

1 **The complete genome of carbapenem-resistant *Escherichia coli* ST410 harbored *bla*_{NDM-5}**
2 **isolated from reservoir water in Singapore.**

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22 **Abstract:**

23 *Objective:* *Escherichia coli* ST410 with *bla*_{NDM-5} has been increasingly detected as multidrug
24 resistance pathogens globally, even though there are very few reports of infections caused by
25 *bla*_{NDM-5} producing *E. coli* in Singapore[1]. And significantly limit sequencing information of
26 *bla*_{NDM-5} carried *E. coli* strain from Singapore. In 2018, our group obtained a carbapenem
27 resistance *E. coli* ST410 strain SrichA-1 isolated from reservoir water in Singapore,
28 determined to harbor the NDM-5 gene. (BioSample Accession: SAMN18579051).

29 *Methods:* The susceptibility test to antimicrobials was performed with microdilution
30 minimum inhibitory concentration (MIC) test and interpreted according to the Clinical And
31 Laboratory Standards Institute (CLSI) -M100 standards. The genomic DNA of this strain
32 was extracted and send for Whole-genome sequencing(WGS) with the Illumina platform. The
33 WGS analysis was processed with the Center for Genomic Epidemiology (CGE, DTU) server.

34 *Results:* During the minimum inhibitory concentration (MIC) test, SrichA-1 has shown strong
35 resistance to all the beta-lactams, including cephalosporin and carbapenem, which can not be
36 inhibited by the clavulanic acid. Further whole genome sequencing analysis has shown that
37 the strain harboring five beta-lactamase genes covers all class A to D, including the
38 carbapenemase genes as *bla*_{NDM-5}.

39 *Conclusion:* Here, we reported the complete chromosome sequence of this isolate as well as
40 the sequence of a cycler plasmid. The pSGNDM-5 plasmid was furtherly identified to carry
41 four beta-lactamase genes, including *bla*_{NDM-5}, *bla*_{CTX-M-15}, *bla*_{TEM-1B}, *bla*_{OXA-1}, while a *bla*_{CMY-}
42 ₂ was detected to be located on the chromosome.

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44 *E. coli* ST410 isolate was obtained during the beta-lactamase-producing bacteria
45 investigation from reservoir water in Singapore by Nanyang Food Technology Centre

46 (NAFTEC). A bottle of 800 ml reservoir water was filtered with a 0.45 µm filter membrane
47 and further enriched with the nutrition medium. The microbiota culture was then streaked on
48 a Brilliance™ ESBL Agar (Thermofisher, the USA) following with purified on Thermo
49 Scientific™ Mueller-Hinton (MH) Agar with 5% Sheep Blood (Thermo Scientific, the USA).
50 The purified strain was further confirmed as carbapenem-resistant with micro-broth dilution
51 methods using Sensititre™ Extended Spectrum Beta-lactamase Plate (Thermofisher, the
52 USA). As determined by the MIC test, the isolate SrichA-1 is resistant to all the tested
53 cephalosporin, carbapenem, and even ciprofloxacin but sensitive to gentamicin. The SrichA-1
54 strain has been further characterized with whole-genome sequencing (WGS) analysis. The
55 genomic DNA was extracted with the QIAamp DNA Blood Mini Kit (Qiagen, Germany) and
56 sequenced with the Pacbio RS II system (Pacbio, the USA). The Multilocus sequence type
57 (MLST) was determined as ST410, serotype as H9, fimH24. Besides, 14 AMR genes were
58 detected using CGE ResFinder 4.0 (<https://cge.cbs.dtu.dk/services/ResFinder/>) with the
59 default setting[2]. The AMR genes belonged to eight classes, including macrolide, beta-
60 lactams, phenicol, sulphonamide, quinolone, tetracycline, trimethoprim were detected. The
61 sequence of a closed *IncF* plasmid type [F:-A1: B49] was obtained after assemble. All the
62 resistance genes except *mdf(A)* and *bla_{CMY-2}* were found on the plasmid.

