Polyclonal Dissemination of NDM-1- and NDM-9-Producing *Escherichia coli* and *Klebsiella pneumoniae* in French Polynesia

Saoussen Oueslati,^b Cécile Emeraud,^{a,b,c} Victor Groperrin,^b Marc Levy,^d Garance Cotellon,^c Elodie Creton,^c Lauraine Gauthier,^{a,b,c} Rémy A. Bonnin,^{b,c} Thierry Naas,^{a,b,c} Laurent Dortet^{a,b,c}

^aBacteriology-Hygiene Unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, Le Kremlin-Bicêtre, France

^bFaculty of Medicine, INSERM UMR 1184, RESIST Unit Paris-Saclay University, Le Kremlin-Bicêtre, France

^cAssociated French National Reference Center for Antibiotic Resistance: Carbapenemase-Producing Enterobacteriaceae, Le Kremlin-Bicêtre, France

^dBacteriology-Hygiene Unit, French Polynesia Hospital, Tahiti

ABSTRACT The whole-genome sequencing analysis revealed a polyclonal dissemination of NDM-1 and NDM-9 variants in *Escherichia coli* ($n=20$) and *Klebsiella pneumoniae* ($n=2$) in Tahiti since 2015 via interspecies transfer of three different *bla*_{NDM}-carrying plasmids (IncR, IncHI2, and IncF) and patient-to-patient cross-transmission. It highlights the potential risk of importation of NDM producers in France, where French Polynesia is not considered *stricto sensu* a foreign country from which repatriated patients have to be screened.

KEYWORDS NDM-1, NDM-9, epidemiology, *Escherichia coli*, Tahiti, NDM-1, whole-genome sequencing

NDM-1 (New Delhi metallo- β -lactamases) was first reported in Sweden in 2008, produced by a *Klebsiella pneumoniae* isolate recovered from a patient repatriated from India (1). Since then, NDM-producing *Enterobacteriales* isolates have disseminated worldwide, representing a serious challenge for treatment, infection control, and public health. In *Enterobacteriales*, the current spread of the *bla*_{NDM-1} gene was not related to the spread of specific clones. Indeed, this gene has been identified in a large variety of enterobacterial species (e.g., *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Salmonella*) carried on many different plasmids (IncF, IncA/C, IncL/M, IncH, IncN, and IncX3) (2). To date, 29 variants of NDM have been reported (3), among which NDM-1 is the most prevalent (4–6). However, NDM-5 was found to be increasingly prevalent in *E. coli* isolates in several countries, including India (7), Myanmar (8), China (9), and Italy (10). Overall, NDM-producing *Enterobacteriales* infections are considered endemic to the Indian subcontinent. In addition, many sporadic cases have been described in China and Australia (11). However, the epidemiology of the NDM producer in French Polynesia, a hot spot for tourism, remains partially unknown.

In the Tahiti hospital (440 beds), 9,000 rectal swab screenings are performed each year. This screening policy, which aims to detect extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriales*- and carbapenemase-producing *Enterobacteriales* (CPE)-colonized patients, is performed once per week for all hospitalized patients. The average rate of ESBL is relatively low, at 5%. In addition, from 2015 to 2019, only 29 carbapenemase-producing Gram-negative isolates, including the 22 NDM-producing *Enterobacteriales* and 7 carbapenemase-producing *Acinetobacter baumannii* (4 OXA-23 and 3 NDM-1) cases (12), have been detected from screening and clinical samples. The 22 NDM-producing *Enterobacteriales* isolates (20 *E. coli* and 2 *K. pneumoniae*) have been sent to the Associated French National Reference Center (NRC) of Antimicrobial Resistance for expertise on resistance mechanism. These NDM producers were isolated

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Address correspondence to Laurent Dortet, laurent.dortet@aphp.fr.

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TABLE 1 Characteristics of NDM-producing *E. coli* and *K. pneumoniae*

