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First whole genome sequence of *Peanalcaligenes suwonensis* bearing *bla*$_{VIM-5}$ Metallo-β-lactamase : a clinical isolate responsible for acute gastroenteritis

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Keywords: Carbapenemase gene, VIM-5, Metallo-β-lactamase (MBL), Whole genome sequence, *Peanalcaligenes suwonensis*, *Alcaligenes faecalis.*

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Abstract

Carbapenemase-producing *Alcaligenes* species has been described in only few studies, with none so far from the African continent. Here, we report the whole genome sequence of *Peanalcaligenes suwonensis* bearing $bla_{\text{VIM-5}}$ metallo-β-lactamase and first detection of carbapenemase producing *Alcaligen*es *faecalis* isolated from patients attending tertiary healthcare facilities in Nigeria. The isolates were identified by MALDI-TOF Mass Spectrometry. Antibiotic susceptibility assay, modified Carba NP test and genomic investigation revealed that two isolates of *Alcaligenes faecalis* and an isolate of *Peanalcaligenes suwonensis* harboured $bla_{\text{VIM-5}}$ gene. The genome sequence analysis of the *P. suwonensis* 191B isolate, responsible for acute gastroenteritis, reveal the presence of 18 antibiotic resistance genes coding for resistance to five different classes of antibiotics. Three of the genes ($bla_{\text{OXA-368}}$, $bla_{\text{CARB-4}}$ and $bla_{\text{VIM-5}}$) codes for resistance to β-lactam antibiotics. To our best knowledge, we describe here the first genome sequence of *P. suwonensis* species and the first detection of class B carbapenemase $bla_{\text{VIM-5}}$ in a clinical isolate of *P. suwonensis* species and *Alcaligenes faecalis* in Nigeria. The finding of this study is of concern, as lateral dissemination of the genes into clinically important Gram-negative pathogens is highly likely.

Introduction

The *Alcaligenaceae* family comprises a large group of related bacteria species characterized by their ability to produce an alkaline reaction in certain medium (Austin, 2013). The most prominent member of the family is *Alcaligenes faecalis,* which are aerobic Gram-negative, non-fermentative, non-encapsulated, oxidase-positive bacterium (Hasan et al., 2019). *Alcaligenes faecalis* is an opportunistic pathogen occasionally implicated in infections in various anatomical sites particularly among the critically ill patients (Brady & Leber, 2018; Tena et al., 2014). Though, often referred to as environment contaminants, clinical infections by *A. faecalis* and even by pan-drug resistant strains are now increasingly reported (Hasan et al., 2019).

Few reports of infections by carbapenemase producing *A. faecalis* and its related species *Achromobacter* (formerly *Alcaligenes*) *xylosoxidans* have been documented in the literature (Kanellopoulou et al., 2004). Verona-integron encoded-metallo-β-lactamase (VIM) is the commonest carbapenemase enzymes in *A. faecalis* (Khajuria et al., 2013; Kreiswirth, 2017; Masseron et al., 2019; Riccio et al., 2001). To the best of our knowledges, only few studies have described bla_{VIM} producing *Alcaligenes* species with none from the African continent so far **(Suppl. Table S1)**. Here, we report the whole genome sequence of *Peanalcaligenes suwonensis* bearing $bla_{\mathrm{VIM\text{-}5}}$ Metallo-β-lactamase and first detection of carbapenemase producing *Alcaligen*es *faecalis* isolated from patients attending tertiary healthcare facilities in Nigeria.

