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Cross-border emergence of *Escherichia coli* producing the carbapenemase NDM-5 in Switzerland and Germany

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Running title: NDM-5 *E. coli* isolates in Switzerland and Germany

KEYWORDS

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ABSTRACT

A series of clinical NDM-5-producing *E. coli* isolates obtained from two surveillance networks of carbapenem-producing Enterobacterales from 2018-2019, namely Switzerland (NARA) and Germany (SurvCARE), were analyzed. The 33 NDM-5-producing *E. coli* isolates were highly resistant to β-lactams including the novel ß-lactam/ß-lactamase inhibitor combinations (ceftazidime/avibactam, imipenem/relebactam and meropenem/vaborbactam), and remained susceptible to fosfomycin, colistin, and tigecycline. Those isolates were assigned to different sequence types (STs) and indicated a predominance of isolates exhibiting the sequence type (ST) ST167 in Switzerland and in Germany (n=10, phylogenetic group C), followed by ST405 (n=4, phylogenetic group E), ST1284 (n=4, phylogenetic group C) and ST361 (n=4, phylogenetic group C). The *bla*$_{\text{NDM-5}}$ gene was predominantly present on an IncF-type plasmid (n=29), and to a lesser extent on the narrow-host range IncX3 plasmid (n=4). Sequence analyses of eight NDM-5 plasmids indicated that NDM-5-encoding F-type plasmids varied in size between 86 to 132 kb**.** The two IncX3 plasmids pCH8NDM5 and pD12NDM5 were 46 and 45 kb in size, respectively. The highly conserved *bla*$_{\text{NDM-5}}$ genetic surrounding structures [ΔIS*Aba125*-*bla*$_{\text{NDM-5}}$-*ble*$_{\text{MBL}}$-*trpT*-*dsbD*-IS*26*] of both the F-type and IncX3 plasmids suggested a common genetic origin. The emergence of the NDM-5 carbapenemase was evidenced in particular for the *E. coli* ST167 clone, which is a successful epidemic clone, known to be associated to both multi-resistance and virulence traits and is therefore of high public health concern. The occurrence of clonally related NDM-5-producing *E. coli* isolates in Switzerland and Germany further indicates the international spread of this multidrug-resistant superbug at least throughout Europe.

Introduction

Carbapenemase-producing Enterobacterales (CPE) are one of the most clinically relevant threats in medical healthcare worldwide (1). Carbapenem-hydrolyzing ß-lactamases of the New Delhi Metallo-β-lactamase (NDM) type belong to the class B of ß-lactamases. These enzymes have rapidly emerged in Gram-negatives during the last decade, being mainly identified among Enterobacterales. As early as in 2010, spread of NDM-1 producers had been identified among European countries (2). Worryingly the corresponding genes are located on diverse plasmid scaffolds, some of them exhibiting broad-host range and disseminating very efficiently (3). A large series of NDM variants have been identified so far, with currently 24 known NDM derivatives. Those variants efficiently hydrolyze all β-lactams with the exception of the monobactam aztreonam. The corresponding genes are often identified in isolates co-producing multiple resistance determinants, which enables pathogens to become multidrug resistant (4)**.** NDM-5 had been primarily identified in a multidrug-resistant *E. coli* in the UK (5). It differs from NDM-1 by two amino acid substitutions at positions 88 (Val3Leu) and 154 (Met3Leu) leading to an enhanced hydrolytic activity against carbapenems (5). A diversity of *E. coli* sequence types (STs) (i.e. clonal background), were shown to produce NDM-5. The ST167 clonal lineage is actually common among NDM-5-producing *E. coli*, being identified in many different countries including China, Italy, Egypt and Switzerland (6-14). With respect to the plasmid location of the $bla_{\text{NDM-5}}$ gene, it has been identified on many different scaffolds, including IncF, IncX3, IncL/M and IncH, and IncA/C2 replicon types, further enhancing its dissemination capacity (10, 15, 16). Our goal here was to analyze the genetic features of NDM-5-producing *E. coli* isolates recovered from infections or colonizations of hospitalized patients in two different European countries, namely Germany and Switzerland.

Material and Methods

Strain collection, susceptibility testing, molecular analyses. The survey was performed during over a two-year period (2018-2019) among the CPE producers in the entire Switzerland and the Hesse region in Germany. A total of 33 NDM-5-producing *E. coli* isolates were obtained from the two carbapenemase-dedicated surveillance networks, one located in Switzerland (National Reference Center for Emerging Antibiotic Resistance (NARA), 8.4 million inhabitants) and the other located in the state of Hesse, Germany (Surveillance of Carbapenem-Resistant Enterobacterales (SurvCARE) 6.2 million inhabitants) (Table 1). The NARA reference center collected enterobacterial strains displaying a decreased susceptibility to carbapenems for a total of 17 NDM-5-producing *E. coli* strains). Those isolates had been recovered from patients hospitalized in different parts of Switzerland (Basel, Bellinzona, Bern, Geneva, Schaffhausen, Lausanne, Sion, and Zurich). For SurvCARE 13 NDM-5-producing *E. coli* isolates were collected by the laboratories of the different participating hospitals in Darmstadt, Hanau, Frankfurt, Rüsselsheim, Kassel, and Bad Wildungen during the surveillance period in 2019. Resistance phenotype and species determination were first made using the EnteroPluri-Test® (Liofilchem S.R.L., Roseto degli, Italy) and either by disk diffusion or the VITEK2® system (bioMérieux, Nürtingen, Germany). In addition, three other NDM-5 producers were included, i.e. two environmental and one animal isolates from Switzerland (river, sewer, and dog) for comparison (Table1, strains ECH1 to ECH3). Overall, clinical specimens from which NDM-5-producing isolates were obtained included rectal swabs (n=19), urine (n=4), skin (n=3) with one urethral swab, colonostomy swab, central venous catheter and bronchoalveolar lavage, each.

