Molecular Characterization of *Enterobacterales* Carried IncX3 Plasmid Harboring bla_{NDM} in Tokyo

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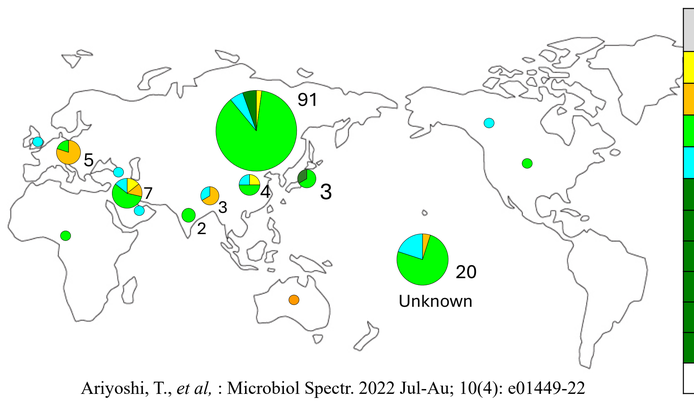
RES-100

Background

Metallo-beta-lactamase (NDM)-producing *Enterobacterales* were mostly detected in overseas cases in Japan until around 2017.

However, in recent years, the number of NDM-producing *Enterobacterales* from patients with no history of overseas travel increased, in particular, *Enterobacterales* carried IncX3 plasmid harboring bla_{NDM} became conspicuous. This trend is spreading worldwide, primarily in Asia.

Detection of *Enterobacterales* carried IncX3 plasmid harboring bla_{NDM} around the world



bla_{NDM} variants	Number of data
NDM-1	4
NDM-4	9
NDM-5	107
NDM-7	16
NDM-16b	1
NDM-17	1
NDM-19	1
NDM-20	1
NDM-21	1
NDM-33	1
Total	142

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Materials & Methods

【Materials】

Among 731 carbapenem-resistant *Enterobacterales* strains collected through national surveillance between 2015 and 2024, 17 strains carried IncX3 plasmid harboring bla_{NDM}

***Escherichia coli*【n=11】**

No.	Collection Year	NDM variants	Sequence Type
1	2015	NDM-5	410
2	2016	NDM-5	167
3※	2018	NDM-5	648
4	2018	NDM-5	1011
5	2018	NDM-5	617
6	2018	NDM-16b	746
7	2019	NDM-5	617
8	2022	NDM-5	167
9	2023	NDM-5	3076
10	2023	NDM-5	38
11	2024	NDM-5	156

***Klebsiella pneumoniae*【n=4】**

No.	Collection Year	NDM variants	Sequence Type
12	2018	NDM-5	726
13	2019	NDM-5	-
14	2020	NDM-5	-
15	2023	NDM-5	412

***Citrobacter* sp.【n=2】**

No.	Collection Year	NDM variants	species
16	2018	NDM-5	*C. braakii*
17	2023	NDM-5	*C. koseri*

※No.3 patient had a history of overseas travel

【Methods】

◆Antimicrobial susceptibility testing

Eiken DPE1 dry plates (Eiken Chemical Co., Ltd.)

◆Comparative analysis of IncX3 plasmids

MiSeq (Illumina, Inc.)

MinION sequencer (Oxford Nanopore Technologies Ltd.)

DigAlign (Nishimura *et al.*)

Easyfig (Sullivan MJ *et al.*)

◆ Plasmids transforming

Electroporation with *E. coli* DH5α Electro-Cells (TAKARA BIO INC.)

S1-PFGE

Results

◆Antimicrobial susceptibility testing

No.	IPM	MEPM	MINO	CPFX	S/T
1	8	＞8	8	＞4	＞38/2
2	＞8	＞8	≦2	＞4	＞38/2
3	＞8	＞8	≦2	＞4	＞38/2
4	＞8	＞8	＞8	＞4	＞38/2
5	＞8	＞8	8	＞4	≦9.5/0.5
6	8	＞8	4	＞4	＞38/2
7	8	＞8	＞8	＞4	＞38/2
8	＞8	＞8	8	＞4	＞38/2
9	8	＞8	＞8	＞4	＞38/2
10	8	＞8	＞8	＞4	＞38/2
11	8	8	＞8	＞4	＞38/2
12	＞8	＞8	＞8	＞4	＞38/2
13	＞8	＞8	8	＞4	＞38/2
14	＞8	＞8	≦2	≦1※	＞38/2
15	＞8	8	≦2	≦1※	≦9.5/0.5
16	8	＞8	＞8	2	＞38/2
17	8	＞8	≦2	≦1※	≦9.5/0.5

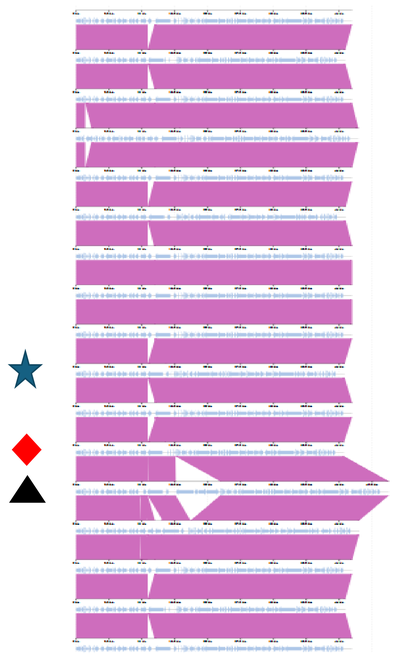
- All strains showed resistance to carbapenem antimicrobials.
- Almost strains also showed no susceptibility to several antimicrobials excepted for carbapenem.