Materials and Methods

Clinical isolates of *A. faecalis* and an isolates of *Peanalcaligenes suwonensis* recovered during diagnostic investigations in the microbiological laboratories of the two

hospitals were collected between the months of January and July, 2019. The isolates were preserved and sent to Microbial Evolution and Phylogeny Infection, Institute Hospital University (IHU), Marseille, France for further characterization. The isolates were subcultured and purified, following the standard laboratory techniques. Well purified colonies of the isolates were identified by Matrix Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) according to the protocol described by the manufacturer (Bruker Daltonics GmbH, Germany). Antibiotic susceptibility test was then carried out on the isolates using the modified Kirby-Bauer disc diffusion method as outlined in the 2019 EUCAST guidelines (EUCAST, 2019). The third generation cephalosporin and carbapenem resistant isolates were tested phenotypically for ESBL and carbapenemase production by the double-disk synergy and modified Carba NP tests, respectively (Bakour et al., 2015; Olowo-okere, 2018). The genomic DNA of the isolates was extracted and purified using the EZ1 Advanced XL robot (Qiagen BioRobot EZ1, Tokyo, Japan), following instructions of the manufacturer. The isolates were screened for the presence of genes encoding ESBLs

($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}) and carbapenemases ($bla_{\text{OXA-48}}$ like, bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{KPC}) using the real-time PCR as previously described (Lalaoui et al., 2018). Among the three VIM-5 positive isolates, one of them i.e. *P. suwonensis* 191B isolate which was obtained from a stool sample of a patient with acute gastroenteritis, for which no genome of this bacterial species is available in public sequence databases, was subjected to whole genome sequencing using Illumina MiSeq technology (Illumina, San Diego, CA, USA). The raw sequence was assembled using the Spades software and annotated by the Prokka bacterial genome annotation pipeline (Bankevich et al., 2012; Seemann, 2014). The Plasmid incompatibility (*Inc*) groups and acquired antibiotic resistance genes were determined using the PlasmidFinder 2.0 platform and ARG-ANNOT, respectively (Carattoli et al., 2014; Gupta

et al., 2014).

Result and Discussion

A total of 19 *A. faecalis* and one *P. suwonensis* isolates were obtained among the multidrug resistant (MDR) clinical isolates collected between January and July 2019. The antibiotics susceptibility testing results revealed that 3 (15 %) of the isolates were resistant to almost all tested antibiotics including to ertapenem and imipenem, but remained susceptible to amikacin, gentamycin, fosfomycin, nitrofurantoin, and colistin (**Table 1**). Phenotypic modified CarbaNP test showed that the three strains were carbapenemase producers. Real-time polymerase chain reaction (RT-PCR) and standard PCR for the detection of ESBL (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) and carbapenemase genes (bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48}, bla_{OXA-58}, bla_{NDM}, bla_{IMP}, and bla_{VIM}) revealed that the three isolates harboured only bla_{VIM-5} gene. The sequenced genome of *P. suwonensis* was estimated to be 3'532'314-bp and assembled into 28 contigs with an average length of 126'521-bp and %GC content of 50.40 %. One complete and circular plasmid (named p191B) was identified with a size of 10'381-bp and 57.80%GC content. Genome annotation of the *P. suwonensis* 191B chromosome with Prokka pipeline resulted to 3'447 genes, 3'372 coding sequences (CDS), 2 rRNA operons, and 49 tRNA sequences. As shown on **Figure 1**, whole-proteome based phylogenetic tree analysis reveals that *P. suwonensis* 191B has a genome highly different with all compared genomes. The two *Peanalcaligenes* species (*suwonensis* and *hominis*), exhibiting the lowest genome size and %CG content, appear also highly distant and share only 68.54% of proteome similarity (**fig. 1**). BlastN analysis of three housekeeping genes of *P. suwonensis* 191B including *rpoB*, 16S rRNA, and 23S rRNA genes reveals respectively 81.68%, 96.61%, and 94.98% identity with best hit genomes including *A. faecalis* BDB4, *A. faecalis* AN70, and *P. hominis* strain 15S00501. As presented in **Table 2**, resistome analysis of the genome sequences reveals a