Antimicrobial susceptibility testing was performed for all isolates by using the disk diffusion method on Muller-Hinton agar plates for amoxicillin, amoxicillin/clavulanic acid, ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, temocillin, ceftazidime, cefotaxime,

cefoxitin, aztreonam, imipenem, ertapenem, meropenem, gentamicin, amikacin, ciprofloxacin, tigecycline, trimethoprim-sulfamethoxazole (SXT), and fosfomycin. The minimal inhibitory concentrations (MICs) were determined using the broth microdilution in cation-adjusted Mueller-Hinton broth (Bio-Rad, Marnes-la-Coquette, France) and results were interpreted according to the latest EUCAST breakpoints (www.eucast.org/clinical_breakpoints) guidelines. For the ceftazidime-avibactam (CAZ-AVI) combination, AVI was tested at a fixed concentration of 4 mg/L. The concentration of relebactam and vaborbactam were fixed at 4 and 8 mg/L, respectively. *E. coli* ATCC 25922 was used as quality control in all testing. Detection of carbapenemase encoding genes, including the $bla_{\text{NDM-5}}$ gene, was first performed by PCR and sequencing of the corresponding amplicons (Microsynth, Balgach, Switzerland) (17).

Phenotypic confirmation of resistance patterns. Carbapenemase production was detected by using the biochemical Carba NP and the NitroSpeed-Carba NP tests (18, 19) as well as the immunochromatographic NG-Test CARBA5 assay (NG Biotech, Guipry, France) that detects the five main types of carbapenemases (IMP, VIM, NDM, KPC, OXA-48) (20). Colistin resistance and fosfomycin resistance were tested by the Rapid Polymyxin NP test (21) and the Rapid fosfomycin NP test (22), respectively.

Plasmid transfer studies. The transferability of the plasmid carrying the NDM-5-encoding gene was evaluated for all NDM-5-producing *E. coli* by mating-out assays using the filter-mating method as described previously (23). NDM-5-positive *E. coli* isolates were used as donors and azide-resistant *E. coli* J53 strain as recipient. Briefly, both donor and recipient strains were cultured separately in LB broth. After incubation, the donor and recipient strains were mixed at a ratio of 1:9 (donor/recipient) and centrifuged; the supernatant was removed, and the pellets were resuspended in 200 µL LB broth, which was plated on a conjugation filter on a LB agar plate. The plates were incubated for 5 h at 37 °C. Transconjugants were selected on LB agar supplemented with sodium azide (100 mg/L) and imipenem (4 mg/L) or

ampicillin (100 mg/L), then re-plated on LB agar supplemented with imipenem (4 mg/L). Transconjugants producing an NDM-type enzyme were confirmed by performing antimicrobial susceptibility testing and by PCR targeting the NDM encoding genes.

Whole-genome sequencing (WGS) and analysis. Total genomic DNA (gDNA) of all isolates was extracted from an overnight bacterial culture using the PureLink Genomic DNA kit (Invitrogen, ThermoFischer, Germany). WGS was performed using a combination of the MiSeq/NextSeq and MinION (Oxford Nanopore Technologies, Oxford, United Kingdom) platforms. For short read sequencing, DNA libraries were constructed using Nextera XT kit (Illumina, Netherlands) according to the manufacturer's instructions and sequenced on MiSeq/NextSeq sequencing machines (read length either 2x300nt or 2x150nt). For MinION sequencing, the long-read sequencing libraries were prepared using the native barcoding kit (EXP-NBD103, Oxford Nanopore Technologies) and 1D chemistry (SQK-LSK108, Oxford Nanopore Technologies, UK). Sequencing was performed on a MinION sequencer (Oxford Nanopore Technologies, UK) using a SpotON Mk I R9 Version flow Cell (FLO-MIN106, Oxford Nanopore Technologies, UK). Post-sequencing quality control, assembly of both Illumina short reads and Nanopore long reads and virulence gene determination was performed using ASA^{3}P pipeline (24). The multilocus sequence typing (MLST) were identified by MLST 2.0 software (https://cge.cbs.dtu.dk/services/MLST/). Antimicrobial resistance genes, plasmid incompatibility groups and pMLST types were determined using the Center for Epidemiology tools (http://www.genomicepidemiology.org/). Serotyping and *fimH* subtyping were performed using SerotypeFinder 2.0 (https://cge.cbs.dtu.dk/services/SerotypeFinder/) and FimTyper 1.0 (https://cge.cbs.dtu.dk/services/FimTyper/), respectively. Phylogenetic analysis was performed using HarvestSuite (25). The phylo-groups of the isolates were determined *in-silico* using the schema of Clermont method (26).

The raw sequence data information had been deposited at GenBank under BioProject ID: PRJNA630933.

Results and Discussion

NDM-producing *E. coli* corresponded to 18% (22/122) and 20% (14/71) of the CPE isolated in Switzerland and in Hesse region in Germany, respectively, during the year of 2019. In addition, NDM-5 producers accounted for 77% (17/22) and 93% (13/14) of the total number of NDM-like producing *E. coli* in Switzerland and the Hesse region, respectively (Table S1). During the period studied (2018-2019), 16.5 % (31/188) and 16.3% (16/98) of the CPE isolated in Switzerland and in Hesse region in Germany, respectively, were NDM-producing *E. coli.* NDM-5 producers accounted for 77.5% (24/31) and 87.5% (14/16) of the total number of NDM-like producing *E. coli* in Switzerland and the Hesse region, respectively (Table S1). Those numbers and ratio indicated an emerging antibiotic resistance phenomenon that was not observed in the previous years. All 33 NDM-5-producing *E. coli* isolates analyzed showed a multidrug-resistant phenotype (Tables S2 and S3). They were resistant to most β-lactam antibiotics, including broad-spectrum cephalosporins, carbapenems, piperacillin/tazobactam, amoxicillin-clavulanic acid, sulfonamides and sulfamethoxazole/trimethoprim, remaining susceptible only to fosfomycin, colistin, and tigecycline. High resistance rates were also observed for aztreonam (79%), nalidixic acid and ciprofloxacin (91%), kanamycin and tobramycin (52%), gentamicin (33%), amikacin (24%), and tetracycline (76%). They were also resistant to the recently developed combinations, namely CAZ/AVI, imipenem/relebactam, and meropenem/vaborbactam (Table S3). As mentioned in the very early reports on NDM producers from collections of strains from India and Pakistan, those strains remained susceptible to colistin, fosfomycin and tigecycline, this latter antibiotic being however not useful for treating urinary tract infections due to its poor diffusion in the urinary tract (4, 27). The carbapenemase production was confirmed by the

positivity of the Rapid Carba NP test, of the NitroSpeed-Carba NP and of the NG-Test CARBA5. This result underlined the value of the newly developed NitroSpeed-Carba NP for identification of any type of carbapenemase producer. As previously reported, two strains co-produced two types of carbapenemases, i.e. NDM-5 and OXA-48 (28-30). None of the strains were positive for the Rapid Polymyxin NP test or the Rapid Fosfomycin NP test, indicating their susceptibility to colistin and fosfomycin (data not shown).

