

# **Chapter Ten**

## **Appendices**

## Appendix A

Table A-1 List of tested isolates collected from the UK, India, France and Chile

Strain code	Strain name	Place of isolation
N1	<i>Klebsiella pneumoniae</i>	London
N2	<i>K. pneumoniae</i>	London
N3	<i>Citrobacter freundii</i>	London
N4	<i>Enterobacter cloacae</i>	London
N5	<i>Enterobacter sp</i>	London
N6	<i>Escherichia coli</i>	London
N7	<i>K. pneumoniae</i>	London
N8	<i>K. pneumoniae</i>	London
N9	<i>K. pneumoniae</i>	London
N10	<i>K. pneumoniae</i>	London
N11	<i>K. pneumoniae</i>	London
N12	<i>K. pneumoniae</i>	London
N13	<i>Citrobacter freundii</i>	London
N14	<i>E. coli</i>	London
N15	<i>E. coli</i>	London
N16	<i>K. pneumoniae</i>	London
N17	<i>K. pneumoniae</i>	London
N18	<i>K. pneumoniae</i>	London
N19	<i>K. pneumoniae</i>	London
N20	<i>E. coli</i>	London
N21	<i>K. pneumoniae</i>	London
N22	<i>K. pneumoniae</i>	London
N23	<i>E. coli</i>	London
N24	<i>Acinetobacter baumannii</i>	London
N25	<i>E. coli</i>	London
N26	Enterobacter sp.	London

N27	<i>K. pneumoniae</i>	London
N28	<i>K. pneumoniae</i>	London
N29	<i>E. coli</i>	London
N30	<i>E. coli</i>	London
N31	<i>Enterobacter cloacae</i>	London
N32	<i>Enterobacter cloacae</i>	London
FF 11	<i>K. pneumoniae</i>	Cardiff
FF101	<i>K. pneumoniae</i>	Cardiff
FF153	<i>K. pneumoniae</i>	Cardiff
FF160	<i>K. pneumoniae</i>	Cardiff
FF177	<i>K. pneumoniae</i>	Cardiff
FF197	<i>K. pneumoniae</i>	Cardiff
FF217	<i>K. pneumoniae</i>	Cardiff
FF264	<i>K. pneumoniae</i>	Cardiff
FF267	<i>K. pneumoniae</i>	Cardiff
FF296	<i>K. pneumoniae</i>	Cardiff
9	<i>E. coli</i>	Cardiff
48	<i>E. coli</i>	Cardiff
59	<i>E. coli</i>	Cardiff
60	<i>E. coli</i>	Cardiff
61	<i>E. coli</i>	Cardiff
63	<i>E. coli</i>	Cardiff
98	<i>E. coli</i>	Cardiff
141	<i>E. coli</i>	Cardiff
166	<i>E. coli</i>	Cardiff
284	<i>E. coli</i>	Cardiff
K15	<i>K. pneumoniae</i>	Haryana
K7	<i>K. pneumoniae</i>	Haryana
IR18K	<i>K. pneumoniae</i>	Chennai
IR28K	<i>K. pneumoniae</i>	Chennai

IR25	<i>K. pneumoniae</i>	Chennai
IR61	<i>K. oxytoca</i>	Chennai
IR5	<i>E. coli</i>	Chennai
IR22	<i>E. coli</i>	Chennai
IR26	<i>E. coli</i>	Chennai
IR29	<i>E. coli</i>	Chennai
OE1	<i>K. pneumoniae</i>	India
OE2	<i>K. pneumoniae</i>	India
OE3	<i>K. pneumoniae</i>	India
OE4	<i>K. pneumoniae</i>	India
OE5	<i>K. pneumoniae</i>	India
OE6	<i>K. pneumoniae</i>	India
OE7	<i>K. pneumoniae</i>	India
OE8	<i>K. pneumoniae</i>	India
OE9	<i>K. pneumoniae</i>	India
OE10	<i>K. pneumoniae</i>	India
OE11	<i>K. pneumoniae</i>	India
OE12	<i>K. pneumoniae</i>	India
OE13	<i>K. pneumoniae</i>	India
OE14	<i>K. pneumoniae</i>	India
OE15	<i>K. pneumoniae</i>	India
OE16	<i>K. pneumoniae</i>	India
OE17	<i>K. pneumoniae</i>	India
OE18	<i>K. pneumoniae</i>	India
OE19	<i>K. pneumoniae</i>	India
OE20	<i>K. pneumoniae</i>	India
OE21	<i>K. pneumoniae</i>	India
OE22	<i>K. pneumoniae</i>	India
OE23	<i>K. pneumoniae</i>	India
OE24	<i>E. coli</i>	India

OE25	<i>E. coli</i>	India
OE26	<i>E. coli</i>	India
OE27	<i>E. coli</i>	India
OE28	<i>E. coli</i>	India
OE29	<i>E. coli</i>	India
OE30	<i>E. coli</i>	India
OE31	<i>E. coli</i>	India
OE32	<i>E. coli</i>	India
OE33	<i>E. coli</i>	India
OE34	<i>E. coli</i>	India
OE35	<i>E. coli</i>	India
OE36	<i>E. coli</i>	India
OE37	<i>E. coli</i>	India
OE38	<i>Salmonella</i>	India
OE39	<i>Salmonella</i>	India
OE40	-----	India
OE41	<i>Salmonella</i>	India
OE42	<i>Enterobacter</i>	India
OE43	<i>Enterobacter</i>	India
OE44	<i>Enterobacter</i>	India
OE45	<i>Salmonella</i>	India
OE46	<i>P. stuarti</i>	India
OE47	<i>Salmonella</i>	India
OE48	<i>E. coli</i>	India
301-5473	<i>Pseudomonas aeruginosa</i>	France
43-14926	<i>P. fluorescens</i>	Chile

**Table A-2 the Antibiotic used**

Antibiotic	Manufacturer	Breakpoint	
		S≤	R>
Ceftazidime	<b>Sigma Aldrich</b>	<b>1</b>	<b>4</b>
Meropenem	<b>Sigma Aldrich</b>	<b>2</b>	<b>8</b>

**Table A-3 Heavy Metals**

Heavy metal	Chemical Structure	Manufacturer
Mercury(II) chloride	<b>HgCL2.H2O</b>	<b>Sigma Aldrich</b>
Copper(II) chloride	<b>Cu2.CL2.H2O</b>	<b>Sigma, life science</b>
Silver chloride	<b>AgCL</b>	<b>Sigma Aldrich</b>
Sodium arsenate dibasic heptahydrate	<b>Na2HAsO4.7H2O</b>	<b>Sigma, life science</b>

**Table A.4 PCR primers used**

Primer	Sequence (5'-3')	PCR product size bp	Target	Reference or source
NDM-1 F	GAAGCTGAGCACCCGCATT	800	NDM-1	Sidjabat <i>et al.</i> , 2010
NDM-1 R	GCCGATGAGCCGTCCGCTAC			Sidjabat <i>et al.</i> , 2010
RepA F	GCACAGCAGACCCGCCGA ACT	840	repA	This work
RepA R	TGCTGTTCTGGCGCTGTCTG			This work
RepB F	CGGGGTGGGTTGTCATCGGC	1000	repB	
RepB R	TGCTGCAATTGCCGGTCCGT			
P1	CAGTTGCGGAGCTTTGAAGC	1300	Fr-NDM-1	This work
P2	CGCGTTAGATTGGCTTACAC		Re-NDM-1	This work
merA F	CTGCGCCGGGAAAGTCCGTT	1035	merA	This work
merA R	GCCGATGAGCCGTCCGCTAC			This work
arsA F	CAGTACCGACCCGGCCTCCA	861	arsA	This work
ArsA R	AGGCCGTGTTCACTGCGAGC			This work
pcoA F	CGGCCAGGTTACGTCCGTC	1371	pcoA	This work
PcoA R	TGCCAGTTGCCGCATCCCTG			This work
SilC F	CGTAGCGCAAGCGTGTCTGGA	1090	silC	This work
SilC R	ATATCAGCGGCCCGCAGCAC			This work
PemK-up	AACGAGAATGGCTGGATGC	232	<i>pemK</i>	Minf <i>et al.</i> , 2010
PemK-low	CCAACGACACCGCAAAGC			Minf <i>et al.</i> , 2010
CcdA-up	AGGAAGGGATGGCTGAGGT	230	<i>ccdA</i>	Minf <i>et al.</i> , 2010
CcdB-low	GGTAAAGTTCACGGGAGAC		<i>ccdB</i>	Minf <i>et al.</i> , 2010
RelE-up	AAAAACCCGATGGCGACAG	370	<i>relE</i>	Minf <i>et al.</i> , 2010
RelE-low	TGATAGACCAGGCGAAAAC		relB	Minf <i>et al.</i> , 2010

Primer	Sequence (5`-3`)	PCR product size bp	Target	Reference or source
ParD-up	ACGGACCAGCAGCACCAG	534	<i>parD</i>	Minf <i>et al.</i> , 2010
ParE-low	AGCCCTTGAGCCTGTCGG		<i>parE</i>	Minf <i>et al.</i> , 2010
VagC-up	GGGACCTGGATTTTGATGG	210	<i>vagC</i>	Minf <i>et al.</i> , 2010
VagD-low	GAGCAGATGTTGGTGTCG		<i>vagD</i>	Minf <i>et al.</i> , 2010
Hok-up	AGATAGCCCCGTAGTAAGTT	203	<i>hok</i>	Minf <i>et al.</i> , 2010
Sok-low	GATTTTCGTGTCAGATAAGTG		<i>sok</i>	Minf <i>et al.</i> , 2010
PndC-up	TCAATCAACCAGGGCTCT	140	<i>pndC</i>	Minf <i>et al.</i> , 2010
PndA-low	CCTCACCATCCAGACAAAA		<i>pndA</i>	Minf <i>et al.</i> , 2010
SrnB-up	ACTGATTGTAGCCTCTTCTTT	171	<i>srnB</i>	Minf <i>et al.</i> , 2010
SrnC-low	CACCACTGTATTTCCCCTGT		<i>srnC</i>	Minf <i>et al.</i> , 2010
VAF	GCCTGTTCGGTTCGTAAGCT	3000	<i>Int1</i>	Levesque <i>et el.</i> , 1994
TnicR	GCTCAAGAAGCGTCTCTAGC	2500	<i>qacG</i>	Toleman <i>et al.</i> , 2007
blaVIM2FF	CGGTCTAGACTTGCTCAAGC	3000	<i>Oxa-2</i>	This work
3F-blaOXA-2FF	GGAGGCAATCGTGCGGGCAA	2500	<i>aacA4</i>	This work
M2-aadbR	CGAGCCTGTAGGACTCTATG	1500	<i>AadB &amp; qacG</i>	This work