total of 18 antimicrobial resistance genes including 3 resistance genes to β-lactams (bla_{OXA368}, bla_{CARB-4}, and bla_{VIM-5}), 4 aminoglycoside resistance genes (*aadA*14, *ant*(2")-Ia, *aph*(3)Ib, and *aph*(6)-Id), 3 resistance genes to trimethoprim/sulfamethoxazole (*sul*1, *sul*2/*folP*, *dfrA*22, and *dfrE*), a *ermD* macrolide resistance gene, 3 tetracyclines resistance genes (*tetA*, *tetG*, and *tetR*), and 3 multidrug efflux pumps (*cat3*, *mexI*, and *mdtB*). Some of these resistance determinants were located on the identified plasmid p191B (**fig. 2A**). Interestingly, as shown on **Figure 2B**, the genetic environment analysis of the bla_{VIM-5} gene shows that the gene is located on an integron inserted into a chromosomal hotspot (*orfX* gene) in which other described resistance genes in different bacterial species (e.g. *Shigella sonnei*, *Citrobacter freundii*, and *Escherichia coli*) are observed.

Conclusion

To our best knowledge, we describe here the first genome sequence of *P. suwonensis* species and the first detection of class B carbapenemase bla_{VIM-5} in a clinical isolate of *P. suwonensis* species. As compared to the literature, very few studies have reported *Alcaligenes* species-producing carbapenemases. Indeed, as shown on **suppl. Table S1**, to the best of our knowledge, only bla_{VIM-1}, bla_{VIM-4}, bla_{VIM-6}, and bla_{NDM} genes have been reported in *Alcaligenes* species so far. Thus, our finding is of concern, as lateral dissemination of the genes into clinically important Gram-negative pathogens is highly likely. It therefore highlights the need to improve laboratory capacity for surveillance and prevention of infection by MDR bacteria in our hospitals.

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Conflict of interest:

We declare no conflicts of interest.

Whole Genome sequence accession:

The whole genome sequences (WGS) of the *Peanalcaligenes suwonensis* 191B isolate have been deposited in the GenBank database under accession numbers: JAANPV000000000.

Figure legends

Figure 1: Whole-proteome based phylogenetic tree of *Peanalcaligenes suwonensis* 191B and pairwise comparison with closely related species.

Figure 2: (A): Circular representation of the *P. suwonensis* plasmid p191B and its comparison with most similar plasmids from the NCBI database. The figure is drew using the "CGView Comparison Tool". From the outside to the inside: ring 1 and 2: ORFs in forward and reverse direction; ring 3 to 9: the 7 compared genome sequences ; ring 10 and 11 : the %GC content and GC skew, respectively, of the reference plasmid. **(B):** Genetic environment of the $bla_{\text{VIM-5}}$ of *P. suwonensis* 191B isolate compared to other genomic sequences from NCBI. The $bla_{\text{VIM-5}}$ appears located into an integron integrated through a hotspot integration site (*ofrX* gene). The predicted functions of genes shown on the figure are given on the suppl. Table S2.

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212 **Table 1:** Antibiotic susceptibility testing of isolated *Alcaligenes*. spp. strains