In addition to the *bla*$_{\text{NDM-5}}$ gene, isolates harbored many other resistance genes such as those conferring resistance to aminoglycosides, β-lactams, macrolides, sulfonamides, trimethoprim, fluoroquinolones, phenicol, and tetracycline, in agreement with their multidrug resistance pattern (Table 2). We found that 63% of the isolates (21/33) co-harbored a *bla*$_{\text{TEM}}$-like gene (*bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-10}}$, *bla*$_{\text{TEM-30}}$ or *bla*$_{\text{TEM-166}}$) and more than half of the isolates (17/33) harbored a *bla*$_{\text{CTX-M}}$-like ESBL gene (*bla*$_{\text{CTX-M-15}}$ or *bla*$_{\text{CTX-M-24}}$), and two isolates harbored OXA-48 derivatives (*bla*$_{\text{OXA-181}}$ or *bla*$_{\text{OXA-244}}$). In addition, the CMY-42 AmpC encoding gene was detected in seven isolates. The *rmtB* gene, encoding a 16S rRNA methylase, conferring resistance to all aminoglycosides, was detected in six isolates (6/33). Also, a total of 10 isolates (30.3%) co-harbored the *aac(6′)-Ib-cr* gene encoding an acetyltransferase conferring reduced susceptibility to quinolones in addition to resistance to aminoglycosides.

Mating-out assays were performed for the 33 isolates with the aim to transfer the *bla*$_{\text{NDM-5}}$ gene. In total, this mating-out assay was successful for 27 out of those 33 isolates. By testing all *E. coli* transconjugants for antibiotic susceptibility, co-resistance to aminoglycosides (kanamycin, tobramycin, gentamicin, amikacin) was observed (n=3), as well as resistance to trimethoprim/sulfamethoxazole (n=22), tetracycline (n=10), and sulfonamides (n=20). Resistance to aminoglycosides, when observed in those *E. coli* transconjugants, correlated with the acquisition of the 16S RNA methylase encoding gene *rmtB*. Interestingly, one of those NDM-5-producing *E. coli* transconjugant showed a multidrug-resistant phenotype

including all β-lactams (including aztreonam), aminoglycosides, sulfonamides, and quinolones, corresponding to the occurrence of the *bla*$_{\text{NDM-5}}$, *bla*$_{\text{CTX-M-15}}$, *aac(6')Ib-cr, rmtB,* and *sul1* genes on the same IncF-type plasmid (Table 3).

The multilocus sequence typing (MLST) analysis of all the isolates revealed a total of eleven different sequence types (ST) genetic backgrounds. ST types being identified both in Switzerland and Germany were ST167 (n=10, phylogenetic group C), ST405 (n=4, phylogenetic group E), ST1284 (n=4, phylogenetic group C) and ST361 (n=4, phylogenetic group C). Other STs identified included ST10, ST46, ST2851, ST940, ST648, ST354 and ST1588, present either only in Switzerland or in Germany (Fig. 1). Phylogenetic analysis revealed that the NDM-5 producer *E. coli* isolates clustered into two clades (I, II). Clade I compromises isolates of ST167, 1284, 361, 940, 10, 46 and 2851, while clade II the isolates of ST648, 354, 1588 and 405 (Fig. 1). Clade I isolates are either of phylogenetic groups C or B1, and contain the recently described ST167 worldwide high-risk clone (31)**.** Isolates of clade II belong to the phylogenetic groups D and E (Fig. 1). The ten ST167 *E. coli* strains shared a common resistance gene pattern including the *bla*$_{\text{NDM-5}}$, *dfrA12*, *sul1*, *tet(A)*, *aadA*, and *mph(A)* genes, but other resistance genes were differently identified among the strains tested.

The *E. coli* ST167 clone is a globally disseminated strain being identified both in humans and animals, and often associated with a multidrug resistance phenotype. The occurrence of *bla*$_{\text{NDM}}$ genes in so-called high-risk clones is notorious (31-33). Previous studies from different countries, such as China (8-10), Italy (11)**,** Egypt (12) and Switzerland (13, 14) have reported ST167 producing *bla*$_{\text{NDM-5}}$ (6, 7). Recently, *bla*$_{\text{NDM-5}}$-harbouring *E. coli* strains belonging to ST167 have been identified in both humans and dogs from Finland (33). In our study**,** *bla*$_{\text{NDM-5}}$-harbouring *E. coli* ST167 clone was also identified from a dog, and from a river sample from Switzerland suggesting its diffusion in the environment.

Previous studies have illustrated the diversity of plasmid types harboring the *bla*$_{NDM-5}$ gene including the IncF, IncFII, IncN and IncX3 incompatibility groups (10). All isolates in our study harbored the *bla*$_{NDM-5}$ gene either on an IncFI or FII plasmid and 61% of those strains have in addition at least one Col-like type plasmid. For 20% (7/33) of all isolates, an IncX-type plasmid was present. Other Inc types identified included IncI-1I, IncI, IncHI, IncQ1, IncQ2, IncY and pO111. Hence, most isolates harbored several plasmids of different Inc types (Table 2, Fig.1).

