Text S1 – Methodology

Genes investigated by PCR

bla$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{BKC}}$, *bla*$_{\text{CTX-group}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *aac(3)-Ia*, *aac(3)-IIa*, *aac(6')-Ih*, *aph(3')-VI*, *ant(2')-Ia*, *aph(3')-Ia*, *aac(6')-Ib*.

Nicoletti AG, Marcondes MF, Martins WM, Almeida LGP, Nicolás MF, Vasconcelos ATR, et al. Characterization of BKC-1 class A carbapenemase from *Klebsiella pneumoniae* clinical isolates in Brazil. Antimicrob Agents Chemother 2015;59(9):5159-5164.

Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010;65(3):490-495.

Akers KS, Chaney C, Barsoumian A, Beckius M, Zera W, Yu X, et al. Aminoglycoside resistance and susceptibility testing errors in Acinetobacter baumannii-calcoaceticus complex. J Clin Microbiol 2010;48(4):1132-1138.

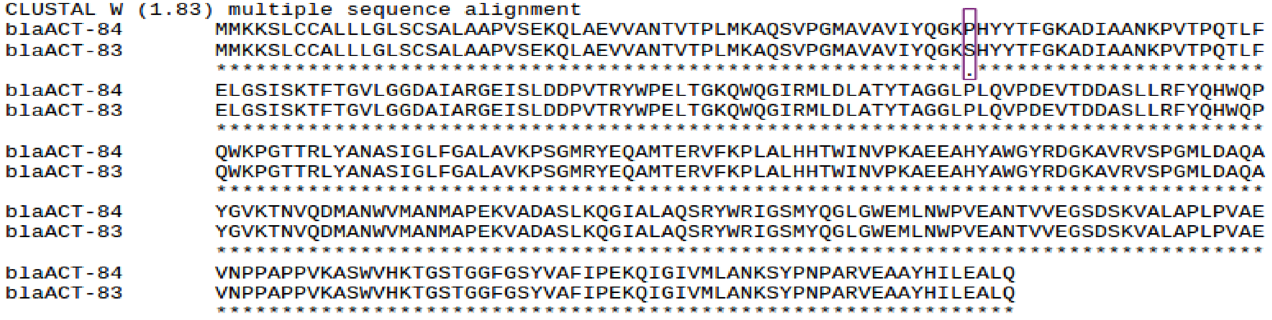
ANI analysis

The following strains were used in the ANI analysis: *E. asburiae* (CP011863.1), *E. cloacae* subsp. *cloacae* ATCC13407 (NC_014121.1), *E. hormaechei* subsp. *steigerwaltii* (NZ_CP017179.1), *E. hormaechei* subsp. *oharae* (NZ_CP017180), *E. hormaechei* subsp. *xiangfangensis* (NZ_CP017183.1), *E. hormaechei* subsp. *hoffmannii* (CP017186.1), *E. hormaechei* subsp. *hormaechei* (MKEQ01000001-MKEQ01000004), *E. bungandensis* (LT992502.1), *E. ludwigii* (CP017279.1), *E. kobei* (NZ_CP017181.1), and *E. cancerogenus* (NZ_CP045769.1).

Fig S1. Plasmid-mediated ARG detected in Ec61. The plasmid location of each gene is demonstrated between the parentheses.

	Ec61 plasmids		
	pEc61A	pEc61B	pEc61C
General Features	149,802 bp 52% GC IncFIB	77,651 bp 53.5% IncC	8382 bp 59.4% IncQ1
Antimicrobial Resistance Genes	mcr-9 (28747.. 30366)	aac(6')-Ib3 (39040..39594) aadA1 (38179..38970) blaSHV-5 (30232..31092) aac(6')-Ib-cr (39040..39558) sul1 (36835..37674) sul2 (49991..50806) tet(C) (42887..44077) tet(D) (21060..22244)	aph(3')-Via (2562..3341) blaBKC-2 (3498..4439)

Fig S2. Clustal W alignment of protein sequences. Amino acid substitutions are highlighted on purple boxes.