Species names	Isolate names	Sources	AMX	AMC	FEP	TZP	KF	CRO	ERT	IMP	AK	CN	FF	F	SXT	CIP	DO	CT	Detected bla_{VIM} gene
P. suwonensis	**191B**	Stool	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S	**R**	S	**R**	**R**	**R**	I	S	bla_{VIM-5}
A. faecalis	455	Urine	**R**	**R**	**R**	**R**	**R**	**R**	**R**	I	S	S	**R**	S	**R**	**R**	I	S	bla_{VIM-5}
A. faecalis	131	Urine	**R**	**R**	**R**	**R**	**R**	**R**	**R**	I	S	**R**	**R**	S	**R**	**R**	S	S	bla_{VIM-5}
A. faecalis	11	Urine	**R**	**R**	**R**	S	**R**	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	I	S	-
A. faecalis	132	Urine	**R**	**R**	**R**	S	**R**	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	I	S	-
A. faecalis	134	Wound swab	**R**	**R**	**R**	S	**R**	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	I	S	-
A. faecalis	318	Wound swab	**R**	**R**	**R**	S	**R**	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	S	S	-
A. faecalis	203	Stool	**R**	**R**	**R**	S	**R**	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	I	S	-
A. faecalis	305	Stool	**R**	**R**	**R**	S	I	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	I	S	-
A. faecalis	157	Urine	**R**	**R**	**R**	S	I	**R**	S	S	S	**R**	**R**	**R**	**R**	**R**	I	S	-
A. faecalis	250	Urine	**R**	**R**	**R**	S	I	**R**	S	S	S	**R**	**R**	S	**R**	**R**	I	S	-
A. faecalis	483	Stool	**R**	**R**	**R**	S	I	**R**	S	S	S	**R**	**R**	S	**R**	**R**	I	S	-
A. faecalis	82	Stool	**R**	**R**	**R**	S	I	**R**	S	S	S	**R**	**R**	S	**R**	**R**	S	S	-
A. faecalis	55B	Stool	**R**	S	S	S	**R**	**R**	S	S	S	S	**R**	**R**	**R**	S	**R**	S	-
A. faecalis	77	Urine	S	S	S	S	**R**	**R**	S	S	S	S	S	**R**	S	**R**	S	S	-
A. faecalis	106	Wound swab	**R**	S	S	S	S	S	S	S	S	S	**R**	**R**	**R**	S	S	S	-
A. faecalis	230	Sputum	**R**	S	S	S	S	S	S	S	S	S	S	**R**	**R**	**R**	I	S	-
A. faecalis	222	Urine	S	S	S	S	I	S	S	S	S	S	**R**	S	**R**	**R**	I	S	-
A. faecalis	68	Stool	I	S	**R**	S	I	S	S	S	S	S	**R**	S	S	S	I	S	-
A. faecalis	178	Wound swab	I	S	**R**	S	I	S	S	S	S	S	**R**	S	S	S	S	S	-

213 AMX, Amoxicillin; AMC, Amoxicillin-clavulanic acid; FEP, Cefepime; TZP, Piperacillin-tazobactam; KF, Cefalotin; CRO, Ceftriaxone; ETP,

214 Ertapenem; IPM, Imipenem; AK, Amikacin; CN, Gentamicin; FF, Fosfomycin; F, Nitrofurantoin ; SXT, Trimethoprim-Sulfamethoxazole; CIP,
215 Ciprofloxacin; DO, Doxycycline; CT, Colistin; R, resistant; S, Susceptible ; I, Intermediary. Susceptibility testing results were
interpreted 216 according to the 2019 EUCAST breakpoints for *Enterobacteriaceae* (there is no breakpoint yet for members of *Alcaligenes* species).

218 **Table 2:** Antibiotic resistance determinants identified in the clinical multidrug resistant *P. suwonensis* 191B isolate

Antibiotic Classes	Gene Name	Location	Predicted Functions	% Nt identity	Accession
β-lactams	*bla*$_{OXA-368}$	Chromosome	Oxacillinase OXA-368	65,14	KT736121.1
	bla$_{CARB-4}$	Chromosome	Carbapenemase CARB-4	100	U14749
	bla$_{VIM-5}$	Chromosome	Metallo-β-lactamase VIM-5	99,88	AY144612
Aminoglycosides	*aadA*14	Chromosome	Aminoglycoside nucleotidyltransferase AaD14	72,66	AJ8847266
	ant(2")-Ia	Chromosome	O-Adenyltransferases (ANT)	99,25	AF078527
	aph(3")-Ib	**Plasmid p191B**	Aminoglycoside phosphotransferase protein	100.00	AF024602
	aph(6)-Id	**Plasmid p191B**	Aminoglycoside phosphotransferase APH(6)-Id	100.00	M28829
Trimethoprim / Sulfamethoxazole	*sul*1	Chromosome	Sulfonamide-resistant dihydropteroate synthase Sul1	100	JF969163
	sul2/folP	**Plasmid p191B**	Sulfonamide-resistant dihydropteroate synthase Sul2	99.86	GQ421466
	*dfrA*22	Chromosome	Dihydrofolate reductase DfrA22	100	HM173356
	dfrE	Chromosome	Dihydrofolate reductase DfrE	65,67	AF028811
Macrolides	*ermD*	**Plasmid p191B**	Erythromycin resistance gene ErmD (rRNA methylase protein)	91,75	CP045008.1
Tetracyclines	*tetA*	Chromosome	Tetracycline-resistance gene TetA	100	AJ517790
	tetG	Chromosome	Tetracycline efflux MFS transporter Tet(G)	64,84	AF133140
	tetR	Chromosome	Tet Repressor proteins TetR	100	HF545434
Multidrug efflux pumps	*catB3*	Chromosome	Chloramphenicol acetyltransferase 3	70,64	NC_019368.1
	mexI	Chromosome	Inner membrane transporter of the efflux complex MexGHI-OpmD, MexI	67,38	NC_002516
	mdtB	Chromosome	MdtABC-TolC efflux complex.	65,78	U00096