Characteristic ^a	<i>E. coli</i> isolate name										
	81E2	84C4	889H1	91I3	94C4	98I2	103C9	104C8	116J2	130B8	130H5
Sample Isolation date	Rectal swab May 2015	Rectal swab July 2015	Rectal swab Sept. 2015	Rectal swab Sept. 2015	Rectal swab Oct. 2015	Rectal swab Dec. 2015	Rectal swab Feb. 2016	Rectal swab Feb. 2016	Rectal swab Aug. 2016	Urine Jan. 2017	Rectal swab Jan. 2017
Patient characteristic											
Age (yr)	50	20	70	47	67	54	44	57	48	79	66
Sex	F	M	F	M	F	M	M	F	M	M	M
Hospital unit	Visceral surgery	Hematology	Nephrology	Hematology	Pneumology	Visceral surgery	Visceral surgery	Cardiology	Internal medicine	outpatient	Neurology
Carba NP test	+	+	+	+	+	+	+	+	+	+	+
Carbapenemase ESBL	NDM-1	NDM-1	NDM-1 CTX-M-3	NDM-1 CTX-M-3	NDM-1	NDM-1 CTX-M-14	NDM-9	NDM-1 CTX-M-3	NDM-1 CTX-M-14	NDM-1	NDM-1
Antimicrobial susceptibility testing, by drug ^b											
Amoxicillin	R	R	R	R	R	R	R	R	R	R	R
Amoxicillin + CLA	R	R	R	R	R	R	R	R	R	R	R
Ticarcillin	R	R	R	R	R	R	R	R	R	R	R
Ticarcillin + CLA	R	R	R	R	R	R	R	R	R	R	R
Piperacillin	R	R	R	R	R	R	R	R	R	R	R
Piperacillin + TZB	R	R	R	R	R	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	R	R	R	R	R	R
Ceftazidime	R	R	R	R	R	R	R	R	R	R	R
Ceftazidime + AVI	R	R	R	R	R	R	R	R	R	R	R
Cefepime	R	R	R	R	R	R	R	R	R	R	R
Ceftolozane + TZB	R	R	R	R	R	R	R	R	R	R	R
Aztreonam	S	S	R	R	S	R	S	R	R	S	S
Meropenem	R	R	R	R	R	R	R	R	R	R	R
Imipenem	R	R	R	R	R	R	R	R	R	R	R
Ertapenem	R	R	R	R	R	R	R	R	R	R	R
Gentamicin	R	R	R	R	R	R	R	R	R	R	R
Amikacin	S	S	S	S	S	S	S	S	S	S	S
Tobramycin	R	R	R	R	R	R	R	R	R	R	R
Colistin	S	S	S	S	S	S	S	S	S	S	S
SXT	R	R	R	R	R	R	R	R	R	R	R
Levofloxacin	R	S	S	R	S	R	R	R	R	R	R
Ciprofloxacin	R	S	S	R	S	R	R	S	R	R	R
Fosfomycin	R	S	S	R	R	S	R	S	S	R	R

PCLA, clavulanate; TZB, tazobactam; AVI, avibactam; SXT, trimethoprim-sulfamethoxazole.
R, resistant; S, susceptible.

Antimicrobial susceptibility testing of the 22 NDM-producing isolates was performed by disk diffusion, and MICs of colistin were determined by broth microdilution. Results were interpreted according to EUCAST guidelines updated in 2020 (<http://www.eucast.org>). Results are recapitulated in Table 1. All isolates were resistant to all β -lactams, including carbapenems, except for aztreonam for 14 *E. coli* isolates that do not express any associated ESBL (Table 1). All NDM-producing isolates remained susceptible to colistin. Amikacin also remained a therapeutic option for most (21/22) of the isolates.

Mating-out assays were performed for all isolates as previously described using agar plates supplemented with cefoxitin (10 μ g/ml) and sodium azide (100 μ g/ml) for the selection of transconjugants (16). Plasmid typing was determined from WGS data of transconjugants using the PlasmidFinder tool available on the Center for Genomic Epidemiology server. Transconjugants were obtained for all clinical isolates. WGS of transconjugants and Kieser (17) plasmid extraction revealed that the *bla*_{NDM-1} gene is carried on an ~50-kb plasmid of IncR type, whereas the *bla*_{NDM-9} gene is carried on an ~160-kb plasmid of IncHI2 type or an ~130-kb plasmid of IncF type. These three of

plasmids are not specific to a single ST, demonstrating *in vivo* transfer of these plasmids. Accordingly, the concomitant outbreaks of NDM-1- and NDM-9-producing *E. coli* relied on a mix of plasmid transfer and bacteria cross-transmission from patient to patient. Regarding the two NDM-producing *K. pneumoniae* isolates, *bla*_{NDM-1} and *bla*_{NDM-9} are both carried on an IncF-type plasmid.

Our study highlighted the cooccurrence of NDM-1 and NDM-9 in Tahiti. Contrary to NDM-1, which is disseminated worldwide, the NDM-9 variant was reported only a few times in Asia (China, South Korea, Taiwan, India) (9, 18–20) and one time in Switzerland in *K. pneumoniae* isolated from wastewater (21). Of note, epidemiological investigations revealed that no patients colonized with NDM producers have travelled outside of French Polynesia, suggesting possible dissemination in the community or contamination through a hospital environmental source, since patients are transferred weekly between the Tahiti and Raiatea hospitals (250 km away). In addition, such polyclonality has already been observed in Tahiti for carbapenemase-producing *Acinetobacter* spp. recovered from environmental sources in the Tahiti hospital (12). However, implementation of a cohorting unit in the Tahiti hospital associated with the systematic screening of patients and specific decontamination of the potential environmental sources of dissemination (mattress, siphon) around each CPE-colonized patient might be powerful in limiting the spread of NDM-producing *Enterobacteriales* in French Polynesia, as well as in countries where patients are regularly repatriated (mainly Metropolitan France).

Here, we highlight the potential (low but existing) risk of importation of NDM-1 and NDM-9 producers from French Polynesia. This risk is of particular importance in France, since French Polynesia is not *stricto sensu* considered as a foreign country from which repatriated patients have to be screened for CPE colonization.

Data availability. Sequencing reads from the 22 sequenced NDM-producing isolates have been deposited in the GenBank database under BioProject number [PRJNA660947](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA660947).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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