**Table A-5 PCR Programs Used for Amplification**

NO.	GENE	CYCLES	ANNEALING Temp./Time
1.	PcoA	35	58°C/1 min
2	SilC	35	58°C/1 min
3	MerA	35	58°C/1 min
4	ArsA	35	58°C/1 min
5	Acca4F/qacG	35	58°C/2 min
6	repA	35	56°C/1 min
7	repB	35	56°C/1 min
8	NDM-1	35	60°C/1 min
9	P1	35	60°C/1 min
10	P2	35	60°C/1 min
11	AacA4FF	35	58°C/2 min
12	Pemk	35	54°C/1min
13	CcdA/B	35	54°C/1min
14	relE	35	57°C/1min
15	ParD/E	35	58°C/1min
16	VagC/D	35	53°C/1min
17	Hok/sok	35	54°C/1min
18	PndC/A	35	52°C/1min
19	SrnB/C	35	54°C/1min
20	CTX-M15	35	52°C/1min
21	Inc. typing	35	60°C/1min

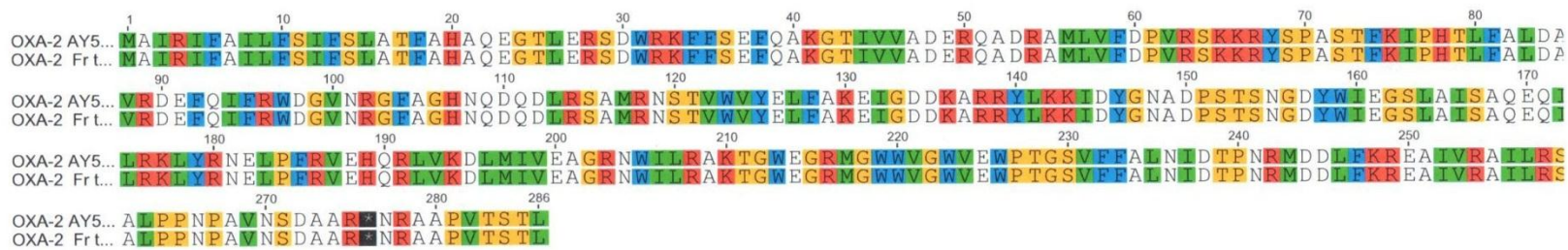
Table A -6 Primers used in PCR-based replicon typing (Carattoli et al., 2005)

Name	DNA sequence	Target site	Amplicons size(bp)
HI1 FW	5`-ggagcgatggattacttcagtac-3`	parA-parB	471
HI1 RW	5`-tgccgtttcacctcgtgagta-3`		
HI2 FW	5`-tttctctgagtcacctgttaacac-3`	iterons	644
HI2 RW	5`-ggctcactaccgttgatcct-3`		
I1 FW	5-cgaaagccggacggcagaa-3`	RNAI	139
I1 RW	5`-tcgtcgttccgccaagttcgt-3`		
X FW	5`-aaccttagaggctatttaagttgctgat-3`	ori <sub>γ</sub>	376
X RW	5`-tgagagtcgaattttatctcatgttttagc-3`		
L/M FW	5`-ggatgaaaactatcagcatctgaag-3`	repA,B,C	785
L/M RW	5`-ctgcaggggcgattctttagg-3`		
N FW	5`-gtctaacgagcttaccgaag-3`	repA	559
N RW	5-gtttcaactctgccaagttc-3`		
FIA FW	5`-ccatgctggttctagagaaggtg-3`	iterons	462
FIA RW	5`-gtatctcttactgcttccgcag-3`		
FIB FW	5`-ggagtctgacacacgattttctg-3`	repA	702
FIB RW	5`-ctcccgtcgttcagggcatt-3`		
W FW	5`-cctaagaacaacaagcccccg-3`	repA	242
W RW	5`-ggtgcgcggcatagaaccgt-3`		
Y FW	5`-aattcaacaacactgtgcagcctg-3`	repA	765
Y RW	5`-gcgagaatggacgattacaaaacttt-3`		
P FW	5`-ctatggcctgcaaacgcgccagaaa-3`	iterons	534
P RW	5`-tcacgcgccagggcgcagcc-3`		
FIC FW	5`-gtgaactggcagatgaggaagg-3`	repA2	262
FIC RW	5`-ttctctcgtcgccaaactagat-3`		
A/C FW	5`-gagaaccaaagacaagacctgga-3`	repA	465
A/C RW	5`-acgacaaacctgaattgcctcctt-3`		
T FW	5`-ttggcctgtttgtgcctaaacct-3`	repA	750
T RW	5`-cgttgattacacttagctttggac-3`		
FIIS FW	5`-ctgtcgtgaagctgatggc-3`	repA	270
FIIS RW	5`-ctctgccacaaacttcagc-3`		
FrepB FW	5`-tgatcgttaaggaatttg-3`	RNAI/repA	270
FrepB RW	5`-gaagatcagtcacaccatcc-3`		
K/B FW	5`-gcggtccggaaagccagaaaac-3`	RNAI	160
K/B RW	5`-tctttcacgagcccgcaaa-3`		
B/O RV	5`-tctgcgttccgccaagttcga-3`	RNAI	159

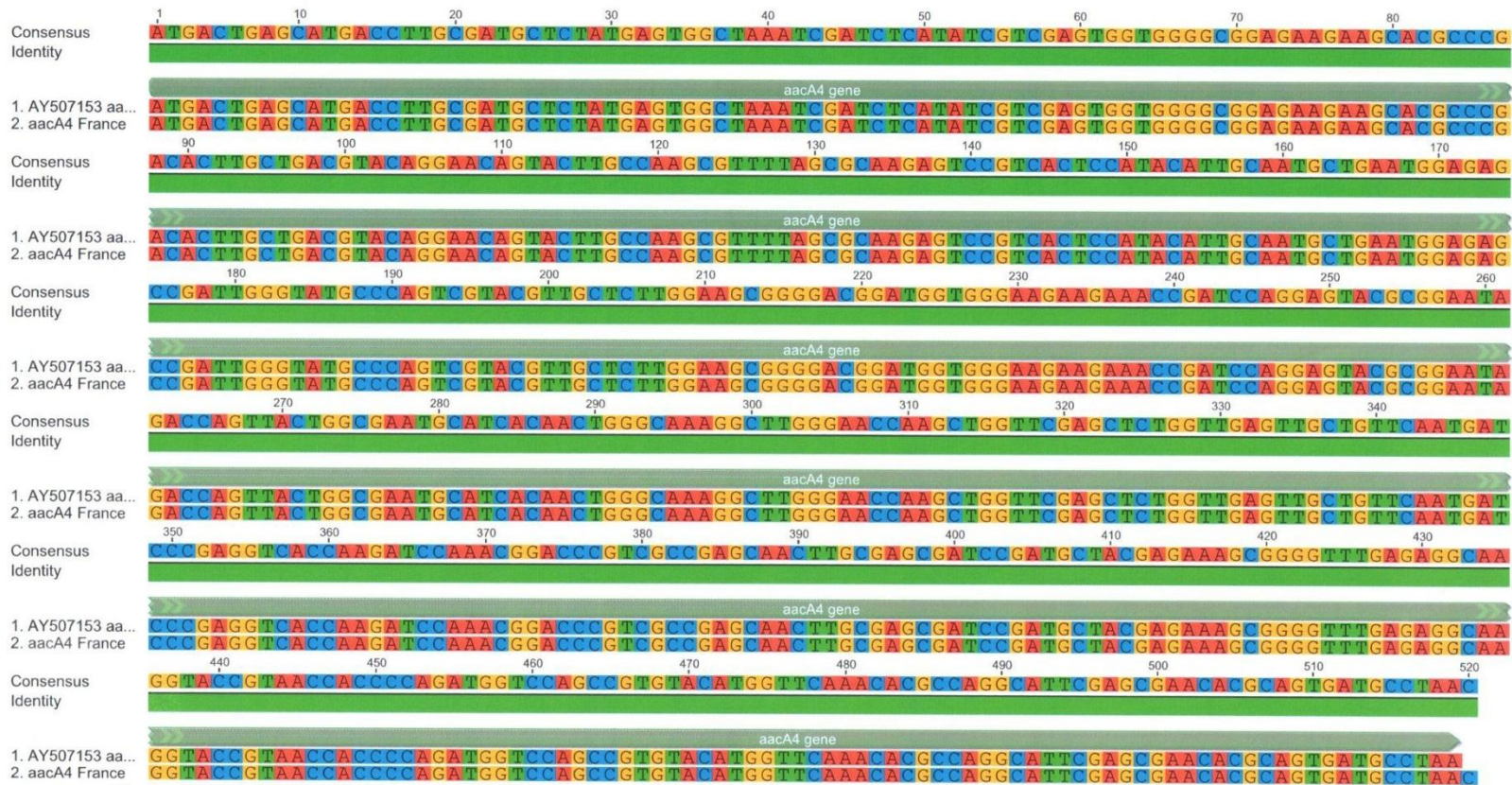
## **Appendix B**



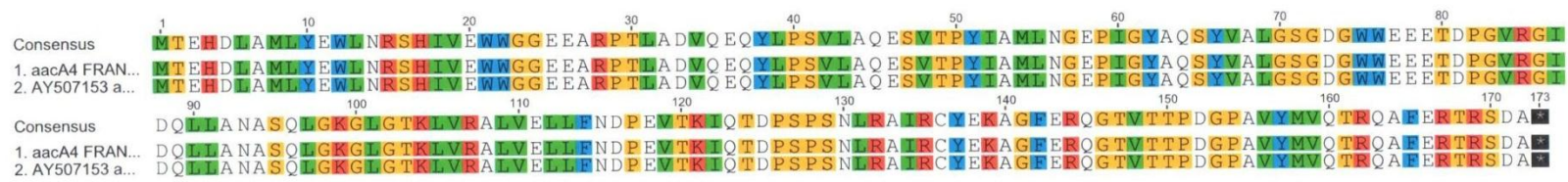
Figure B.1 Nucleotide alignment of *bla*<sub>OXA-2</sub> gene of *P. aeruginosa* (301-5473) to *bla*<sub>OXA-2</sub> gene from *P. aeruginosa* (Accession no. AY507153). Length:828 bp, Identical sites: 828 bp (100% identity).