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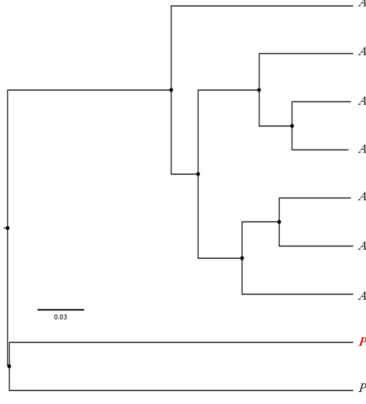
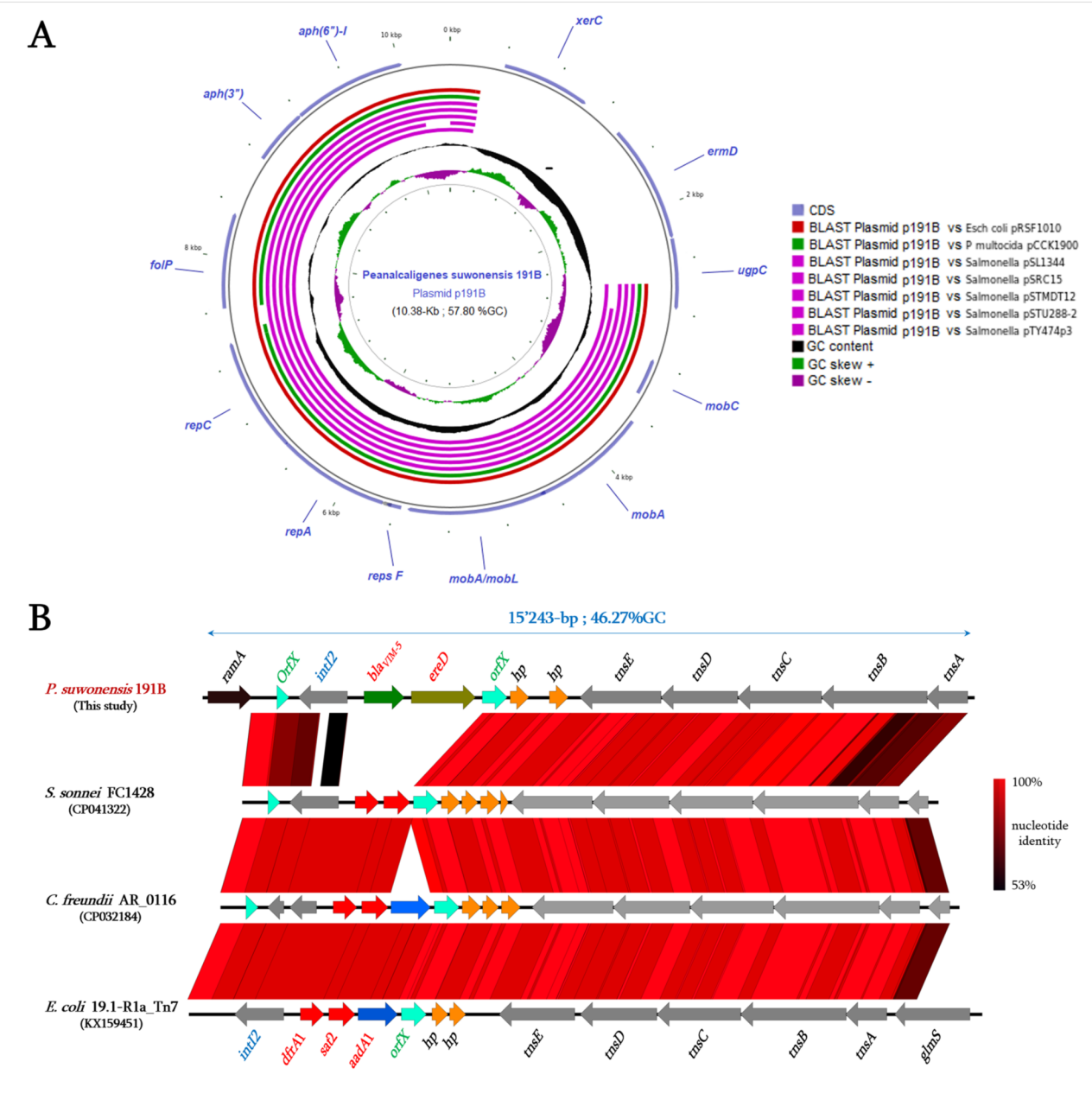


