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.

**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*<sub>NDM</sub> only and sequencing identified *bla*<sub>NDM</sub>-1. The gene resided in a remnant of the Tn125 transposon, shown by PCR mapping to include the 3’ part of the upstream ISAba125 element, the *bla*<sub>NDM</sub>-1-<sub>ble</sub>_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
showed bla\textsubscript{OXA-48} to be the only carbapenemase gene found. PCR mapping revealed that the gene was located in the \textit{Tn1999.2} transposon, with the upstream IS\textsubscript{999} element disrupted by IS\textsubscript{1R} \cite{10}. The isolate was found to be ST101.

Ten days after admission, MBL-positive \textit{K. pneumoniae} and \textit{Escherichia coli} isolates were cultured from wound and rectal swabs. Isolates from the wound were analysed by molecular methods: both the \textit{K. pneumoniae} and \textit{E. coli} isolates were identified as New Delhi metallo-beta-lactamase-1 (NDM-1) producers; no other carbapenemases were found. The \textit{Tn125}-like elements with their \textit{bla\textsubscript{NDM-1}} genes produced the same PCR mapping pattern as that of the \textit{K. pneumoniae} isolate from Patient A. The \textit{K. pneumoniae} isolate belonged to ST147, whereas \textit{E. coli} was classified by MLST \cite{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) \cite{12}. The isolates showed extensive resistance patterns, all being susceptible only to colistin, and all \textit{K. pneumoniae} isolates to chloramphenicol (Table). Amikacin minimum inhibitory concentrations (MICs) of the four isolates indicated either susceptibility or intermediate resistance (EUCAST breakpoints: S \leq 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 \textit{K. pneumoniae} and \textit{E. coli}. Their multiple emergence in many European countries has been often attributed to imported cases from the Indian subcontinent \cite{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 \textit{Acinetobacter} species \cite{13-15}.

Partial molecular analysis revealed that the NDM-1-producing isolates from both Polish patients probably had the same genetic context of the \textit{bla\textsubscript{NDM-1}} gene, and both NDM-1-producing \textit{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 Tn9992.2 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; AP 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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