection. Following a new course of antimicrobials, the patient was discharged in good conditions after 61 days of hospitalisation.

The link between NDM acquisition and healthcare exposure abroad has been extensively described [1,4]. This situation necessitates a series of infection control measures as soon as possible. At a local level, patients with a history of travel or originating from high-risk countries should be screened for NDM-producing bacteria [20]. Notably, the patients in the current study were isolated on admission owing to their previous hospitalisation in a country considered at-risk for CRE and were screened by rectal swabs. This procedure in combination with the early detection of NDM determinants prevented the development of onward transmission and potential outbreaks and helped to optimise antibiotic therapy. No further cases occurred in the hospital setting. Limiting patient infections and surveillance of patients colonised with carbapenemase-producing Enterobacteriaceae are of paramount importance to hospital epidemiology and patient safety. In this context, optimal therapeutic options are very limited. Early recognition of carbapenemase expression is the key for appropriate treatment. Ensuing availability of drugs based on specific carbapenemase inhibitors (e.g. ceftazidime/avibactam, which is not effective against class B carbapenemases) is a new reason for precise identification of carbapenem resistance determinants. Finally, it is worth noting that Italy is subject to strong migration flows from African nations and plays a pivotal role in limiting the dissemination of high-risk clones among European countries.

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Competing interests

None declared.

Ethical approval

Not required.

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