In most isolates, the *bla*$_{NDM-5}$ gene was located exclusively on an F-type plasmid (n=29), and a minority on the narrow-host range IncX3 plasmid (n=4, with three independent isolates from a single patient) which are commonly associated with the global spread of *bla*$_{NDM-5}$ (9). NDM-encoding IncX3 plasmids have been also identified in Germany (*bla*$_{NDM-7}$) (33), India (*bla*$_{NDM-5}$), and the United Arab Emirates (*bla*$_{NDM-1}$) (36)**.** Yang et al. (2014) reported two nearly identical *bla*$_{NDM-5}$-carrying plasmids found in China and India separately, indicating international transfers of a same IncX3 plasmid. The F-type plasmids were multiple replicons and belonged to various pMLST types clustering into four type-groups, FII, FII-FIA, FII-FIB or FII-FIA-FIB (Table S4, Fig. 1). The plasmid backbone comprises regions conferring stability, fertility inhibition, establishment in a new host as well as the DNA transfer region starting at *lts* (the specialized lytic transglycosylase gene) and ending at *finO*. For the plasmid pD9NDM5, the DNA transfer region (about 40 kb in size) has been lost (Fig. 2). Detailed sequence analysis of the six NDM-5 F-type plasmids indicated that they varied in size between 86 to 132 kb (Table 3, Fig. S1). The two IncX3 plasmids pCH8NDM5 and pD12NDM5 harbored almost identical sequences; pD12NDM5 lacks a copy of the IS*Aba*125 insertion sequence (Fig. 2, Fig. 3).

The combination of resistance and virulence may play an important role in the global expansion of this NDM-5-producing *E. coli* ST167 clone. Very recently, a novel capsular

synthesis gene cluster (K48-like capsular synthesis cluster), was identified in phylogenetically related *E. coli* strains of the ST167 clone (31). In our study, none of the strains carried KL48-like capsular synthesis cluster, excepting ES2 isolate of ST167 recovered from dog. Further analysis of the WGS data for ST167 isolates indicated acquisition of other capsule determinants i.e. KL9, KL30, KL103, KL115, Kl123 and KL124 from *Klebsiella* species (Table S5 and Fig. S2). In another study, a *E. coli* K30-like capsular synthesis cluster was identified in 8/343 genomes of the NDM-5-Producing *E. coli* Sequence Type 167 (31). Moreover, the O antigen gene cluster, O89 was found to be present in all ST167 *E. coli* strains as recently reported (37). The co-occurrence of the $bla_{\text{NDM-5}}$ gene in serotype O89 harboring ST167 clone with fitness and virulence factors such the capsule synthesis clusters could explain its global dissemination as a high-risk clone**.** The presence of capsule loci in *E. coli* ST167 normally present *Klebsiella* species suggest their common existence in presently unknown environments. Isolates of other STs, such as ST940, ST10 and ST1248 however harbor K30 (accession no.AF104912) or K30-like (accession no. CP024859) capsule biosynthesis gene cluster from other *E. coli* isolates.

In-depth analysis of the structures surrounding the $bla_{\text{NDM-5}}$ gene in the IncX3 and IncF plasmids showed that a remnant of insertion sequence IS*Aba125* was systematically present upstream of the $bla_{\text{NDM-5}}$ gene (Fig. 3) as known for the structures containing bla_{NDM} genes (38). The presence of this very peculiar insertion sequence identified only in *Acinetobacter* spp. has suggested that this species may play the role of an intermediate host between a still unknown natural reservoir and Enterobacterales (39). A sequence fragment consisting of a ble_{MBL} gene (encoding bleomycin resistance), a *trpT* gene (encoding a tRNA), and a *dsbD* gene (encoding a thiol-disulfide interchange protein) was systematically identified downstream of the $bla_{\text{NDM-5}}$ gene, regardless its location either on IncF-like or IncX3 plasmids (Fig. 3, Fig. S1). A complex class 1 integron, consisting of an IS*CR1* and [*sul1-aadA2-*

dfrA12] gene cassettes, was identified and inserted between the *dsbD* gene and the IS*26* on IncF plasmids. Also, the insertion of IS*CR3* with the *qepA* gene (fluoroquinolone resistance) was observed on IncF-type plasmids (Fig. 3, Fig. S1). The highly conserved $bla_{\text{NDM-5}}$ genetic surrounding structures [ΔIS*Aba125*-$bla_{\text{NDM-5}}$-ble_{MBL}-*trpT*-*dsb*-IS*26*] of the F-type plasmid and IncX3 plasmid indicating their common genetic origin (Fig. 3, Fig. S1). Those results underline the variety of genetic events that drive the spread of the $bla_{\text{NDM-5}}$ gene.

In conclusion, this study showed the cross-border spread of NDM-5-producing *E. coli* strains in Switzerland and Germany, involving a series of successful epidemic clones (ST167, ST405, ST1284, ST361), with a predominance of the ST167 clone. Isolates were found not only in humans but also in a companion animal and in the environment. Our data indicate that NDM-5 gene is carried on commonly occurring IncFII and IncX3 plasmids, suggesting the expansion of the serotype O89 ST167 clone is being facilitated by the acquisition of specific "epidemic"-associated plasmid types. The spread of those NDM-5 producers is of concern since they occur in *E. coli* that is a well-known enterobacterial species as a source of hospital but also community-acquired infections. In addition, the multidrug resistance pattern of the strains leaves very limited therapeutic alternatives for treating infections due to those NDM-5 producers. Those strains are resistant to the novel antibiotics (CAZ/AVI, imipenem/relebactam, meropenem/vaborbactam) remaining susceptible to "old "antibiotics such as fosfomycin and colistin where efficacy still remains debatable for treating bloodstream infections.

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REFERENCES

1- Logan LK, Weinstein RA. 2017. The epidemiology of carbapenem-resistant *enterobacteriaceae*: the impact and evolution of a global menace. J Infect Dis 215: S28-S36.

2- Struelens MJ, Monnet DL, Magiorakos AP, Santos O'Connor F, Giesecke J; European NDM-1 Survey Participants. 2010. New Delhi metallo-ß-lactamase 1-producing Enterobacteriaceae: emergence and response in Europe. Euro Surveill 15:19716.

3- Brinkac LM, White R, D'Souza R, Nguyen K, Obaro SK, Fouts DE. 2019. Emergence of New Delhi metallo-β-Lactamase (NDM-5) in *Klebsiella quasipneumoniae* from neonates in a Nigerian hospital. mSphere 4:e00685-18.

4- Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 10:597-602.

5- Hornsey M, Phee L, Wareham DW. 2011. A novel variant, NDM-5, of the New Delhi metallo-β-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. Antimicrob Agents Chemother 55:5952-5954.

6- Li X, Fu Y, Shen M, Huang D, Du X, Hu Q, Zhou Y, Wang D, Yu Y. 2018. Dissemination of $bla_{\text{NDM-5}}$ gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. Antimicrob Resist Infect Control 7:59.