63 The chromosome is 4806160 bp, detected to contain 4786 genes. The closest genome found
64 based on both cgMLST and cgSNP is strain SIEC197 (Accession: SAMN11399743) from
65 Thailand, which carries four same beta-lactamase genes as our isolate. As the SIEC197 is a
66 pathogen isolated from patients, a pathogenetic prediction with Virulence Finder and
67 Pathogen Finder was also performed and identified Srich A-1 as a potential human pathogen
68 with a probability of 0.936[3]. Virulence genes include *fyuA*, *gad*, *irp2*, and *terC* were
69 detected on the chromosome. However, based on the phylogenetic analysis of SrichA-1, no
70 close related clinical isolates or infection cases have been found in Singapore.

71 The circular plasmid, which is 84257 bp, was further aligned with other close neighbors
72 found by NCBI Blastn from different sources and other countries. The pSGNDM-5 plasmid
73 has shown higher identification compare to the plasmid pAMA1167-NDM-5 from Denmark,
74 which was also reported from an ST410 isolate[4]. Except for the beta-lactamase, seven other
75 AMR genes have been detected. Two integrases *IntI* leading gene cassette were detected on
76 the upstream and downstream of *bla*_{TEM-1}, respectively. A partial converse gene cluster
77 contains *ISEcpI*, the *bla*_{CTX-M-15}, and *WubC*, which has been reported by our group to be
78 located on the chromosome of other *E. coli* isolates, was also find on this plasmid[5]. But
79 the first 1375 bp of *ISEcpI* was replaced by a partial reverse transposase (Access:
80 EIL37998.1), which may as a consequence of recombination during transfer.

81 To our knowledge, this is the first complete chromosome and the plasmid sequence report of
82 the *E. coli* ST410 strain carry *bla*_{NDM-5} from Singapore.