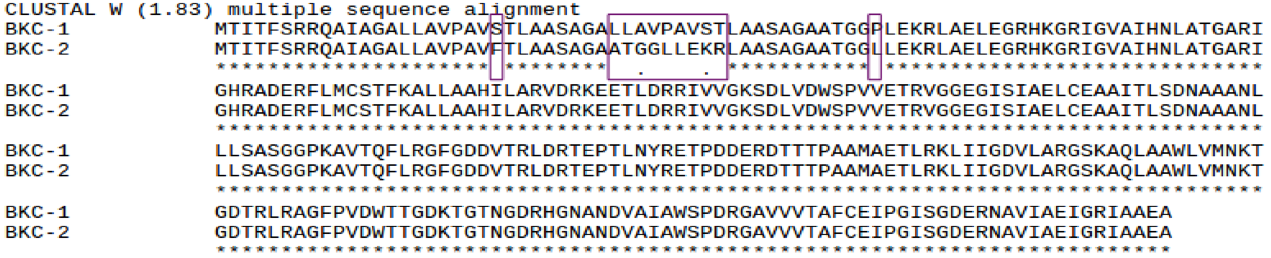
```
CLUSTAL W (1.83) multiple sequence alignment
blaACT-84       MMKKSLCCALLLGLSCSALAAPVSEKQLAEVVANTVTPLMKAQSVPGMAVAVIYQGKPHYYTFGKADIAANKPVTPQTLF
blaACT-83       MMKKSLCCALLLGLSCSALAAPVSEKQLAEVVANTVTPLMKAQSVPGMAVAVIYQGKSHYYTFGKADIAANKPVTPQTLF
                *********************************************************.**********************

blaACT-84       ELGSISKTFTGVLGGDAIARGEISLDDPVTRYWPELTGKQWQGIRMLDLATYTAGGLPLQVPDEVTDDASLLRFYQHWQP
blaACT-83       ELGSISKTFTGVLGGDAIARGEISLDDPVTRYWPELTGKQWQGIRMLDLATYTAGGLPLQVPDEVTDDASLLRFYQHWQP
                ********************************************************************************

blaACT-84       QWKPGTTRLYANASIGLFGALAVKPSGMRYEQAMTERVFKPLALHHTWINVPKAEEAHYAWGYRDGKAVRVSPGMLDAQA
blaACT-83       QWKPGTTRLYANASIGLFGALAVKPSGMRYEQAMTERVFKPLALHHTWINVPKAEEAHYAWGYRDGKAVRVSPGMLDAQA
                ********************************************************************************

blaACT-84       YGVKTNVQDMANWVMANMAPEKVADASLKQGIALAQSRYWRIGSMYQGLGWEMLNWPVEANTVVEGSDSKVALAPLPVAE
blaACT-83       YGVKTNVQDMANWVMANMAPEKVADASLKQGIALAQSRYWRIGSMYQGLGWEMLNWPVEANTVVEGSDSKVALAPLPVAE
                ********************************************************************************

blaACT-84       VNPPAPPVKASWVHKTGSTGGFGSYVAFIPEKQIGIVMLANKSYPNPARVEAAYHILEALQ
blaACT-83       VNPPAPPVKASWVHKTGSTGGFGSYVAFIPEKQIGIVMLANKSYPNPARVEAAYHILEALQ
                *************************************************************
```



