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Short Communication

First case of NDM-1 producing *Klebsiella pneumoniae* in Caribbean islands

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SUMMARY

Objectives: Characterize a NDM-1 producing *K. pneumoniae* isolate recovered from a patient hospitalized in Guadeloupe, French West Indies, after its transfer from Cuba
Methods: Antibiotic susceptibilities were determined by the disk diffusion method, and E-test. Carbapenemase production was assessed using the Carba NP test. Antibiotic resistance determinants and their surrounding structures were characterized by PCR mapping and DNA sequencing. Transfer of the β-lactam resistance marker was attempted by liquid mating-out assays
Results: Here we reported the first NDM-1 producing enterobacterial isolate recovered from Caribbean islands. This *K. pneumoniae* isolate belongs to a new sequence type (ST1649). The bla_{NDM-1} gene together with the *aacA4* gene were carried on a self conjugative IncR plasmid of c.a. 80 kb.
Conclusion: This study describes the first identification of a NDM-1 producer in Caribbean islands. The uncommon incompatibility group of the bla_{NDM-1} carrying plasmid and the uncommon ST type of the *K. pneumoniae* strain suggest a possible local emergence of NDM producers.

The metallo-β-lactamase (MBL) group of enzymes are carbapenemases that inactivate most of the β-lactam molecules except aztreonam, and are frequently associated with genes conferring resistance to several other classes of antibiotics. Among the MBLs, the New Delhi metallo-β-lactamase-1 (NDM-1) was first described in a *Klebsiella pneumoniae* isolate.[1] Initially, the spread of NDM-1 was mostly identified from the Indian sub-continent.[2] Since then, NDM-1 producers have been described worldwide.[2] Here, we report the emergence of a NDM-1 producing *K. pneumoniae* recovered from a patient hospitalized in Guadeloupe, French West Indies after its transfer from Cuba.

In June 2014, a 67-year-old German traveler was hospitalized in La Havana, Cuba, for an ischemic cerebrovascular failure. On day 8, the patient developed a pneumonia that was empirically treated with ceftazidime, ciprofloxacin, and metronidazole. The clinical status of the patient worsened necessitating intubation and mechanical ventilation on day 11, and his transfer on day 15 to the intensive care unit of the University hospital of Pointe-à-Pitre, Guadeloupe, France. As recommended for the detection of carbapenemase producers,[3] a rectal swab was collected at admission, leading to the isolation of the multidrug resistant *K. pneumoniae* KHU strain.

Antimicrobial drug susceptibilities were determined by the disk diffusion method and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines as updated in 2014.[4] The minimal inhibitory concentrations (MICs) were determined by using E-test (bioMérieux, La Balme-les-Grottes, France) on Mueller-Hinton agar at 37 °C. The *K. pneumoniae* KHU

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Table 1
MICs of β-lactams for *K. pneumoniae* KHU clinical isolate and the transconjugant *E. coli* J53 harboring the natural plasmid from *K. pneumoniae* KHU

β-Lactam (s)[a]	*K. pneumoniae* KHU	Tc[b] *E. coli* J53
Amoxicillin	> 256	> 256
Amoxicillin +CLA	> 256	> 256
Ticarcillin	> 256	> 256
Ticarcillin + CLA	> 256	> 256
Piperacillin	> 256	128
Piperacillin + TZB	> 256	128
Ceftazidime	> 256	> 256
Cefotaxime	> 256	> 256
Cefepime	> 256	64
Cefoxitin	> 512	> 512
Aztreonam	0.12	0.12
Imipenem	16	6
Meropenem	32	6
Ertapenem	32	4
Doripenem	12	6

[a] CLA, clavulanic acid; TZB, tazobactam, at 4 μg/ml.
[b] transconjugant.

was resistant to all β-lactams except aztreonam, including imipenem (MIC = 16 μg/mL), meropenem (MIC = 32 μg/mL), ertapenem (MIC = 32 μg/mL) and doripenem (MIC = 12 μg/mL) (Table 1). It was also resistant to all aminoglycosides except to amikacin, to fluoroquinolones, to trimethoprim-sulphamethoxazole, to doxycycline, and to tetracycline but remained susceptible to colistin, fosfomycin, and tigecycline. In addition, three other multidrug resistant strains were isolated as a carrier stage, an extended-spectrum β-lactamase (CTX-M-15) producing *Escherichia coli*, a methicillin-resistant *Staphylococcus aureus* and an OXA-23 producing *Acinetobacter baumannii*. To the contrary of the NDM-1 producing *K. pneumonia*, the latter two bacteria also were isolated in the respiratory secretions.

Using the Carba NP test,[5] a carbapenemase activity was rapidly detected. Then PCR amplification followed by sequencing on whole-cell DNA as described,[6] identified the bla_{NDM-1} gene together with the *aacA4* gene encoding the AAC(6')-Ib acetyltransferase that confers high-level resistance to aminoglycosides, except to amikacin. PCR for the bla_{CTX-M}, bla_{TEM}, bla_{OXA-1}, bla_{OXA-9} and bla_{OXA-10} genes were negative. Multi-locus sequence typing (MLST) analysis showed that the *K. pneumoniae* KHU isolate belonged to a new sequence type (ST) variant, ST1649, that is single locus variant of ST129 that has never been associated with NDM producers.

Plasmid DNA of *K. pneumoniae* KHU was extracted and analyzed using the Kieser method, as described.[7] Two plasmids were identified, being ca. 80-kb and 160-kb in size. Transfer of the β-lactam resistance marker into *E. coli* J53 was attempted by liquid mating-out assays at 37 °C. *E. coli* transconjugants were obtained being resistant to all β-lactams except aztreonam, including to carbapenems (MICs of imipenem, meropenem, ertapenem, and doripenem were 6, 6, 4, and 6 μg/mL, respectively) (Table 1). They harbored a ca. 80-kb plasmid carrying the bla_{NDM-1} and *aacA4* genes. PCR-based replicon typing method was performed as described,[8] and showed that this bla_{NDM-1}-positive plasmid belonged to the IncR incompatibility group. Although bla_{NDM-1} carrying is usually located on IncA/C-, IncF-, IncL/M-, IncN2-, IncH1B- or untypable-type plasmids,[3] an IncR bla_{NDM-1} carrying plasmid has been recently reported in Czech Republic.[9] Genetic structures surrounding the bla_{NDM-1} gene, performed by PCR mapping as previously described,[6] identified a truncated insertion sequence IS*Aba125* and the bleomycin resistance gene ble_{MBL} upstream and downstream of the bla_{NDM-1} gene, respectively. The same genetic environment is known for most NDM-1-producers in *Enterobacteriacae*.[6]

This study describes the identification of a NDM-1 producer in Caribbean islands. As the patient was a traveler from Germany, an intestinal colonization before travelling to Cuba cannot be excluded. However, the patient had never been hospitalized before, which is the most important risk factor for multidrug resistant bacteria acquisition.[2] No apparent link of this patient with the Indian subcontinent was established. Combined with the uncommon incompatibility group of the bla_{NDM-1} carrying plasmid and the uncommon ST of the *K. pneumoniae* strain, all these elements suggest a local emergence of NDM-1 producers. This is of major concern as Cuba is considered a major hub for all forms of human migrations, to and from the United States, Europa and other Caribbean islands. Furthermore, few hospitals in Cuba can implement all the measures necessary to prevent nosocomial infections but also act as a barrier between the community and the hospital.[10] Further work shall determine the prevalence rate of NDM and carbapenemase producers in Cuba to search for a possible endemicity of this multidrug resistance trait.

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