1 The complete genome of carbapenem-resistant *Escherichia coli* ST410 harbored *bla*_{NDM}-
2 *5* 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 cyclizer 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

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

The chromosome is 4806160 bp, detected to contain 4786 genes. The closest genome found based on both cgMLST and cgSNP is strain SIEC197 (Accession: SAMN11399743) from Thailand, which carries four same beta-lactamase genes as our isolate. As the SIEC197 is a pathogen isolated from patients, a pathogenetic prediction with Virulence Finder and Pathogen Finder was also performed and identified Srich A-1 as a potential human pathogen with a probability of 0.936[3]. Virulence genes include *fyuA*, *gad*, *irp2*, and *terC* were detected on the chromosome. However, based on the phylogenetic analysis of SrichA-1, no 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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Table 1. The minimum inhibitory concentration (MIC) tested with microdilution method.

Antimicrobials	SrichA-1 (µg/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