7- Zong Z, Fenn S, Connor C, Feng Y, McNally A. 2018. Complete genomic characterization of two *Escherichia coli* lineages responsible for a cluster of carbapenem-resistant infections in a Chinese hospital. J Antimicrob Chemother 73:2340-2346.

8- Huang Y, Yu X, Xie M, Wang X, Liao K, Xue W, Chan EW, Zhang R, Chen S. 2016. Widespread dissemination of carbapenem-resistant *Escherichia coli* sequence type

167 strains harboring *bla*$_{NDM-5}$ in clinical settings in China. Antimicrob Agents Chemother 60:4364-4368.

9- Yang P, Xie Y, Feng P, Zong Z. 2014. *bla*$_{NDM-5}$ carried by an IncX3 plasmid in *Escherichia coli* sequence type 167. Antimicrob Agents Chemother 58:7548-7552.

10- Zhu YQ, Zhao JY, Xu C, Zhao H, Jia N, Li YN. 2016. Identification of an NDM-5-producing *Escherichia coli* sequence type 167 in a neonatal patient in China. Sci Rep 6:29934.

11- Giufrè M, Errico G, Accogli M, Monaco M, Villa L, Distasi MA, Del Gaudio T, Pantosti A, Carattoli A, Cerquetti M. 2018. Emergence of NDM-5-producing *Escherichia coli* sequence type 167 clone in Italy. Int J Antimicrob Agents 52:76-81.

12- Ramadan H, Gupta SK, Sharma P, Ahmed M, Hiott LM, Barrett JB, Woodley TA, Frye JG, Jackson CR. 2020. Circulation of emerging NDM-5-producing *Escherichia coli* among humans and dogs in Egypt. Zoonoses Public Health 67:324-329.

13- Endimiani A, Brilhante M, Bernasconi OJ, Perreten V, Schmidt JS, Dazio V, Nigg A, Gobeli Brawand S, Kuster SP, Schuller S, Willi B. 2020. Employees of Swiss veterinary clinics colonized with epidemic clones of carbapenemase-producing *Escherichia coli*. J Antimicrob Chemother 75:766-768.

14- Peterhans S, Stevens MJA, Nüesch-Inderbinen M, Schmitt S, Stephan R, Zurfluh K. 2018. First report of a *bla*$_{NDM-5}$-harbouring *Escherichia coli* ST167 isolated from a wound infection in a dog in Switzerland. J Glob Antimicrob Resist 15:226-227.

15- Pitart C, Solé M, Roca I, Román A, Moreno A, Vila J, Marco F. 2015. Molecular characterization of *bla*$_{NDM-5}$ carried on an IncFII plasmid in an *Escherichia coli* isolate from a nontraveler patient in Spain. Antimicrob Agents Chemother 59:659-662.

16- Sadek M, Poirel L, Nordmann P, Nariya H, Shimamoto T, Shimamoto T. 2020. Genetic characterization of NDM-1 and NDM-5-producing Enterobacterales from retail chicken meat in Egypt. J Global Antimicrob Resist S2213-7165.

17- Poirel L, Walsh TR, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70:119-23.

18- Nordmann P, Poirel L, Dortet L. 2012. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 18:1503-1507.

19- Nordmann P, Sadek M, Demord A, Poirel L. 2020. NitroSpeed-Carba NP test for rapid detection and differentiation between different classes of carbapenemases in *Enterobacterales* [published online ahead of print, 2020 Jun 24]. J Clin Microbiol JCM.00932-20.

20- Hopkins KL, Meunier D, Naas T, Volland H, Woodford N. 2018. Evaluation of the NG-test CARBA 5 multiplex immunochromatographic assay for the detection of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases. J Antimicrob Chemother 73:3523-3526.

21- Nordmann P, Jayol A, Poirel L. 2016. Rapid detection of polymyxin resistance in *Enterobacteriaceae*. Emerg Infect Dis 22:1038-1043.

22- Nordmann P, Poirel L, Mueller L. 2019. Rapid detection of fosfomycin resistance in *Escherichia coli*. J Clin Microbiol 57:e01531-18.

23- Aires-de-Sousa M, Ortiz de la Rosa JM, Goncalves ML, Costa A, Nordmann P, Poirel L. 2020. Occurrence of NDM-1-producing *Morganella morganii* and *Proteus mirabilis* in a single patient in Portugal: probable in vivo transfer by conjugation. J Antimicrob Chemother 75:903-906.

24- Schwengers O, Hoek A, Fritzenwanker M, Falgenhauer L, Hain T, Chakraborty T, Goesmann A. 2020. ASA3P: An automatic and scalable pipeline for the assembly,

annotation and higher-level analysis of closely related bacterial isolates. PLoS Comput Biol 16:e1007134.

25- Treangen TJ, Ondov BD, Koren S, Phillippy AM. 2014. The harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15:524.

26- Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58-65.

27- Zahedi Bialvaei A, Samadi Kafil H, Ebrahimzadeh Leylabadlo H, Asgharzadeh M, Aghazadeh M. 2015. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iran J Microbiol 7:226-246.

28- Dortet L, Poirel L, Al Yaqoubi F, Nordmann P. 2012. NDM-1, OXA-48 and OXA-181 carbapenemase-producing *Enterobacteriaceae* in Sultanate of Oman. Clin Microbiol Infect 18:E144-E148.

29- Jhang J, Wang HY, Yoo G, Hwang GY, Uh Y, Yoon KJ. 2018. NDM-5 and OXA-48 Co-producing uropathogenic *Escherichia coli* isolate: first case in Korea. Ann Lab Med 38:277-279.

30- Haller S, Kramer R, Becker K, Bohnert JA, Eckmanns T, Hans JB, Hecht J, Heidecke CD, Hübner NO, Kramer A, Klaper K. 2019. Extensively drug-resistant *Klebsiella pneumoniae* ST307 outbreak, north-eastern Germany, June to October 2019. Euro Surveill 24:1900734.

31- Garcia-Fernandez A, Villa L, Bibbolino G, Bressan A, Trancassini M, Pietropaolo V, Venditti M, Antonelli G, Carattoli A. 2020. Novel insights and features of the NDM-5-producing *Escherichia coli* sequence type 167 high-risk clone. MSphere 5:e00269-20.

