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Extensive clonal spread of *Klebsiella pneumoniae* ST11 NDM-1 in Poland

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Background: Since the end of 2012 Poland has been experiencing rapid dissemination of NDM-producing Enterobacteriaceae. The number of new cases has triplicated consecutively year-by-year, and in 2016 it exceeded 1,500. The previous analysis of isolates from 2012-14 revealed an interregional outbreak of *Klebsiella pneumoniae* ST11 NDM-1 and several imports. The aim of this study was to update this view, especially after March 2015 when Polish victims of a terrorist attack in Tunisia arrived in Warsaw hospitals being colonized with *K. pneumoniae* ST147 NDM-1.

Material/methods: The study comprised 1342 Enterobacteriaceae isolates from all NDM cases in Poland from April 2015 to August 2016 (1334 *K. pneumoniae*, 6 *Escherichia coli*, 1 *Klebsiella oxytoca*, 1 *Citrobacter freundii*). NDMs were detected by Carba NP and PCR. The polymorphism of *bla*_{NDM}-carrying Tn125 transposon derivatives was analysed in all isolates by PCR mapping. Representative isolates were typed by PFGE and MLST, and by S1 plasmid profiling with identification of *bla*_{NDM} plasmids by hybridization. Additional β -lactamases were identified by PCR and sequencing.

Results: Nine different Tn125 derivatives were found in the study material. However, the type Tn125A, indistinguishable from that of the epidemic *K. pneumoniae* ST11 NDM-1 lineage, was observed in ~99% of the isolates (1321 *K. pneumoniae*, 4 *E. coli*, 1 *K. oxytoca*). All randomly-selected *K. pneumoniae* with Tn125A formed one pulsotype with the 2012-14 isolates, belonged to ST11 and produced NDM-1. These isolates represented local outbreaks and sporadic cases from the entire

country, including the large epidemic in Warsaw and its administrative region. The Tn125A-carrying *E. coli* of different STs and *K. oxytoca* ST88 were usually co-identified with *K. pneumoniae* ST11 in Warsaw hospitals. The Tn125B variant, indistinguishable from that of the Tunisian *K. pneumoniae* ST147 NDM-1 genotype resided in seven *K. pneumoniae* ST147 NDM-1 isolates. All these were closely related by PFGE to the isolates from Tunisia, and were mainly from the Warsaw centre in which the attack victims had been treated. The remaining seven Tn125 elements, never observed in Poland before, occurred in nine *E. coli*, *K. pneumoniae* & *C. freundii* isolates from three cities. These were of multiple STs, produced NDM-1 or NDM-5, and much varied in S1 profiles, *bla*_{NDM} plasmids and β -lactamase content (CTX-M-3/-15, CMY-4/-6 and/or TEM-1/-30 enzymes). Only in few cases their country of origin was documented.

Conclusions: The extensive, public health-threatening NDM increase in Poland in 2015-2016 has been largely due to the continuous clonal spread of *K. pneumoniae* ST11 NDM-1. It has heavily affected Warsaw and its region; however, many new outbreaks and sporadic cases have occurred meantime all over the country. The Tunisian import *K. pneumoniae* ST147 NDM-1 has been settled, causing a limited outbreak in so far, followed by several new documented or probable imports.