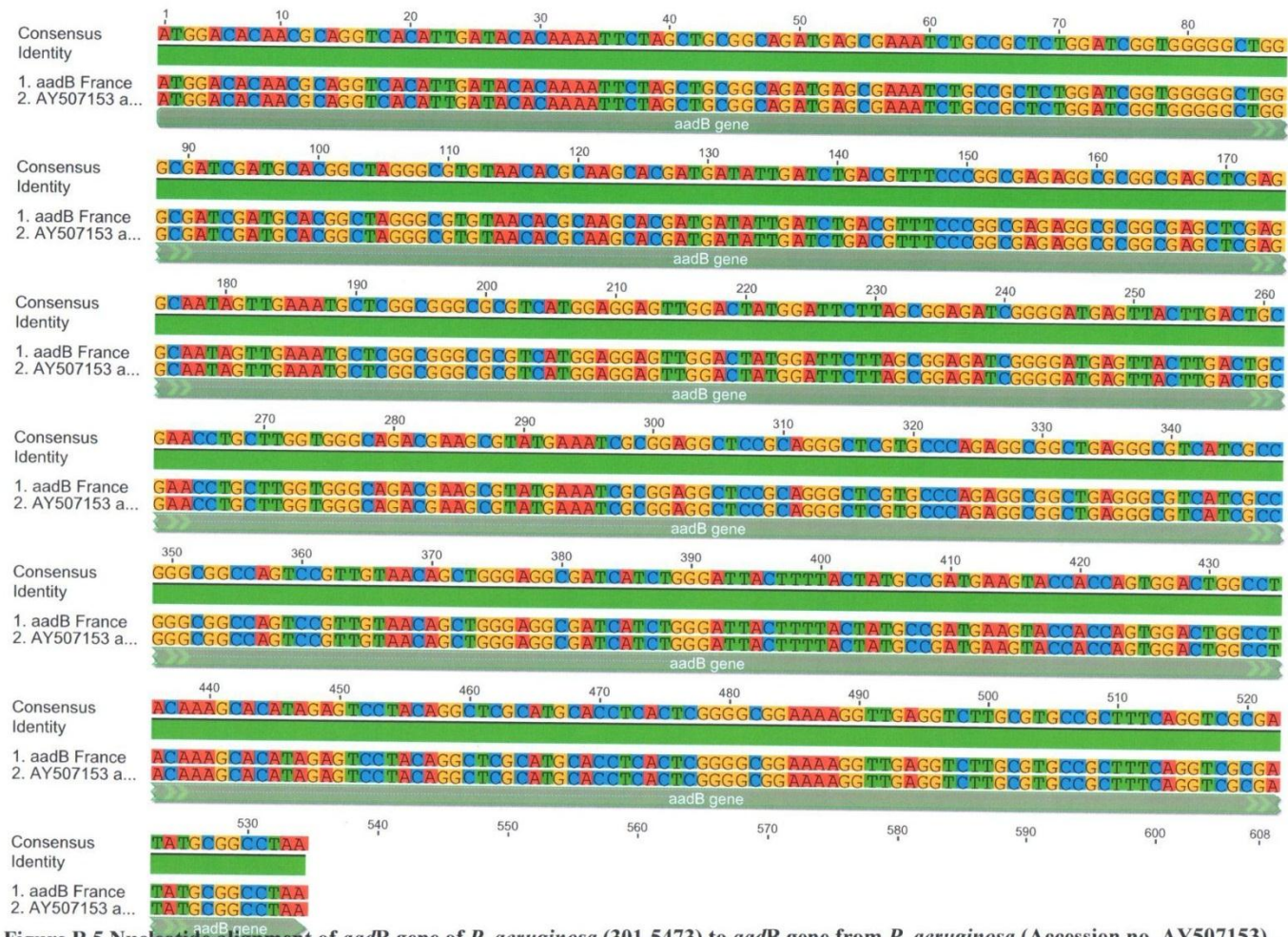
**Figure B.2 Protein alignment of OXA-2 of *P. aeruginosa* (301-5473) to OXA-2 from *P. aeruginosa* (Accession no. AY507153). Length: 286 amino acid, Identical sites: 286 amino acid (100% identity).**



**Figure B.3 Nucleotide alignment of *aacA4* gene of *P. aeruginosa* (301-5473) to *aacA4* gene from *P. aeruginosa* (Accession no. AY507153). Length:519 bp, Identical sites: 519 bp (100% identity).**

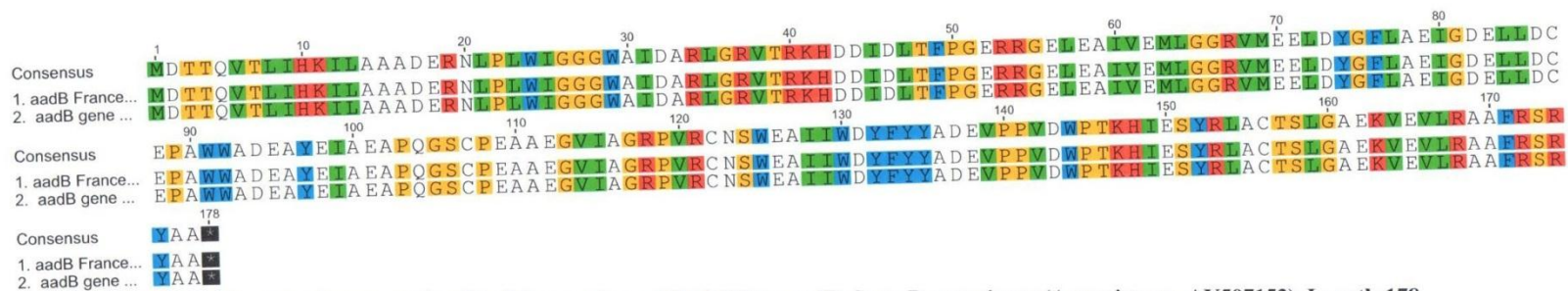


**Figure B.4 Protein alignment of aacA4 of *P. aeruginosa* (301-5473) to aacA4 from *P. aeruginosa* (Accession no. AY507153). Length:173bp, Identical sites: 173 bp (100% identity).**



**Figure B.5 Nucleotide alignment of *aadB* gene of *P. aeruginosa* (301-5473) to *aadB* gene from *P. aeruginosa* (Accession no. AY507153). Length:534 bp, Identical sites: 534 bp (100% identity).**





**Figure B.6 Protein alignment of aadB of *P. aeruginosa* (301-5473) to aadB from *P. aeruginosa* (Accession no. AY507153). Length:178 bp, Identical sites: 178bp (100% identity).**

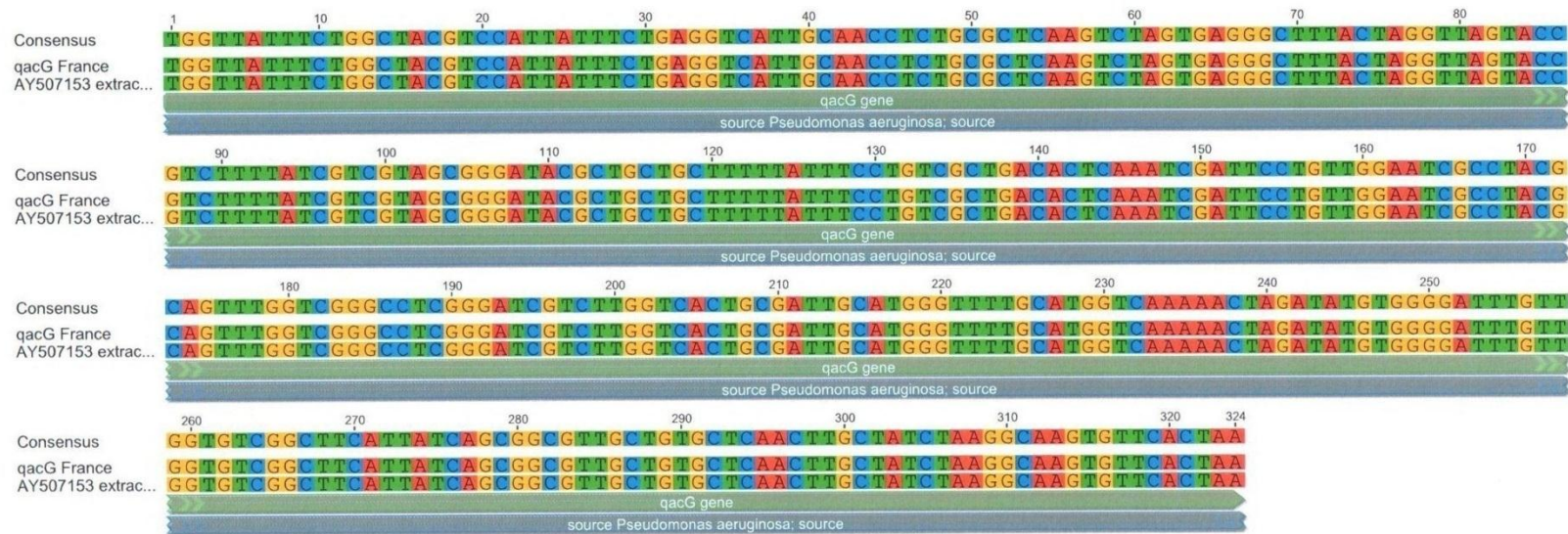


Figure B.7 Nucleotide alignment of *qacG* gene of *P. aeruginosa* (301-5473) to *qacG* gene from *P. aeruginosa* (Accession no. AY507153). Length:324 bp, Identical sites: 342 bp (100% identity).