32- Oteo J, Diestra K, Juan C, Bautista V, Novais Â, Pérez-Vázquez M, Moyá B, Miró E, Coque TM, Oliver A, Cantón R. 2009. Extended-spectrum ß-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. Int J Antimicrob Agents 34:173-176.

33- Schink AK, Kadlec K, Kaspar H, Mankertz J, Schwarz S. 2013. Analysis of extended-spectrum-β-lactamase-producing *Escherichia coli* isolates collected in the GERM-Vet monitoring programme. J Antimicrob Chemother 68:1741-1749.

34- Grönthal T, Österblad M, Eklund M, Jalava J, Nykäsenoja S, Pekkanen K, Rantala M. 2018. Sharing more than friendship-transmission of NDM-5 ST167 and CTX-M-9 ST69 *Escherichia coli* between dogs and humans in a family, Finland, 2015. Euro Surveill 23:1700497.

35- Göttig S, Hamprecht AG, Christ S, Kempf VA, Wichelhaus TA. 2013. Detection of NDM-7 in Germany, a new variant of the New Delhi metallo-β-lactamase with increased carbapenemase activity. J Antimicrob Chemother 68:1737-1740.

36- Sonnevend A, Al Baloushi A, Ghazawi A, Hashmey R, Girgis S, Hamadeh MB, Al Haj M, Pal T. 2013. Emergence and spread of NDM-1 producer *Enterobacteriaceae* with contribution of IncX3 plasmids in the United Arab Emirates. J Med Microbiol 62:1044-1050.

37- Zeng X, Chi X, Ho BT, Moon D, Lambert C, Hall RJ, Baybayan P, Wang S, Wilson BA, Ho M. 2019. Comparative genome analysis of an extensively drug-resistant isolate of avian sequence type 167 *Escherichia coli* strain Sanji with novel *In silico* serotype O89b:H9. MSystems 4:e00242-18.

38- Poirel L, Dortet L, Bernabeu S, Nordmann P. 2011. Genetic features of $bla_{\text{NDM-1}}$-positive *Enterobacteriaceae*. Antimicrob Agents Chemother 55:5403-5407.

39- Bonnin RA, Poirel L, Nordmann P. 2014. New Delhi metallo-β-lactamase-producing *Acinetobacter baumannii*: a novel paradigm for spreading antibiotic resistance genes. Future Microbiol 9:33-41.

Legends of figures

Figure 1: Phylogenomic clustering of NDM-5 producing *E. coli* isolates with the respective ST types and phylogenic group indicated and plasmids harbored.

Figure 2: Fig. 2A and 2B, Circular comparison between different F-type plasmids. The plasmids pD13NDM5 (A) and pECH3NDM5 (B) (the inner circle) was used by the BRIG software as a reference plasmid. **Fig. 2C**, Genome comparison of the IncX3 plasmids pCH8NDM5 and pD12NDM5. Gaps indicate regions that were missing in the respective plasmid compared to the reference plasmid. The outmost rings (black) show the functional genes including the resistance and structural genes. The different colors indicate different plasmids and are listed in the color key.

Figure 3: Structures of $bla_{\text{NDM-5}}$ genetic context of the IncX3 and F-type plasmids.

Table 1: Features of the *E. coli* NDM-5 producing isolates and their origin

Isolate	Month/Year of isolation	Origin	Sample type	Age (years, if not otherwise stated)	Sex	Infection (I)/ Colonization (C)	Carba NP/ NitroSpeed Carba NP	Immunology test (NG-Carba 5 test)
D1	Jun-19	D, Darmstadt	Rectal swab	1 month	M	C	+	NDM
D2	May-19	D, Darmstadt	Rectal swab	11 months	F	C	+	NDM
D3	May-19	D, Hanau	Central venous catheter	15	F	I	+	NDM
CH1	Apr-19	CH, Zurich	Rectal swab	27	F	C	+	NDM
CH2	Mar-19	CH, Geneva	Rectal swab	16	F	C	+	NDM
CH3	Mar-19	CH, Sion	Rectal swab	16	F	C	+	NDM
CH4	Apr-19	CH, Lausanne	Rectal swab	69	M	C	+	NDM
CH5	Apr-19	CH, Zurich	Groin swab	68	F	C	+	NDM
CH7	Jan-19	CH, Lausanne	Rectal swab	14	M	C	+	NDM
ECH1	2018	CH, Zurich	River	N.A.	N.A.	N.A.	+	NDM
ECH2	2018	CH, Zurich	Dog	N.A.	N.A.	N.A.	+	NDM
CH8	May-19	CH, Schaff-hausen	Rectal swab	72	M	C	+	NDM
ECH3	2018	CH, Zurich	Sewer	N.A.	N.A.	N.A.	+	NDM
D4	Oct-19	D, Darmstadt	Urethral swab	38	M	I	+	NDM
D5	Sep-19	D, Frankfurt	Rectal swab	76	F	C	+	NDM
D6	May-19	D, Rüsselsheim	Rectal swab	78	M	C	+	NDM
CH9	Sep-19	CH, Geneva	Rectal swab	31	F	C	+	NDM
CH10	May-19	CH, Basel	Rectal swab	62	M	C	+	NDM+OXA-48
D7	Sep-19	D, Darmstadt	Skin lower leg	42	M	I	+	NDM
D8	Oct-19	D, Darmstadt	Bronchoalveolar lavage	75	M	I	+	NDM
D9	Mar-19	D, Hanau	Urine/ Catheter urine	76	F	I	+	NDM+OXA-48
D10	Nov-19	D, Bad Wildungen	Rectal swab	64	F	C	+	NDM
D11	Oct-19	D, Bad Wildungen	Rectal swab	64	F	C	+	NDM
D12	Sep-19	D, Kassel	Rectal swab	64	F	C	+	NDM
CH11	May-19	CH, Zurich	Rectal swab	55	M	C	+	NDM

Isolate	Month/Year of isolation	Origin	Sample type	Age (years, if not otherwise stated)	Sex	Infection (I)/ Colonization (C)	Carba NP/ NitroSpeed Carba NP	Immunology test (NG-Carba 5 test)
CH12	Oct-19	CH, Bern	Rectal swab	69	M	C	+	NDM
CH13	May-19	CH, Geneva	Urine	56	M	I	+	NDM
CH14	Feb-19	CH, Geneva	Rectal swab	55	M	C	+	NDM
CH15	Oct-19	CH, Geneva	Rectal swab	4	F	C	+	NDM
CH16	Jul-19	CH, Zurich	Groin swab	55	M	C	+	NDM
CH17	Jan-19	CH, Basel	Urine	71	F	I	+	NDM
D13	Jun-19	D, Darmstadt	Urine /Catheter urine	81	M	I	+	NDM
CH18	Feb-19	CH, Ticino	Colonostomy swab	66	M	C	+	NDM

Strains D10, D11, D12 were obtained from a same patient. Abbreviations: CH, Switzerland; D, State of Hesse, Germany; ECH; Environment from Switzerland; N.A., Not applicable.