Pairwise comparison of whole proteomes (% Identity)

Bacterial name	Genome size (Mb)	% GC content	*A. faecalis* AN70	*A. aquatilis* QD168	*A. faecalis* JQ135	*A. aquatilis* BU33N	*A. faecalis* ZD02	*A. faecalis* P156	*A. faecalis* AU14	*Peana. suwonensis* 191B	*Peana. hominis* 15S00501
Alcaligenes faecalis AN70	3,92	57,3	--								
Alcaligenes aqualis QD168	4,32	56,3	94,12	--							
Alcaligenes faecalis JQ135	4,07	56,7	94,13	97,82	--						
Alcaligenes aqualis BU33N	3,84	56,1	94,17	97,95	98,41	--					
Alcaligenes faecalis ZD02	4,25	56,81	94,53	95,98	96,06	96,09	--				
Alcaligenes faecalis P156	4,05	56,7	94,5	95,99	96,03	96,12	98	--			
Alcaligenes faecalis UA14	4,2	56,4	94,61	95,85	95,85	95,93	96,95	97,03	--		
Peanalcaligenes suwonensis 191B	**3,53**	**50,4**	**68,87**	**68,8**	**68,78**	**68,79**	**68,89**	**68,88**	**68,84**	--	
Peanalcaligenes hominis 15S00501	2,69	48,4	67,39	67,31	67,32	67,3	67,3	67,31	67,33	**68,54**	--


A
aph(6")-I
10 kbp
0 kbp
xerC
aph(3")
ermD
2 kbp
8 kbp
folP
ugpC
Peanalcaligenes suwonensis 191B
Plasmid p191B
(10.38-Kb ; 57.80 %GC)
mobC
repC
4 kbp
6 kbp
mobA
repA
reps F
mobA/mobL
CDS
BLAST Plasmid p191B vs Esch coli pRSF1010
BLAST Plasmid p191B vs P multocida pCCK1900
BLAST Plasmid p191B vs Salmonella pSL1344
BLAST Plasmid p191B vs Salmonella pSRC15
BLAST Plasmid p191B vs Salmonella pSTMDT12
BLAST Plasmid p191B vs Salmonella pSTU288-2
BLAST Plasmid p191B vs Salmonella pTY474p3
GC content
GC skew +
GC skew -
B
15'243-bp ; 46.27%GC
ramA
OrfX
intI2
blaVIM-5
ereD
orfX
hp
hp
tnsE
tnsD
tnsC
tnsB
tnsA
P. suwonensis 191B
(This study)
S. sonnei FC1428
(CP041322)
C. freundii AR_0116
(CP032184)
E. coli 19.1-R1a_Tn7
(KX159451)
100%
nucleotide identity
53%
intI2
dfrA1
sul2
aadA1
orfX
hp
hp
tnsE
tnsD
tnsC
tnsB
tnsA
glmS