**Figure B.8 Protein alignment of qacG of *P. aeruginosa* (301-5473) to qacG from *P. aeruginosa* (Accession no. AY507153). Length:108 bp, Identical sites: 180 bp (100% identity).**

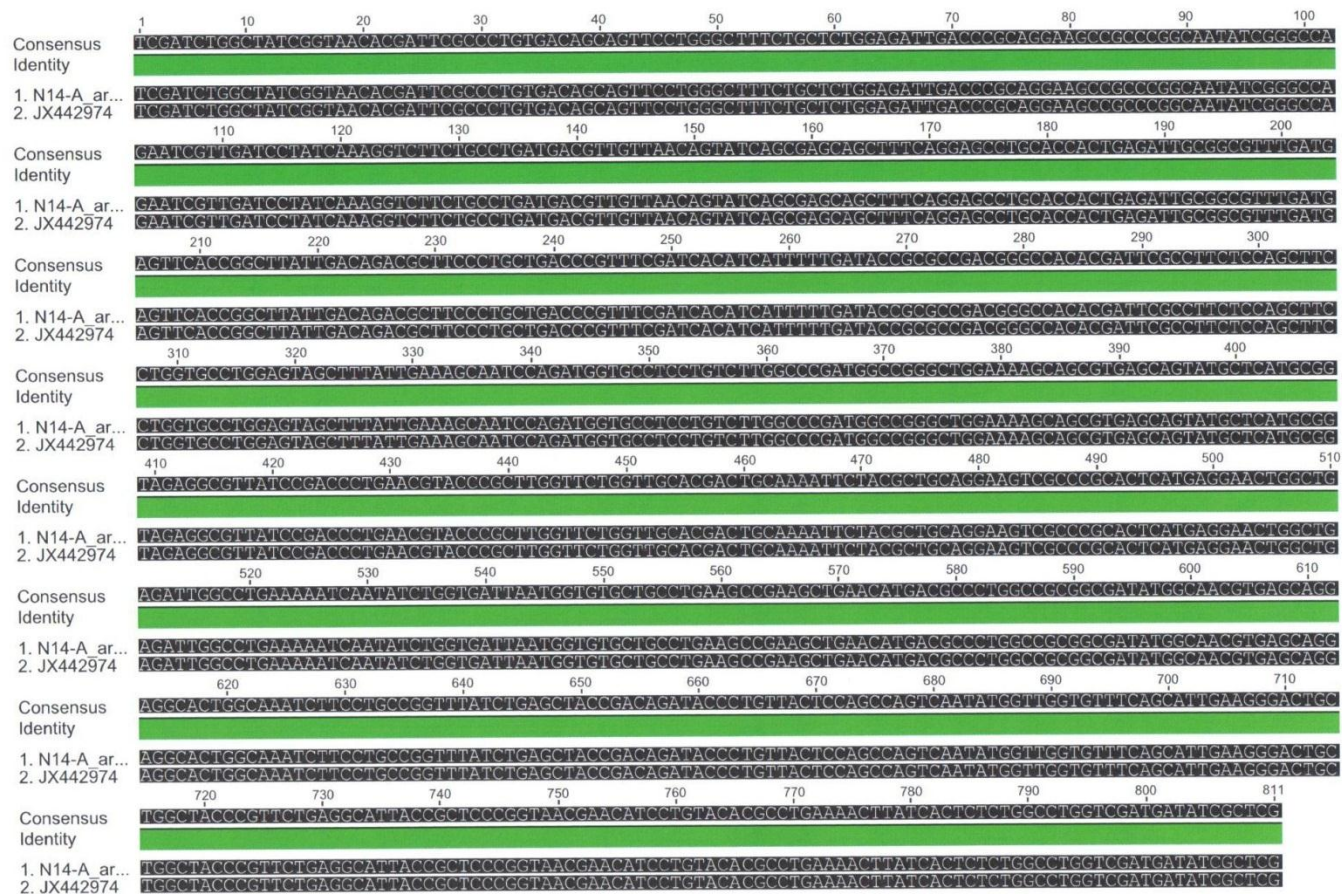
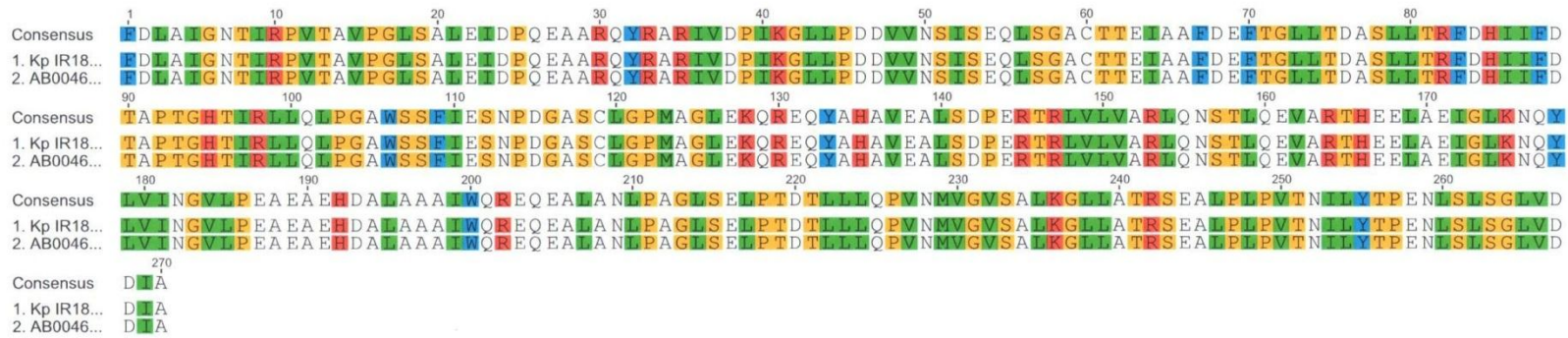


Figure B.9 Nucleotide alignment of *arsA* gene in *E. coli* N14 to *arsA* gene from *K. pneumoniae* plasmid pKN-LS6 (Accession no. JX442974). Length:811 bp, Identical sites: 811 bp (100% identity).

	1	10	20	30	40	50	60	70	80	
Consensus	DLAIGNTIRPVTAVPGLSALEIDPOEAAROYRARIVDPIKGLLPDDVVNSISEOLSGACTTEIAAFDEFTGLLTDASILLTRFDHIFD									
Identity										
1. JX44297...	DLAIGNTIRPVTAVPGLSALEIDPOEAAROYRARIVDPIKGLLPDDVVNSISEOLSGACTTEIAAFDEFTGLLTDASILLTRFDHIFD									
2. N14 arsA...	DLAIGNTIRPVTAVPGLSALEIDPOEAAROYRARIVDPIKGLLPDDVVNSISEOLSGACTTEIAAFDEFTGLLTDASILLTRFDHIFD									
	90	100	110	120	130	140	150	160	170	
Consensus	TAPTGHITIRLLOLPGAWSSFIENPDGASCLGPMAGLEKOREOYAHAVEALSDDPERTRLVIVARLONSTLOEVARTHEELAEITGLKNO									
Identity										
1. JX44297...	TAPTGHITIRLLOLPGAWSSFIENPDGASCLGPMAGLEKOREOYAHAVEALSDDPERTRLVIVARLONSTLOEVARTHEELAEITGLKNO									
2. N14 arsA...	TAPTGHITIRLLOLPGAWSSFIENPDGASCLGPMAGLEKOREOYAHAVEALSDDPERTRLVIVARLONSTLOEVARTHEELAEITGLKNO									
	180	190	200	210	220	230	240	250	260	
Consensus	YLVINGVLPPEAAEHDALAAAIWOREOFALANLPAGLSELPTDTLLLOPVNMVGVSAIKGLLATRSEALPLPVTNILYTPENISISGL									
Identity										
1. JX44297...	YLVINGVLPPEAAEHDALAAAIWOREOFALANLPAGLSELPTDTLLLOPVNMVGVSAIKGLLATRSEALPLPVTNILYTPENISISGL									
2. N14 arsA...	YLVINGVLPPEAAEHDALAAAIWOREOFALANLPAGLSELPTDTLLLOPVNMVGVSAIKGLLATRSEALPLPVTNILYTPENISISGL									
	269									
Consensus	VDDDA									
Identity										
1. JX44297...	VDDDA									
2. N14 arsA...	VDDDA									

**Figure B.10 Protein alignment of arsA in *E. coli* N14 to arsA from *K. pneumoniae* plasmid pKN-LS6 (Accession no. JX442974). Length:269 a.a, Identical sites: 269 a.a (100% identity).**

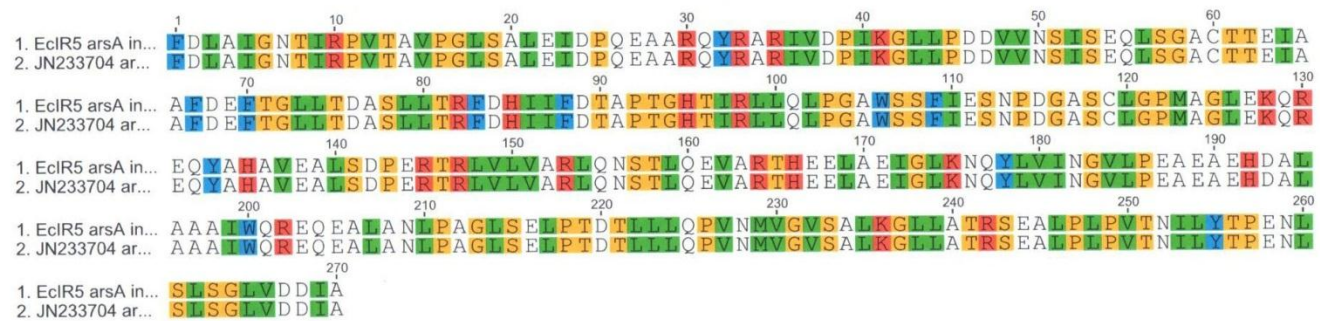




**Figure B.12** Protein alignment of *arsA* in *K. pneumoniae* IR18K to *arsA* from *Acidiphilium multivorum* pkw301 (Accession no. AB004659). Length:270aa, Identical sites: 270aa (100% identity).







**Figure B.14 Protein alignment of arsA in *E. coli* IR5 to arsA from *K. pneumoniae* ST258 (Accession no. JN233704). Length:270aa  
Identical sites: 270 aa (100% identity).**



Figure B.15 Nucleotide alignment of *merA* gene in *K. pneumoniae* E5/14 to *merA* gene from *K. pneumoniae* p KPC- LK30 (Accession no. KC405622.1). Length:992 bp Identical sites: 992 (100% identity).