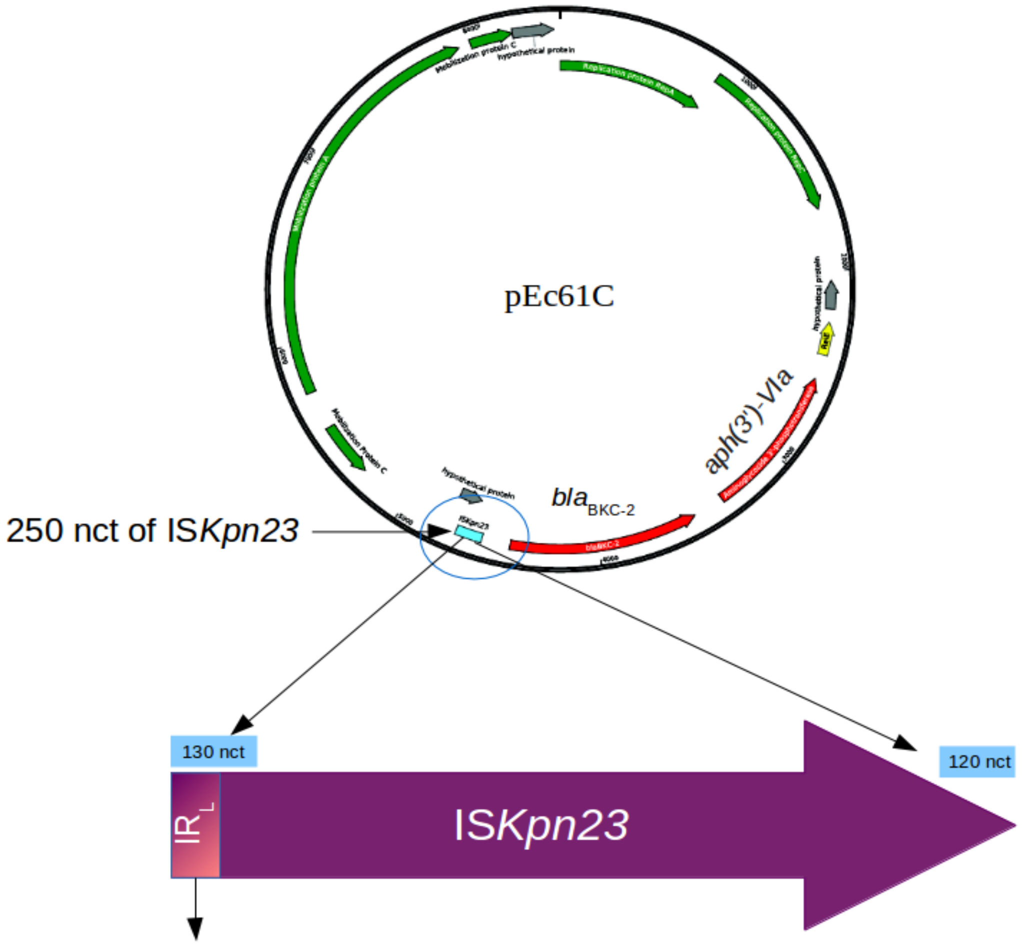
```
CLUSTAL W (1.83) multiple sequence alignment
BKC-1           MTITFSRRQAIAGALLAVPAVSTLAASAGALLAVPAVSTLAASAGAATGGPLEKRLAELEGRHKGRIGVAIHNLATGARI
BKC-2           MTITFSRRQAIAGALLAVPAVFTLAASAGAATGGLLEKRLAASAGAATGGLLEKRLAELEGRHKGRIGVAIHNLATGARI
                ********************* ********  .    . *********** *****************************

BKC-1           GHRADERFLMCSTFKALLAAHILARVDRKEETLDRRIVVGKSDLVDWSPVVETRVGGEGISIAELCEAAITLSDNAAANL
BKC-2           GHRADERFLMCSTFKALLAAHILARVDRKEETLDRRIVVGKSDLVDWSPVVETRVGGEGISIAELCEAAITLSDNAAANL
                ********************************************************************************

BKC-1           LLSASGGPKAVTQFLRGFGDDVTRLDRTEPTLNYRETPDDERDTTTPAAMAETLRKLIIGDVLARGSKAQLAAWLVMNKT
BKC-2           LLSASGGPKAVTQFLRGFGDDVTRLDRTEPTLNYRETPDDERDTTTPAAMAETLRKLIIGDVLARGSKAQLAAWLVMNKT
                ********************************************************************************

BKC-1           GDTRLRAGFPVDWTTGDKTGTNGDRHGNANDVAIAWSPDRGAVVVTAFCEIPGISGDERNAVIAEIGRIAAEA
BKC-2           GDTRLRAGFPVDWTTGDKTGTNGDRHGNANDVAIAWSPDRGAVVVTAFCEIPGISGDERNAVIAEIGRIAAEA
                *************************************************************************
```

Fig S3. Representation of the 250 bp nucleotide distribution of the remaining IS*Kpn23* sequence detected in the pEc61C. The predicted promoter was detected in a region denominated IRL (left inverted repeat).



Predicted promoter region

Start	End	Score	Promoter Sequence
282	327	0.98	gatttacttggggaatgtgggagtaaatagatgatcttaatttggttcac