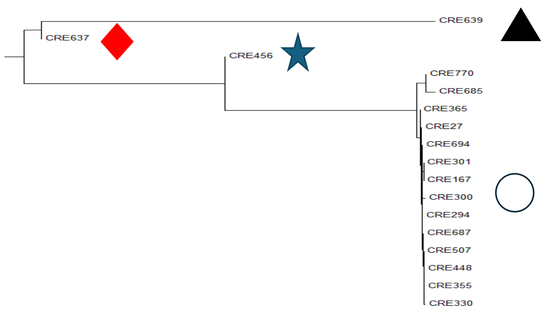
IPM：Imipenem
MEPM：Meropenem
MINO：Minocycline
CPFX：Ciprofloxacin
S/T：Sulfamethoxazole/Trimethoprim

Criteria: CLSI M100-ED35

Red fonts indicates resistance
Green fonts indicates intermediate
Black fonts indicates susceptibility
※Unable to determine

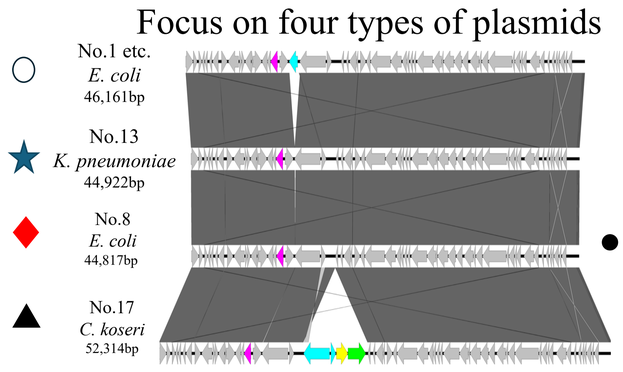
◆ Comparative analysis of IncX3 plasmids





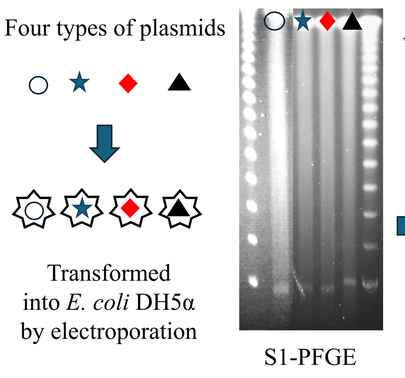
- The complete genome sequences of 17 strains were obtained using NGS.
- Theses strains carried IncX3 plasmids harboring bla_{NDM} ranging from 44,817 to 52,314 bp (median approximately 46,000 bp).
- Comparative analysis of these plasmids using DigAlign resulted in the formation of one major cluster though some strains formed an outgroup.

Focus on four types of plasmids



- The plasmids nucleotide sequences were largely identical, but local indels occurred.

◆ Plasmids transforming



We constructed plates ourselves for a detail antimicrobial susceptibility testing of resistance to carbapenems.
(MIC values ranged from 0.12 to 256 μg/mL)

	IPM	MEPM
DH5α	≦0.12	≦0.12
○	8	4
★	8	8
◆	8	8
▲	8	16

- The plasmid containing a ~6,000 bp gene cassette insertion upstream of bla_{NDM} conferred higher resistance to meropenem compared to the other plasmids.

Conclusion & Future prospects

【Conclusion】

Enterobacterales carried IncX3 plasmid harboring bla_{NDM} detected at medical institutions in Tokyo was predominantly *E. coli* but showed no bias toward specific STs. There was no specific STs bias for other species. The IncX3 plasmid sequences of these strains carried observed similarity with other strains, with approximately 46 kbp of plasmid sizes. Moreover, there were no patients with a history of overseas travel except for one case. This suggests that these IncX3 plasmids may have spread in Tokyo, leading to the detection of various *Enterobacterales* carried IncX3 plasmid harboring bla_{NDM} regardless of species or STs.

The IncX3 plasmids are spreading worldwide, primarily in Asia, regardless of host or species. Among these IncX3 plasmids, some carry $bla_{NDM-16b}$ which is rarely detected globally, while others showed higher resistance to meropenem compared to other plasmids. Because these plasmids may be a serious problem not only a clinical point of view but also One Health increased attention should be paid to the plasmids in the future.

【 Future prospects 】

- Perform more detailed comparative plasmid analysis, such as SNPs analysis.
- We plan to conduct additional analyses on the plasmid showed higher resistance to meropenem, including protein expression levels and fitness costs, to elucidate the mechanism of higher resistance and its molecular characteristics.