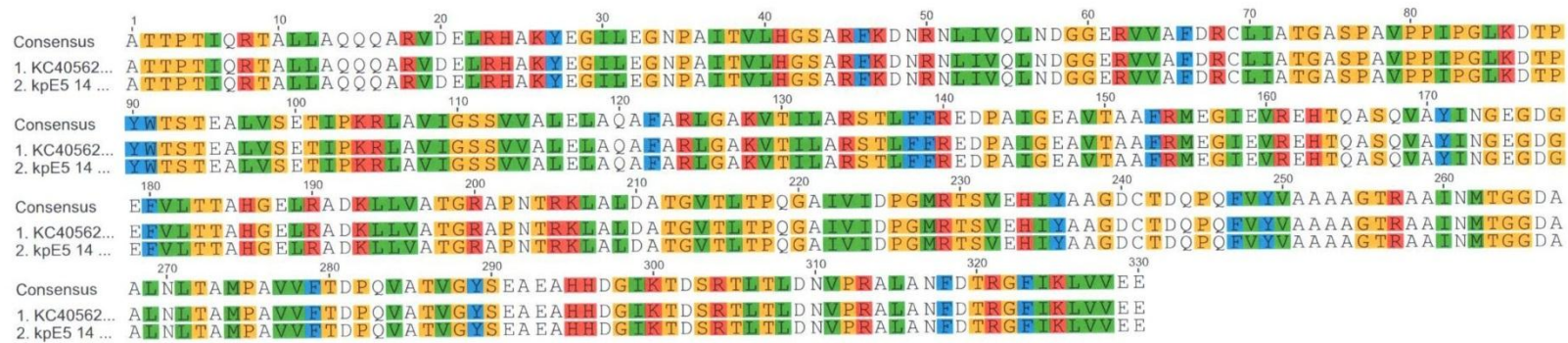


Figure B.16 Protein alignment of merA in *K. pneumoniae* E5/14 to merA from *K. pneumoniae* p KPC- LK30 (Accession no. KC405622.1). Length:330 aa Identical sites: 330 aa (100% identity).

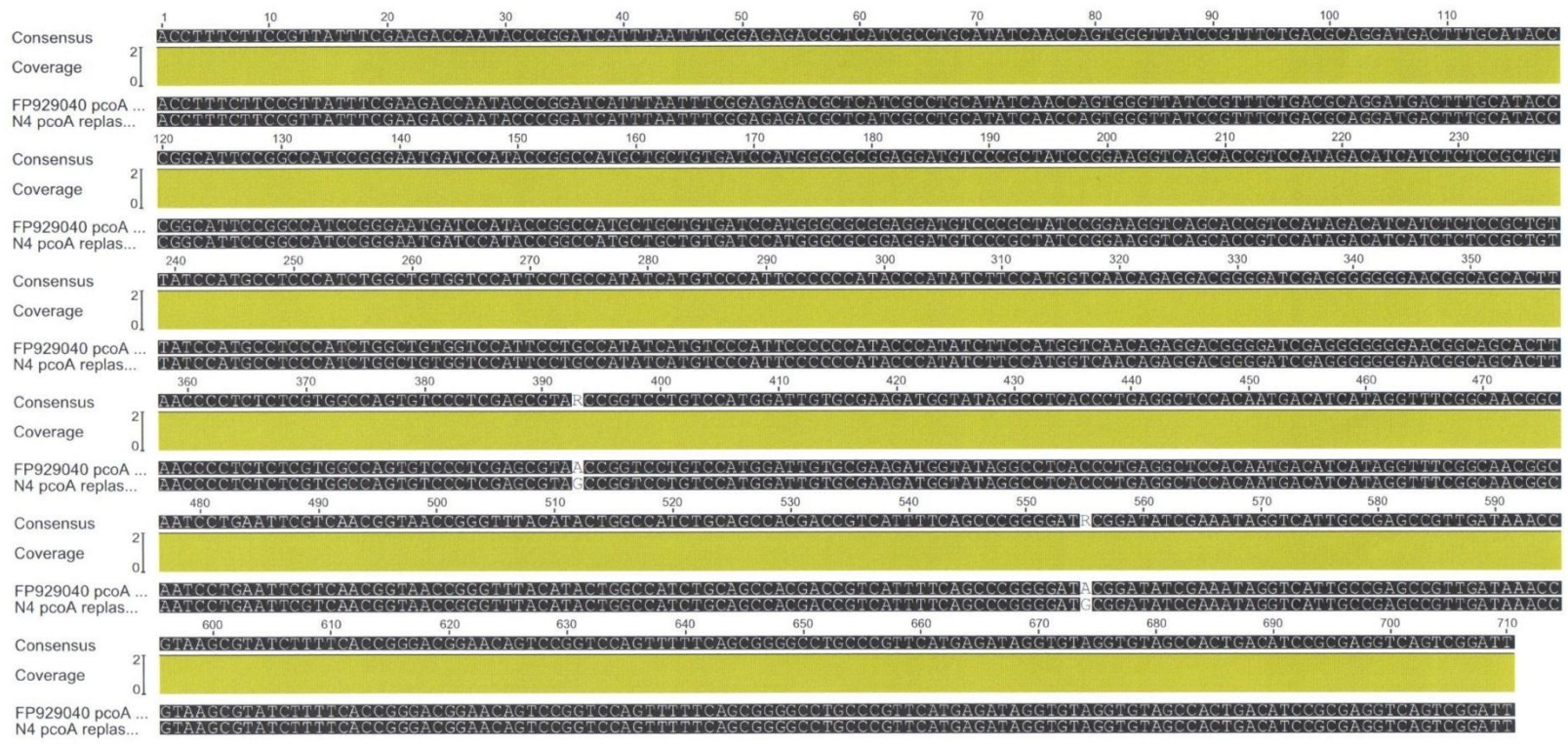


Figure B.17 Nucleotide alignment of *pcoA* gene in *Enterobacter cloacae* N4 to *pcoA* gene from *E. cloacae* NCTC9394 (Accession no. FP929040). Length:710 bp Identical sites: 708 (99.7% identity).



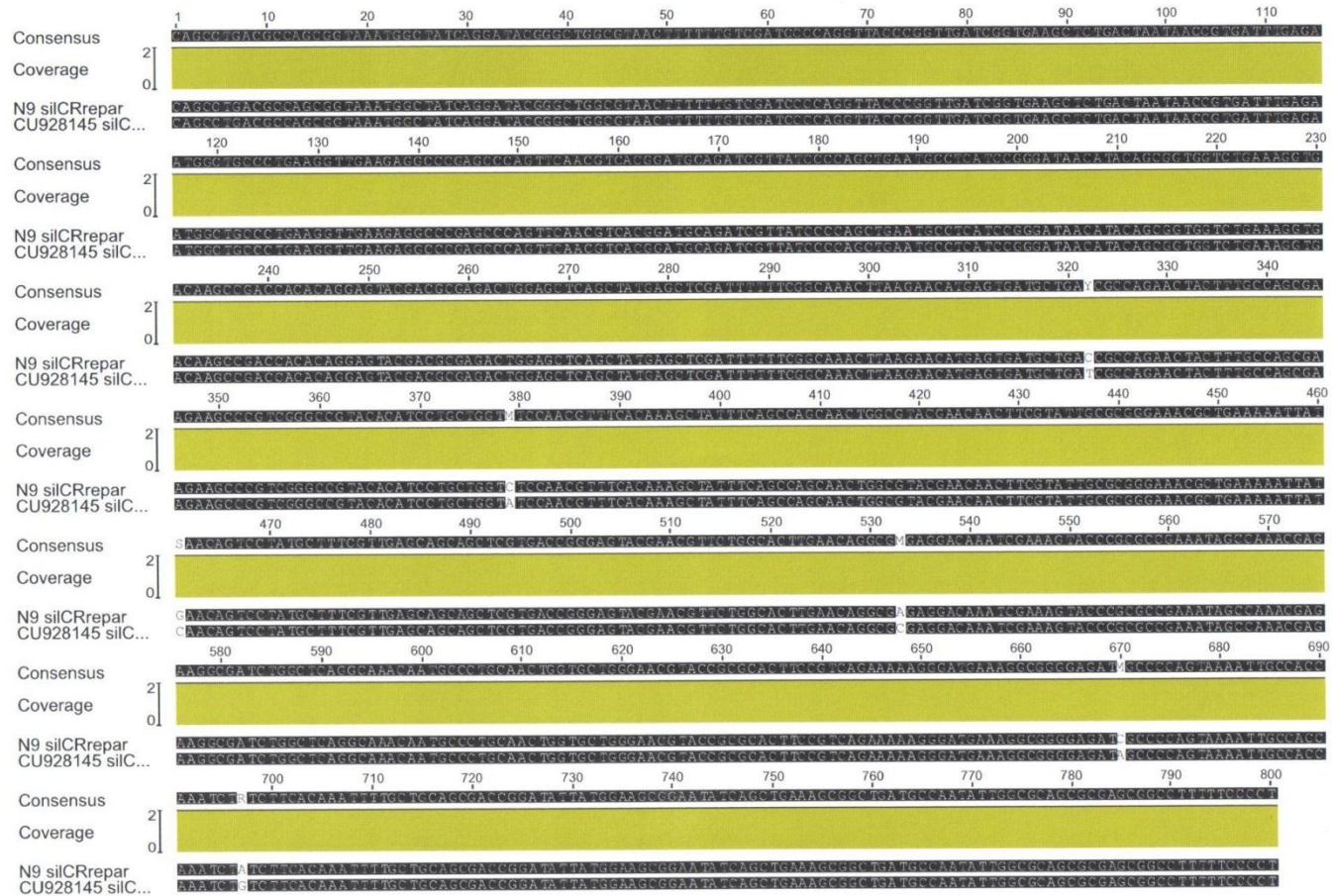


Figure B.19 Nucleotide alignment of *silC* gene in *K. pneumoniae* N9 to *silC* gene from *E. coli* 55989 (Accession no. CU928145). Length:800bp Identical sites: 796 bp(99.3% identity).