Table 2: Plasmid characterization of *bla*$_{NDM-5}$ bearing *E. coli* isolates. All the antibiotic resistance genes are shown. The *bla*$_{NDM-5}$ genes were located on plasmid of four different IncF groups and IncX3 group. The *bla*$_{NDM-5}$ -encoding IncF-type plasmids harbored commonly *aadA2/aadA5*, *sul1*and *dfrA12*, and in addition, some of them contained more or less other genes.

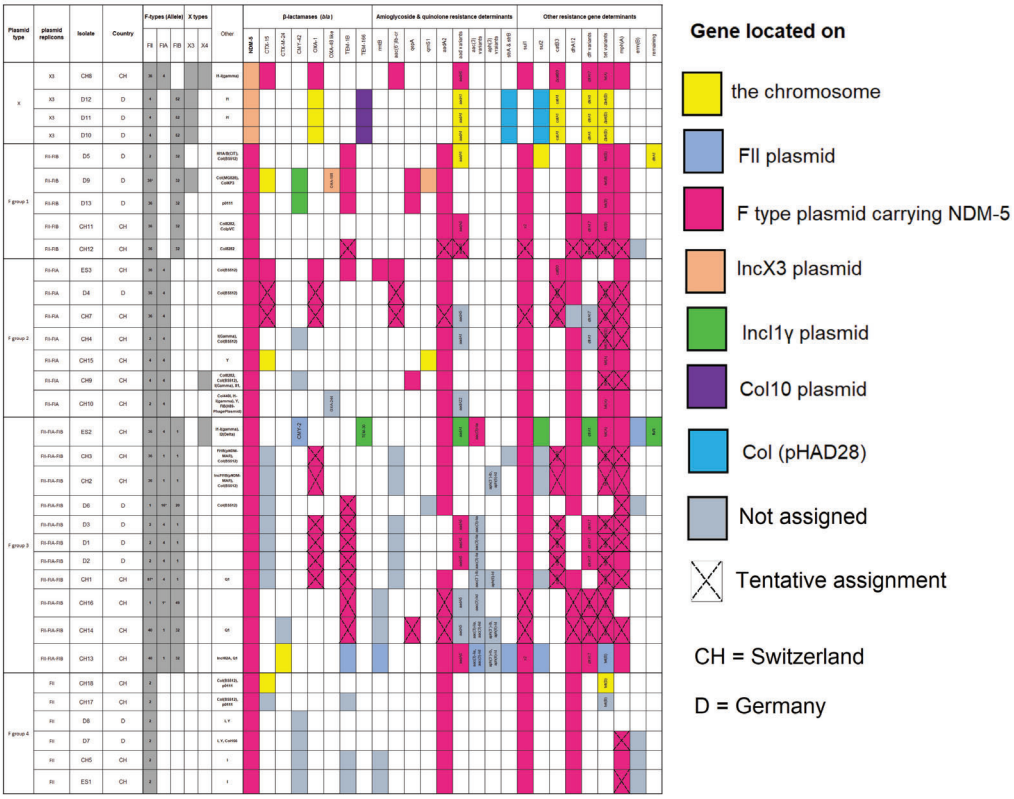

Table 3. Characteristics of chromosome and plasmids of eight NDM-5 producing *E. coli* clinical isolates. *

Sample name	Sample name	Size (bp)	MLST	*fimH*-Type	Serotype	pMLST	Virulence genes	Antibiotic Resistance gene(s)
D12	Chromosome	4,949,001	940	-	:H5		*gad, IpfA, capU*	*aadA1, catA1, tet (B), dfrA1*
	F-like plasmid	134,348				IncF [F4:A-:B52]	-	-
	IncX3- plasmid	45,232					-	bla_{NDM-5}
	IncI1-I (Gamma) [plasmidR64-like]	87,488					-	-
	ColE10	15,077					-	bla_{TEM166}
	Col (pHAD28)	6,200					-	*strA, strB, sul2*
D5	Chromosome	4,674,817	10	H54	O9:H9		*gad*	*aadA1, dfrA1, sul2*
	F-type plasmid	105,195				IncF[F2:A-:B32*]	-	bla_{NDM-5}, bla_{TEM-1}, *aadA2, mph(A), sul1, dfrA12*
	Col (BS512)	2,100					-	-
	FII	93,329					-	-
D13	Chromosome	5,099,687	405	H27	O102:H6		*gad, iha, sat, air*	
	F-like plasmid	127,528				IncF[F36:A-:B32*]	-	bla_{NDM-5}, bla_{TEM-1}, *qepA*, *aadA2, mph(A), sul1, tet (B), dfrA12*
	IncI1-I (Gamma)]plasmidR621a-like]	48,543					-	bla_{CMY42}
	p0111	96,219					-	
D9	Chromosome	4,779,189	2851	H24	O154:H18		*gad, IpfA*	$bla_{CTX-M-15}$
	F-like plasmid	89,595				IncF[F36:A-:B32*]	-	bla_{NDM-5}, bla_{TEM-1}, *gepA*, *aadA2, mph(A), sul1, tet (B), dfrA12*
	IncX3+ColKp3	51,468					-	$bla_{OXA-181}$, *qnrS1*
	IncI1-I (Gamma) [plasmidR621a-like]	26,256					-	bla_{CMY42}
	Col440I	2,213					-	-
	Col (MG828)	1,549					-	-
CH11	Chromosome	5,182,494	648	H27	:H6		*gad, iss, IpfA, eilA, air*	-
	F-like plasmid	124,740				IncF[F36:A-:B32*]	-	bla_{NDM-5}, *aadA2, aadA5, mph(A), sul1, tet (B), dfrA12, dfrA17*
	ColE10	10,122					-	-