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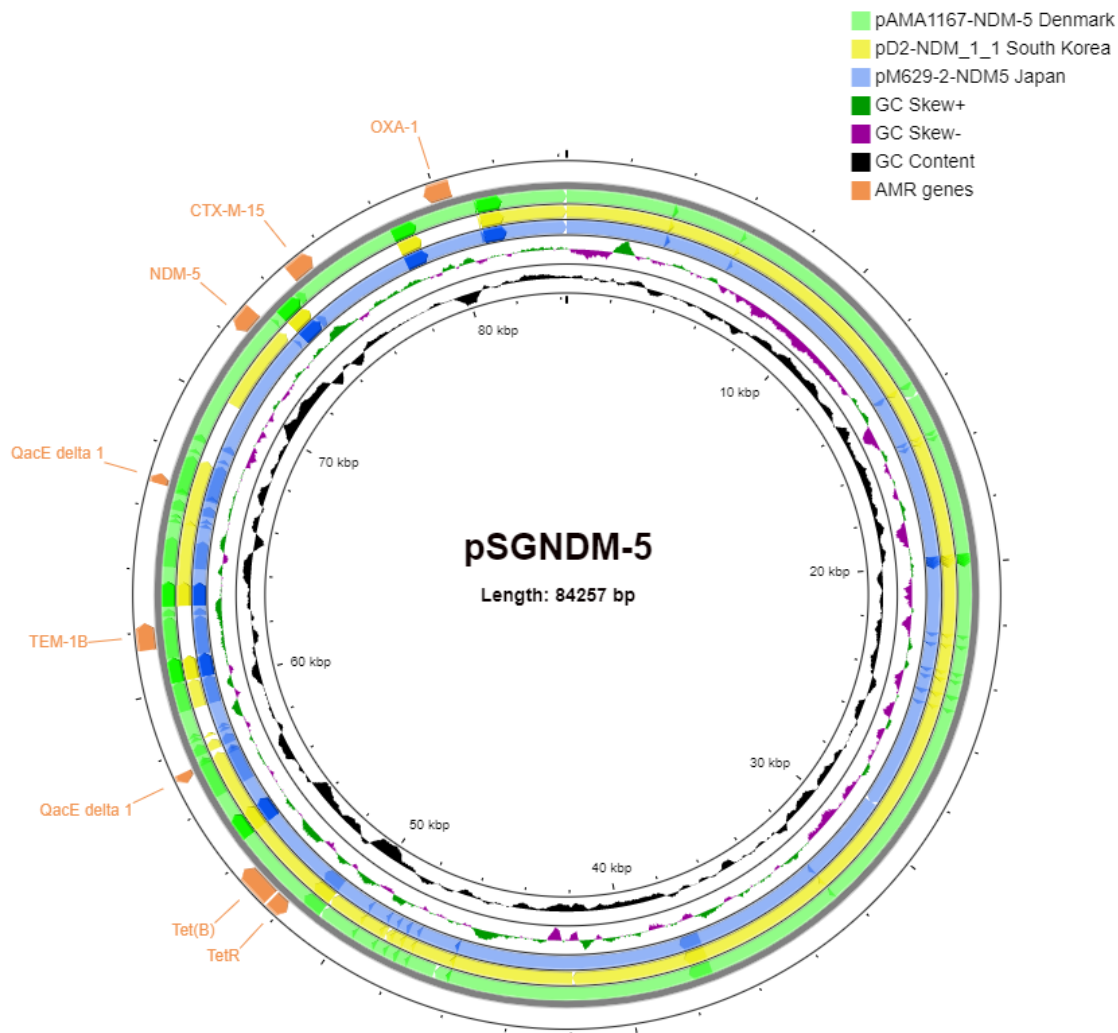
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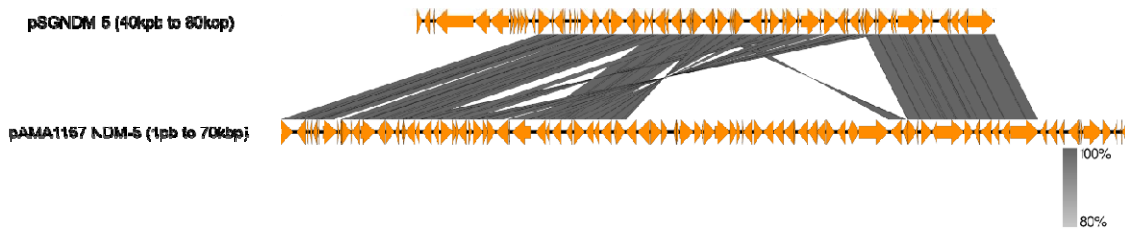
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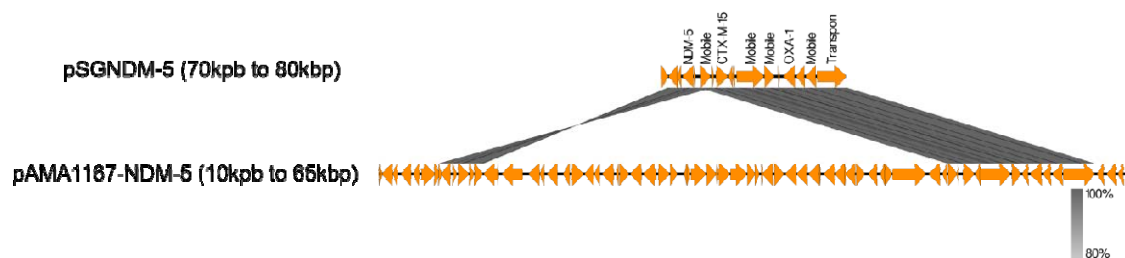
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4 **Fig 1. Comparison of plasmid pSGNDM-5 aligned with three similar plasmids**
5 **harboring *bla*_{NDM-5}.** For the circulation map, the inner circle presents pSGNDM-5 as the
6 reference sequence. Three plasmids that were also bearing *bla*_{NDM-5} were chosen for
7 comparison and presented with different colors as middle circles. The antimicrobial genes
8 were annotated on the outer circle as arrows. A linear alignment was performed between
9 pSGNDM-5 and pAMA1167 NDM-5 (Denmark). Compared to pAMA1167 NDM-5 (111310
10 bp), pSGNDM-5 is missing a 27053 bp fragment, which presents potential recombination of
11 the genetic environment around the *bla*_{NDM-5} gene.

Table 1. The minimum inhibitory concentration (MIC) tested with microdilution method.

Antimicrobials	SrichA-1 ($\mu\text{g/ml}$)
Ceftriaxone	>128
Meropenem	>8
Cephalothin	>16
Cefpodoxime	>32
Ciprofloxacin	>2
Cefotaxime	>64
Gentamicin	<4
Cefotaxime / clavulanic acid	>64/4
Ampicillin	>16
Ceftazidime	>128
Cefazolin	>16
Ceftazidime / clavulanic acid	>128/4
Imipenem	16
Piperacillin / tazobactam constant 4	>64/4
Cefepime	>16
Cefoxitin	>64