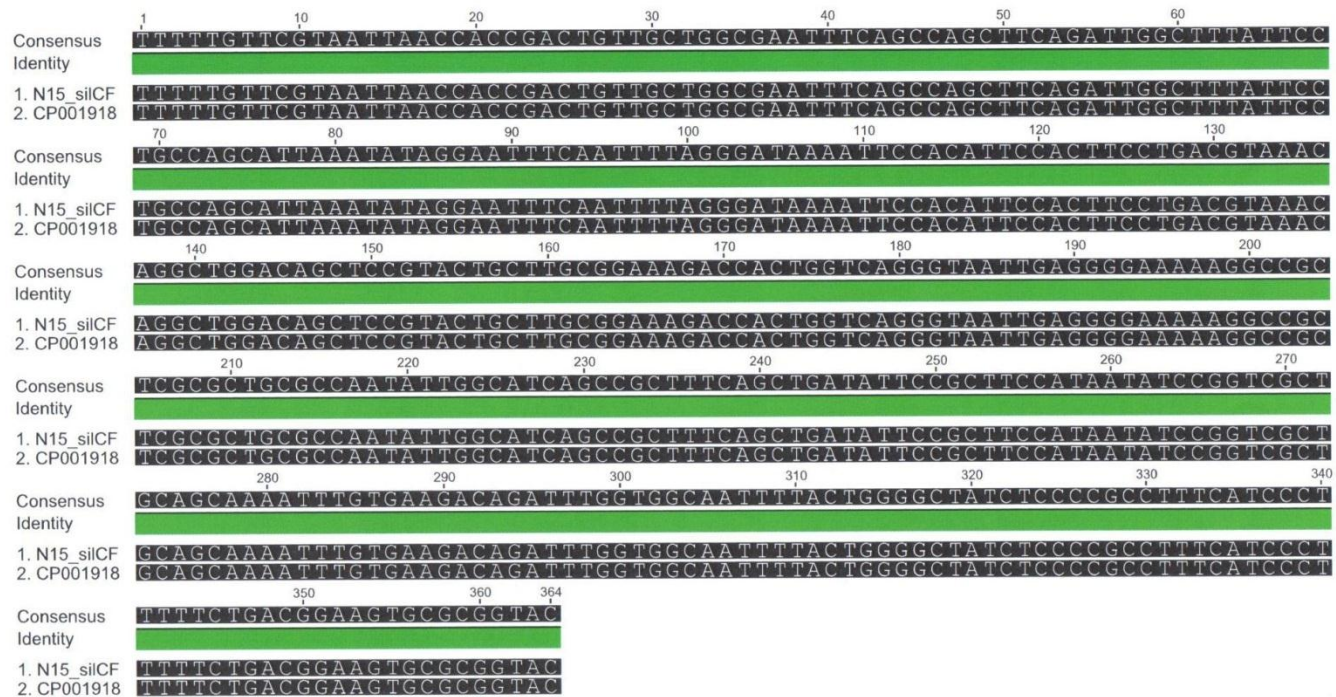
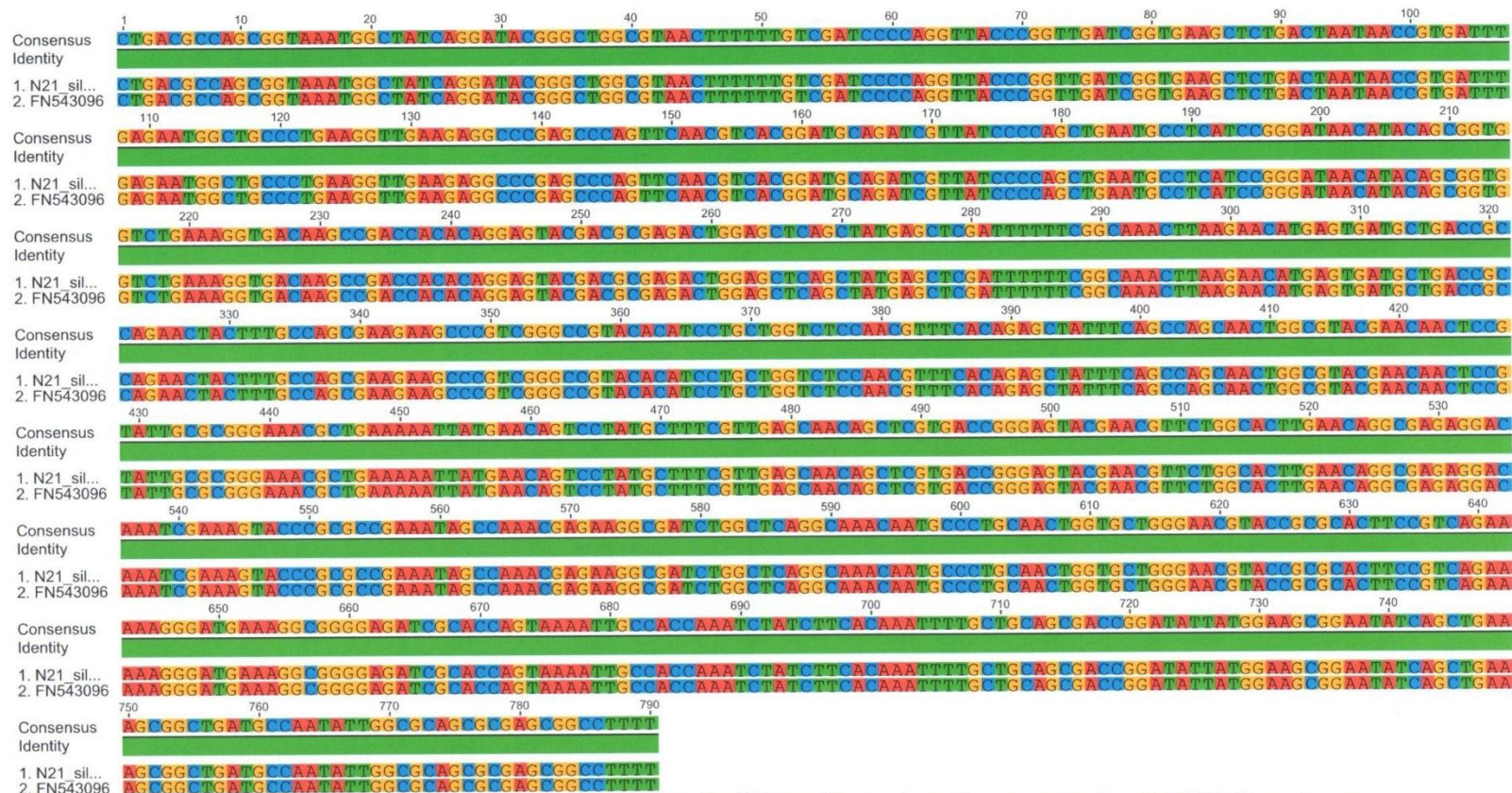


Figure B.21 Nucleotide alignment of *silC* gene in *E. coli* N15 to *silC* gene from *E. cloacae* subsp *cloacae* ATCC13047 (Accession no. CP001918). Length:364bp Identical sites: 364 bp(100% identity).



	1	10	20	30	40	50	60	
Consensus	YRALPSEKGMKGGETAPVKLPPNLSSOILLORPDIMEAEYOLKAADANIGAARA AFFPSITLTSGLSA							
Identity								
1. CP00191...	YRALPSEKGMKGGETAPVKLPPNLSSOILLORPDIMEAEYOLKAADANIGAARA AFFPSITLTSGLSA							
2. N15 silC ...	YRALPSEKGMKGGETAPVKLPPNLSSOILLORPDIMEAEYOLKAADANIGAARA AFFPSITLTSGLSA							
	70	80	90	100	110	121		
Consensus	SSTELSSLEFSGSGMWNEIPKIEIPIFNAGR NKANLKLAEIROOOSVVNYEOK							
Identity								
1. CP00191...	SSTELSSLEFSGSGMWNEIPKIEIPIFNAGR NKANLKLAEIROOOSVVNYEOK							
2. N15 silC ...	SSTELSSLEFSGSGMWNEIPKIEIPIFNAGR NKANLKLAEIROOOSVVNYEOK							

**Figure B.2** Protein alignment of SilC in *E. coli* N15 to SilC from *E. cloacae* subsp *cloacae* ATCC13047 (Accession no. CP001918).  
**Length:**121 a.a **Identical sites:** 121 a.a (100% identity).



**Figure B.23** Nucleotide alignment of *silC* gene in *K. pneumoniae* N21 to *silC* gene from *Cronobacter turicensis* Z3032 (Accession no. FN543096). Length:790 bp, Identical sites: 790 bp (100% identity).

```

1. FN54309...  1  A L K V E E A R A Q T N V T D A D R Y P Q T N A S S G I T Y S G G L K G D K P T T Q E Y D A R L E L S Y E L D F E G K L K N M S D A D R
2. Kp N21 si...  A L K V E E A R A Q T N V T D A D R Y P Q T N A S S G I T Y S G G L K G D K P T T Q E Y D A R L E L S Y E L D F E G K L K N M S D A D R

1. FN54309...  70  Q N Y F A S E E A R R A V H I L L V S N V S Q S Y F S Q Q I A Y E Q L R I A R E T L K N Y E Q S Y A F V E Q Q L V T G S T N V I A L E Q
2. Kp N21 si...  Q N Y F A S E E A R R A V H I L L V S N V S Q S Y F S Q Q I A Y E Q L R I A R E T L K N Y E Q S Y A F V E Q Q L V T G S T N V I A L E Q

1. FN54309...  140  A R G Q L E S T R A E L A K R E G D L A Q A N N A L Q L V L G T Y R A L P S E K G M K G G E I A P V K I P P N L S S Q I L L Q R P D I M
2. Kp N21 si...  A R G Q L E S T R A E L A K R E G D L A Q A N N A L Q L V L G T Y R A L P S E K G M K G G E I A P V K I P P N L S S Q I L L Q R P D I M

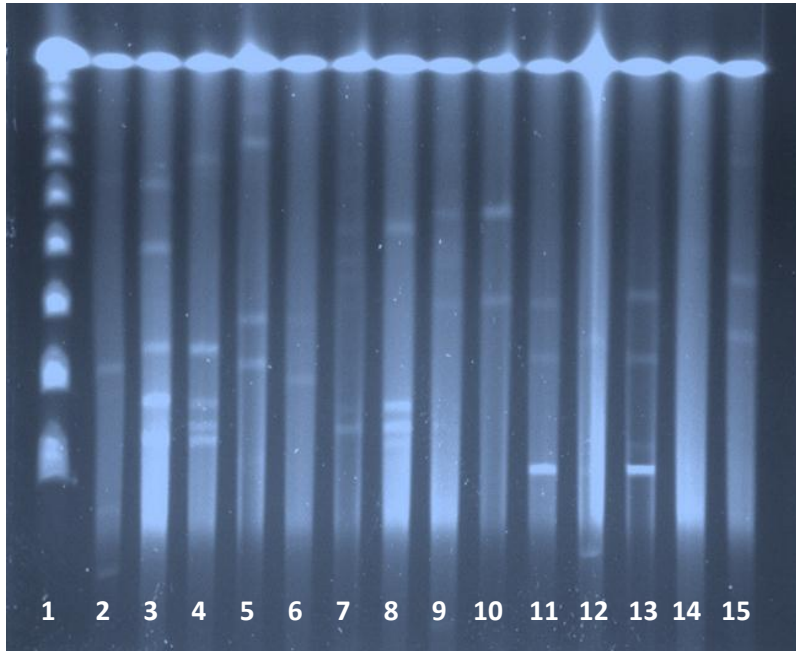
1. FN54309...  210  E A E Y Q L K A A D A N T G A A R A A F
2. Kp N21 si...  E A E Y Q L K A A D A N T G A A R A A F

