NDM-1- or OXA-48-producing Enterobacteriaceae colonising Polish tourists following a terrorist attack in Tunis, March 2015

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We describe the introduction of NDM-1-producing *Klebsiella pneumoniae* ST147 and *Escherichia coli* ST410, and OXA-48-producing *K. pneumoniae* ST101 strains to Poland by two patients transported to the country after hospitalisation in Tunisia. The patients had gunshot wounds following the terrorist attack in the Bardo National Museum in Tunis in March 2015. Our report reinforces the need for microbiological screening of patients returning from travel on admission to healthcare institutions, especially following hospitalisation in countries where carbapenemase-producing Enterobacteriaceae are endemic.

We describe two patients colonised by carbapenemase-producing Enterobacteriaceae (CPE), which were identified on and following admission of the patients to a hospital in Warsaw, Poland, in March 2015. The patients had gunshot wounds as a result of a terrorist attack in Tunis, Tunisia, and were transferred to Warsaw directly from a hospital in Tunis.

following the terrorist attack

On 18 March 2015, visitors at the Bardo National Museum in Tunis were attacked by a group of armed terrorists. A total of 24 people from different countries were killed and around 50 were injured by gunshot. According to the Poland's Ministry of Foreign Affairs, three Polish citizens died and 10 others were wounded, who were treated in several clinical centres in Tunis [1]. On 20 March, eight of the 10 less seriously injured Polish patients were transferred to a hospital in Warsaw (Hospital A), where they stayed in a surgical and then orthopaedic wards; the clinical records of these patients are not available.

The other two seriously injured Polish patients were operated on in the same surgical unit of a hospital in Tunis and stayed in there until 28 March (10 days), at

which point they were transported by air to a surgical ward in Hospital B in Warsaw. The patients were microbiologically screened on admission and later monitored at least once a week during their hospitalisation. The specimens and body sites examined included blood, wounds and rectum, to test for the presence of CPE.

Case description

Patient A, in their late 50s, was shot in the sacrum during the attack. A rectal swab taken on admission yielded a *Klebsiella pneumoniae* isolate, identified by VITEK 2 (bioMérieux, Marcy l'Etoile, France) as carbapenem resistant. The isolate was subsequently tested for metallo-beta-lactamase (MBL)-, carbapenem-hydrolysing oxacillinase OXA-48- and *K. pneumoniae* carbapenemase (KPC)-like carbapenemases using CARBA NP and phenotypic tests [2-5]. The isolate was positive in Carba NP and the MBL EDTA double-disk test, and was resistant to temocillin, suggestive of OXA-48 [5]. Polymerase chain reaction (PCR) analysis for several carbapenemase genes [6] showed that the isolate was positive for *bla*_{NDM} only and sequencing identified *bla*_{NDM-1}. The gene resided in a remnant of the Tn125 transposon, shown by PCR mapping to include the 3' part of the upstream ISAb₁₂₅ element, the *bla*_{NDM-1}-*ble*_{MBL} operon, genes *iso*, *tat*, *dct*, *groES* and *groEL*, and to be truncated downstream of *groEL* [7,8]. By multilocus sequence typing (MLST) [9], the isolate was classified as sequence type (ST) 147. No CPE isolates were recovered from other sites of the patient either on admission or during hospitalisation.

Patient B, in their early 20s, had severe damage of subcutaneous tissue near the trochanter of the femur as a result of being shot. A rectal swab on admission yielded a carbapenem-resistant *K. pneumoniae* isolate that was Carba NP-positive, negative in MBL and KPC tests, but resistant to temocillin. PCR and sequencing

TABLE

Antimicrobial minimum inhibitory concentrations for the carbapenemase-producing *Enterobacteriaceae* isolates from two Polish patients wounded in a terrorist attack in Tunisia, March 2015

Species, sequence type, carbapenemase	Minimum inhibitory concentration (µg/mL)													
	AMX	AMC	PIP	TZP	CTX	CAZ	FEP	ATM	IPM	MEM	ERT	CIP	GEN	AMK
Patient A														
<i>Klebsiella pneumoniae</i> ST147 NDM-1	>256	>256	>256	>256	>256	>256	128	>256	12	>32	>32	>32	64	12
Patient B														
<i>K. pneumoniae</i> ST101 OXA-48	>256	>256	>256	>256	>256	128	>256	>256	>32	32	>32	>32	64	6
<i>K. pneumoniae</i> ST147 NDM-1	>256	>256	>256	>256	>256	>256	96	128	32	>32	>32	>32	128	8
<i>Escherichia coli</i> ST410 NDM-1	>256	>256	>256	>256	>256	>256	>256	48	32	>32	>32	>32	128	12

AMC: amoxicillin-clavulanic acid; AMK: amikacin; ATM: aztreonam; CAZ: ceftazidime; CIP: ciprofloxacin; CML: chloramphenicol; CST: colistin; CTX: cefotaxime; ERT: ertapenem; FEP: cefepime; GEN: gentamicin; IPM: imipenem; MEM: meropenem; NDM: New Delhi metallo-beta-lactamase; PIP: piperacillin; SXT: trimethoprim-sulfamethoxazole; ST: sequence type; ST: sequence type; SXT: trimethoprim-sulfamethoxazole; TET: tetracycline; TGC: tigecycline; TZP: piperacillin-tazobactam.

showed *bla*_{OXA-48} to be the only carbapenemase gene found. PCR mapping revealed that the gene was located in the Tn1999.2 transposon, with the upstream IS1999 element disrupted by IS1R [10]. The isolate was found to be ST101.