	Col440I	4,011					-	-
	Col (MG828)	4,237					-	-
	Col8282	5,601					-	-
CH8	Chromosome	4,947,318	167	-	O89:H5		*gad, iss,capU*	-
	F-like plasmid	125,539				IncF[F36:A4:B-]	-	*aadA5, aac(6´)Ib-cr,* $bla_{CTX-M-15,}$ $bla_{OXA-1,}$ *mph(A), catB3, sul1, tet (A), dfrA17*
	IncX3- plasmid	46,164					-	bla_{NDM-5}
	IncI1-I (Gamma) [plasmidR64-like]	82,754					-	-
	Col (pHAD28)	2,998					-	-
	Col440I	3,684					-	-
	IncX4	33,826					-	-
CH18	Chromosome	5,311,011	405	H29	O102:H6		*gad, iss, eilA, air*	$bla_{CTX-M-15,}$ *tet (B)*
	F-like plasmid	86,176				IncF[F2: A-:B-]	-	bla_{NDM-5}, *aadA2, sul1,dfrA12*
	p0111	71,423					-	-
	Col (BS512)	2,101					-	-
	Col (MG828)	1,552					-	-
	Col156	5,166					-	-
ES3	Chromosome	4,892,070	167	-	O89:H5		*gad, iss,capU*	-
	F-like plasmid	132,483				IncF[F36:A4:B-]	-	bla_{NDM-5}, bla_{TEM-1}, $bla_{OXA-1,}$ $bla_{CTX-M-15,}$ *aadA2, aac(6´)Ib-cr, rmtB, mph(A), catB3, sul1, dfrA12*
	Col440I	3,684					-	-

*, "-" = not present; space = not applicable.

Figure 1

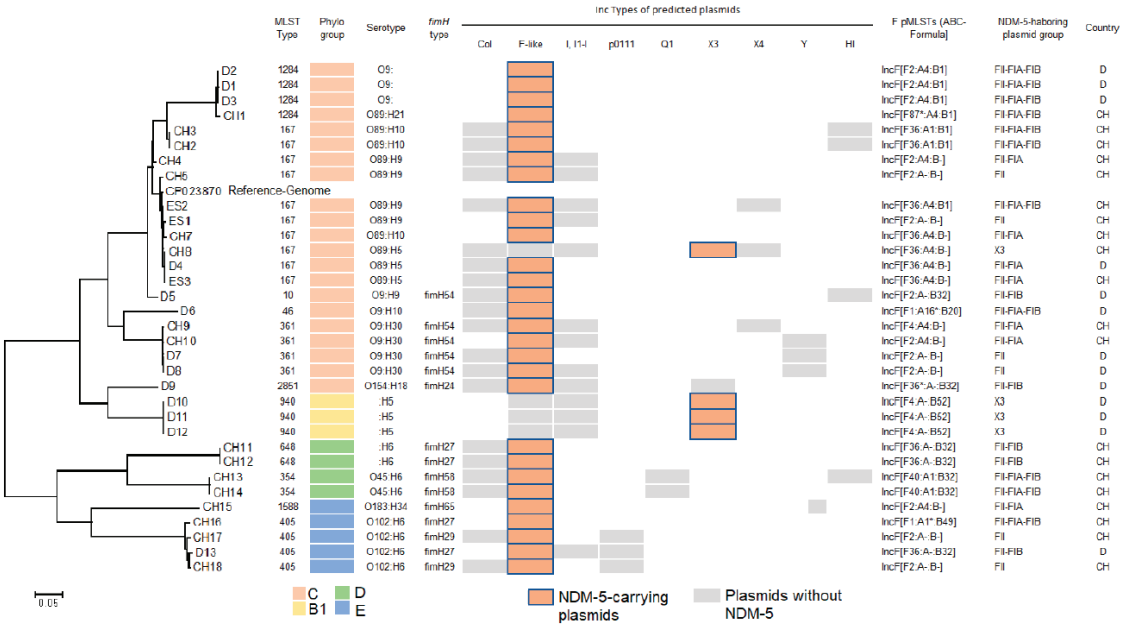

Figure 2

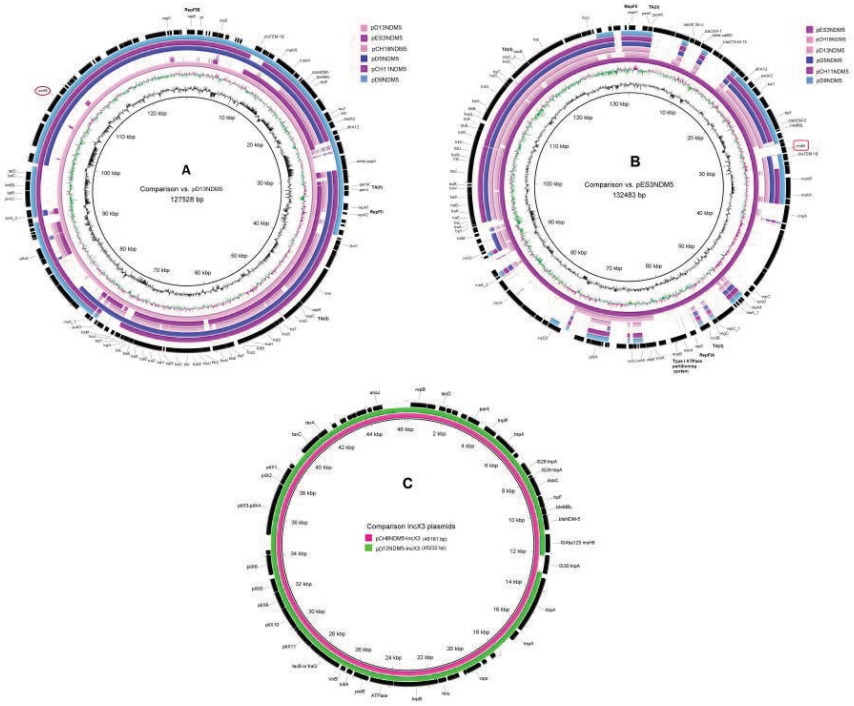

Figure 3