```

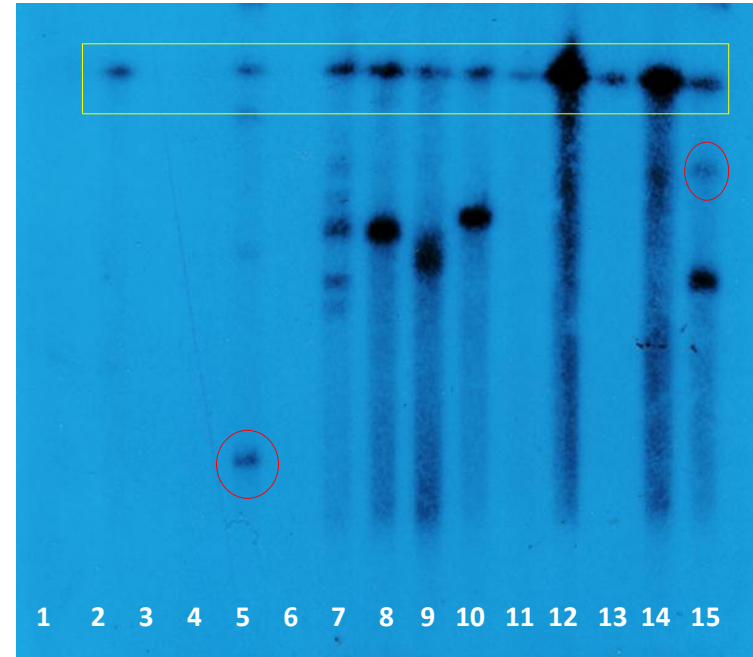
**Figure B.24 Protein alignment of SilC in *K. pneumoniae* N21 to SilC from *Cronobacter turicensis* Z3032 (Accession no. FN543096). Length:225 a.a Identical sites: 225 a.a (100% identity).**

## Appendix C

A

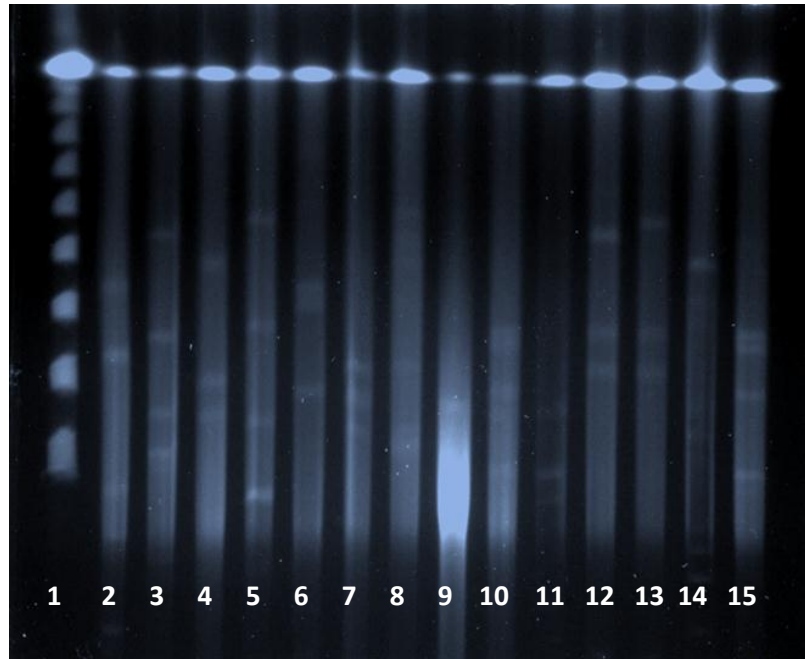


B



**Figure C.1 Pulsed-field gels of genomic DNA of NDM-1 positive strains, isolated from the UK. (A) Plasmid size differentiation by digestion with *S1* nuclease. (B) Hybridization of gel A with *silC* gene probe. Lane 1: Marker. Lane 2,3,8,9,10,11,12= *Klebsiella* spp., lane 4, 14 = *Citrobacter* spp., lane 5,7,13= *E. coli* and lane 6= *Enterobacter* sp.**

A



B

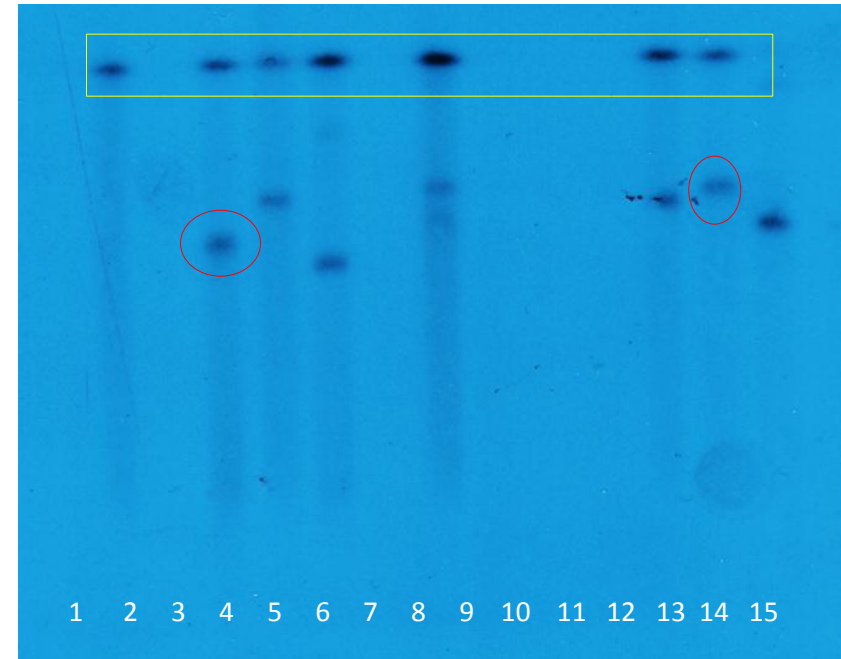
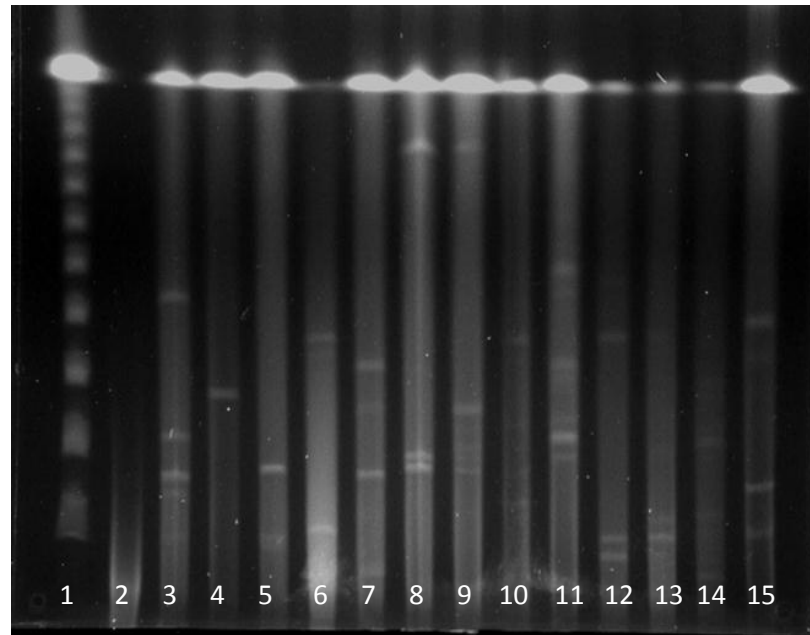
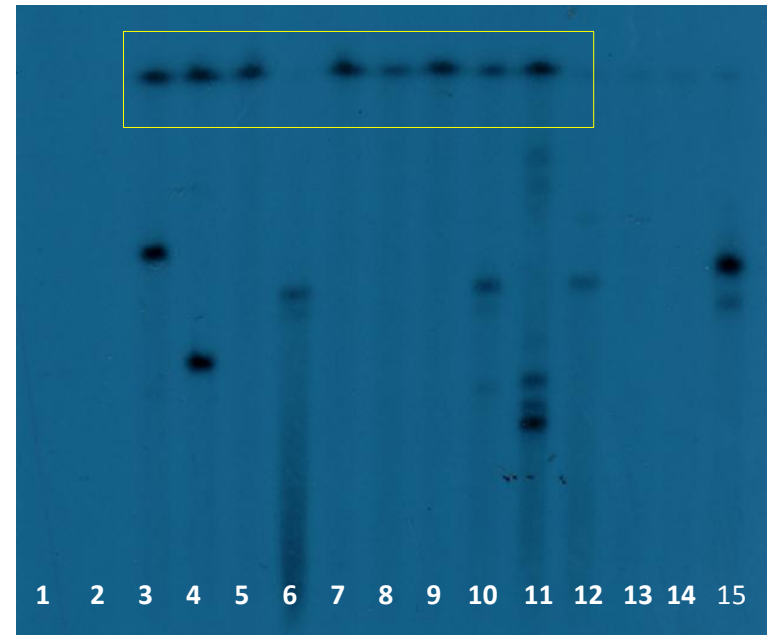