Ten days after admission, MBL-positive *K. pneumoniae* and *Escherichia coli* isolates were cultured from wound and rectal swabs. Isolates from the wound were analysed by molecular methods: both the *K. pneumoniae* and *E. coli* isolates were identified as New Delhi metallo-beta-lactamase-1 (NDM-1) producers; no other carbapenemases were found. The Tn125-like elements with their *bla*_{NDM-1} genes produced the same PCR mapping pattern as that of the *K. pneumoniae* isolate from Patient A. The *K. pneumoniae* isolate belonged to ST147, whereas *E. coli* was classified by MLST [11] as ST410.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the CPE isolates from both patients was tested by MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. The isolates showed extensive resistance patterns, all being susceptible only to colistin, and all *K. pneumoniae* isolates to chloramphenicol (Table). Amikacin minimum inhibitory concentrations (MICs) of the four isolates indicated either susceptibility or intermediate resistance (EUCAST breakpoints: S ≤ 8 µg/mL, R > 16 µg/mL).

Discussion

The global spread of CPE is a public health problem of great concern. MBLs of the NDM type and OXA-48-like oxacillinases are among the most frequently reported carbapenemases in CPE, mainly *K. pneumoniae* and *E. coli*. Their multiple emergence in many European countries has been often attributed to imported cases from the Indian subcontinent [13,14] or the eastern and southern parts of the Mediterranean basin. These parts of the Mediterranean basin have been mostly associated with transmission of OXA-48-positive CPE. The second CPE transmitted from these areas was NDM-producing *Acinetobacter* species [13-15].

Partial molecular analysis revealed that the NDM-1-producing isolates from both Polish patients probably had the same genetic context of the *bla*_{NDM-1} gene, and both NDM-1-producing *K. pneumoniae* isolates were ST147. According to National Reference Centre for Susceptibility Testing records, the four isolates differed genetically from all NDM-1 or OXA-48 producers identified in Poland to date (data not shown). Therefore, the patients were most probably colonised in Tunisia, either during hospitalisation or, less likely, before the attack, outside the hospital setting.

It is unclear why NDM-1 producers from Patient B were recovered only 10 days after admission to a Warsaw hospital. Both patients had shared common care

exposure in the Tunis hospital and it is possible that the fact that the cultures were NDM negative on admission to the hospital in Warsaw was due to limited sensitivity of the screening.

In the Warsaw hospital, a set of enhanced infection control measures were used, including separate rooms with dedicated sanitary facilities, strict contact isolation and dedicated equipment. Nevertheless, transmission from Patient A to Patient B in Warsaw cannot be entirely excluded, especially as both patients were treated by the same personnel. To date, no secondary transmission of the CPE to other patients in the hospital has been observed. To date, Patient A is still hospitalised whereas Patient B was discharged on 22 April 2015. Control measures are in place: all patients admitted to high-risk wards, such as intensive-care units, surgery, haematology and oncology, are screened on admission.

Although a number of reports have indicated North Africa as a reservoir of OXA-48- and NDM-producing organisms, lack of local surveillance data impedes full assessment of the situation there. Some reviews articles have shown these organisms as being of 'sporadic occurrence' in that region [13-17], especially NDM-positive *Enterobacteriaceae*, which have been reported in North African countries only a few times [15], including one NDM-1- and OXA-48-producing *K. pneumoniae* ST11 isolate recovered in Tunisia from a Libyan patient in 2012 [18].

ST101 and ST147 are emerging clones of *K. pneumoniae*, found worldwide with various beta-lactamases, including carbapenemases [19]. *K. pneumoniae* ST101 with OXA-48 encoded by Tn1999.2 was described in Tunisia and other North African countries [16,20,21]. *K. pneumoniae* ST147 with NDM-1, as well as NDM-1-producing pandemic *E. coli* ST410, have been reported in many regions, but to the best of our knowledge not in North Africa [22-26]. Our report once again reinforces the need for microbiological screening of patients returning from travel, especially following hospitalisation in countries where CPE are endemic, as specified, for example, in Polish infection control guidelines [27].

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Conflict of interest

None declared.

Authors' contributions

RI performed the molecular analysis, collected the data and drafted the manuscript; KB performed the microbiological analysis; AB performed the molecular analysis and collected the data; EL performed the microbiological analysis; MH

performed the microbiological analysis; DŻ coordinated the microbiological analysis; AG performed the hospital laboratory analysis and collected the isolates with clinical data; MP coordinated the hospital infection control measures and collected the clinical data; WH consulted the cases and edited the manuscript; MG supervised the research and analysis, coordinated and edited the manuscript.

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