Figure C.2 Pulsed-field gels of genomic DNA of NDM-1 positive strains, isolated from the UK. (A) Plasmid size differentiation by digestion with *S1* nuclease. (B) Hybridization of gel A with *silC* gene probe. Lane 1 = Marker. Lane 2, 3, 5, 6, 9, 10, 11= *Klebsiella* spp., lane 4,7=*E. coli*, lane 12,13 =*Enterobacter* sp, lane 14,15 = unknown strains

A

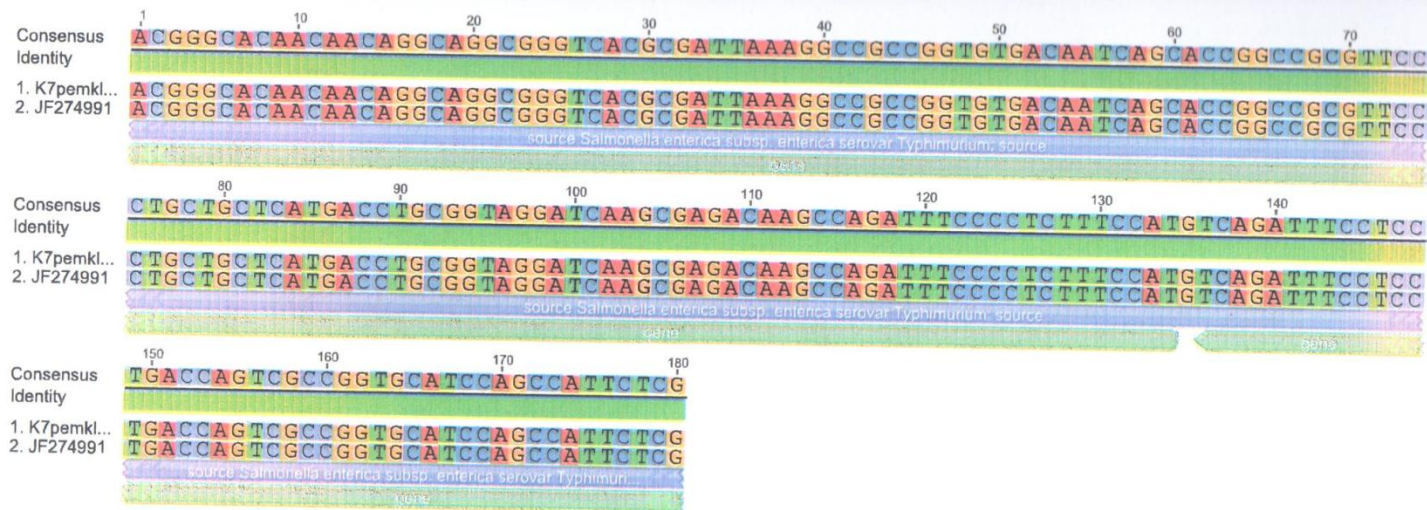


B



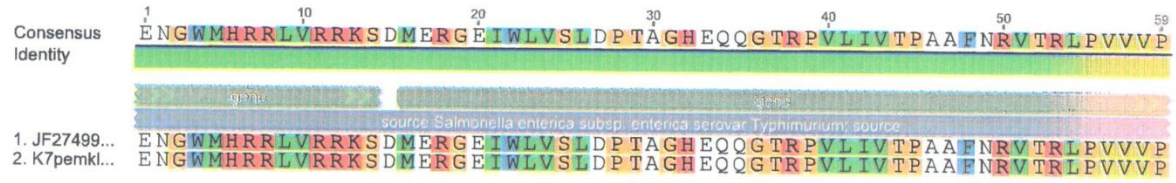
**Figure C.3 Pulsed-field gels of genomic DNA of NDM-1 positive strains, isolated from the UK. (A) Plasmid size differentiation by digestion with *S1* nuclease. (B) Hybridization of gel A with *silC* gene probe. Lane 1 = DNA size marker. Lane 2 to lane 13= *Klebsiella* spp., and lane 14=*K. pneumoniae* K15.**

## Appendix D

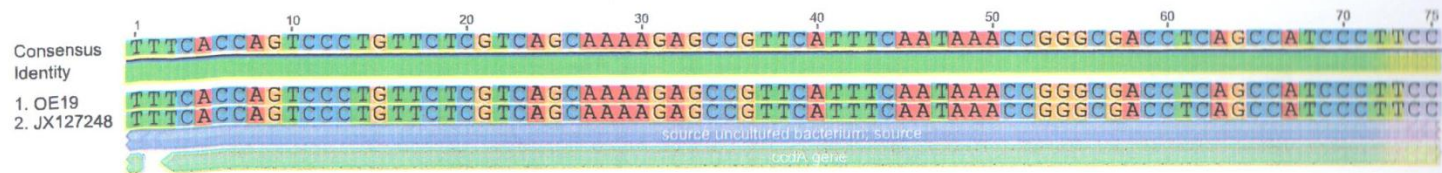


**Figure D.1 Nucleotide Alignment of *pemKI* gene in *K. pneumoniae* N7 to *pemKI* gene in *Salmonella enterica* subsp. *enterica* serovar *typhimurium* (Accession no.JF274991). Length :180 bp, identical site: 180 bp (100%).**

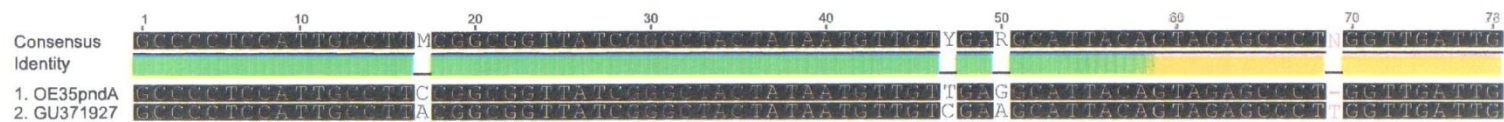




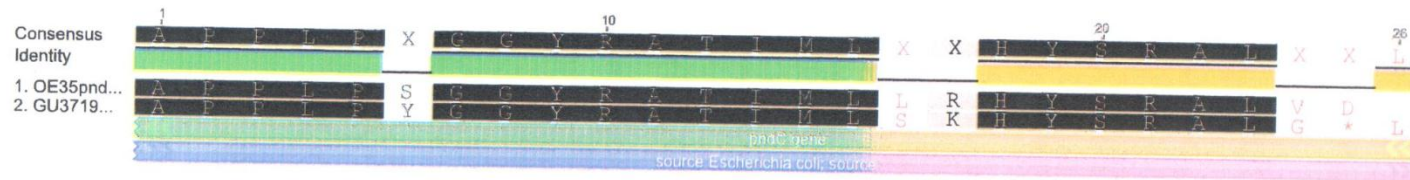
**Figure D.2 Protein Alignment of PemKI in *K. pneumoniae* N7 to PemKI in *Salmonella enterica* subsp. enterica serovar typhimurium (Accession no.JF274991). Length :59 a.a, identical site: 59 a.a (100%).**



**Figure D.3 Nucleotide Alignment of *ccdAB* gene in *K. pneumoniae* E5/19 to *ccdAB* gene in uncultured bacterium (Accession no. JX127248). Length :75 bp, identical site:75 bp (100%).**



**Figure D.5 Nucleotide Alignment of *pndAC* gene in *E. coli* I4/13 to *pndAC* gene in *E. coli* plasmid pEC\_Bactec(Accession no.GU371927).  
Length :78 bp, identical site: 74 bp (98.1%).**



**Figure D.6 Protein Alignment of PndAC in *E. coli* I4/13 to PndAC in *E. coli* plasmid pEC\_Bactec(Accession no.GU371927). Length : 26 a.a, identical site:21 a.a (94.1%).**

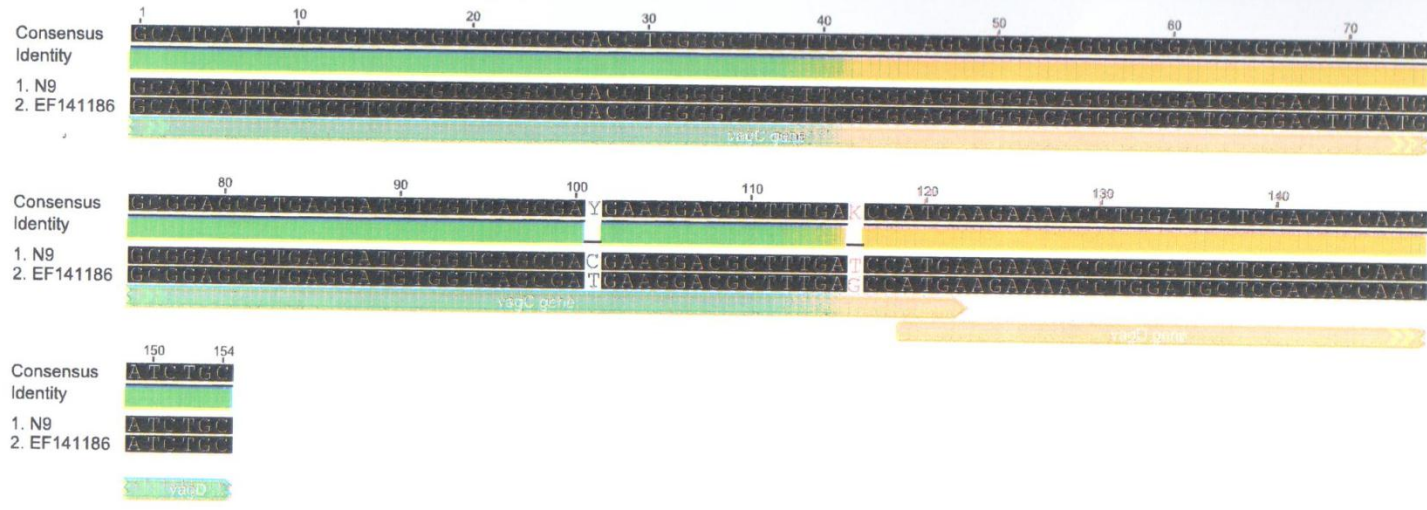


Figure D.7 Nucleotide Alignment of *vagCD* gene in *K. pneumoniae* N9 to *vagCD* gene in IncI1 plasmid source *Salmonella enterica* sub *enterica* serovare typhimurium (Accession no.EF141186). Length : 154 bp, identical site:152 bp (98.7%).

