Novel *mgrB* variants identified in colistin-resistant *Klebsiella pneumoniae*

Basil Britto Xavier[1], Nienke Plantinga[2], Christine Lammens[1], Laurent Poirel[3,4], Patrice Nordmann[3,4], Marc Bonten[2], Herman Goossens[1], Surbhi Malhotra-Kumar[1]

[1]Laboratory of Medical Microbiology, Vaccine & Infectious Disease Institute, Universiteit Antwerpen, Antwerp, Belgium; [2]Department of Medical Microbiology and Epidemiology of Infectious Diseases, Julius Center for Health Sciences & Primary Care, UMC Utrecht, The Netherlands; [3]Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland; [4]INSERM U914 Emerging Resistance to Antibiotics, Faculté de Médecine et Université Paris Sud, Le Kremlin-Bicêtre, France

R-GNOSIS

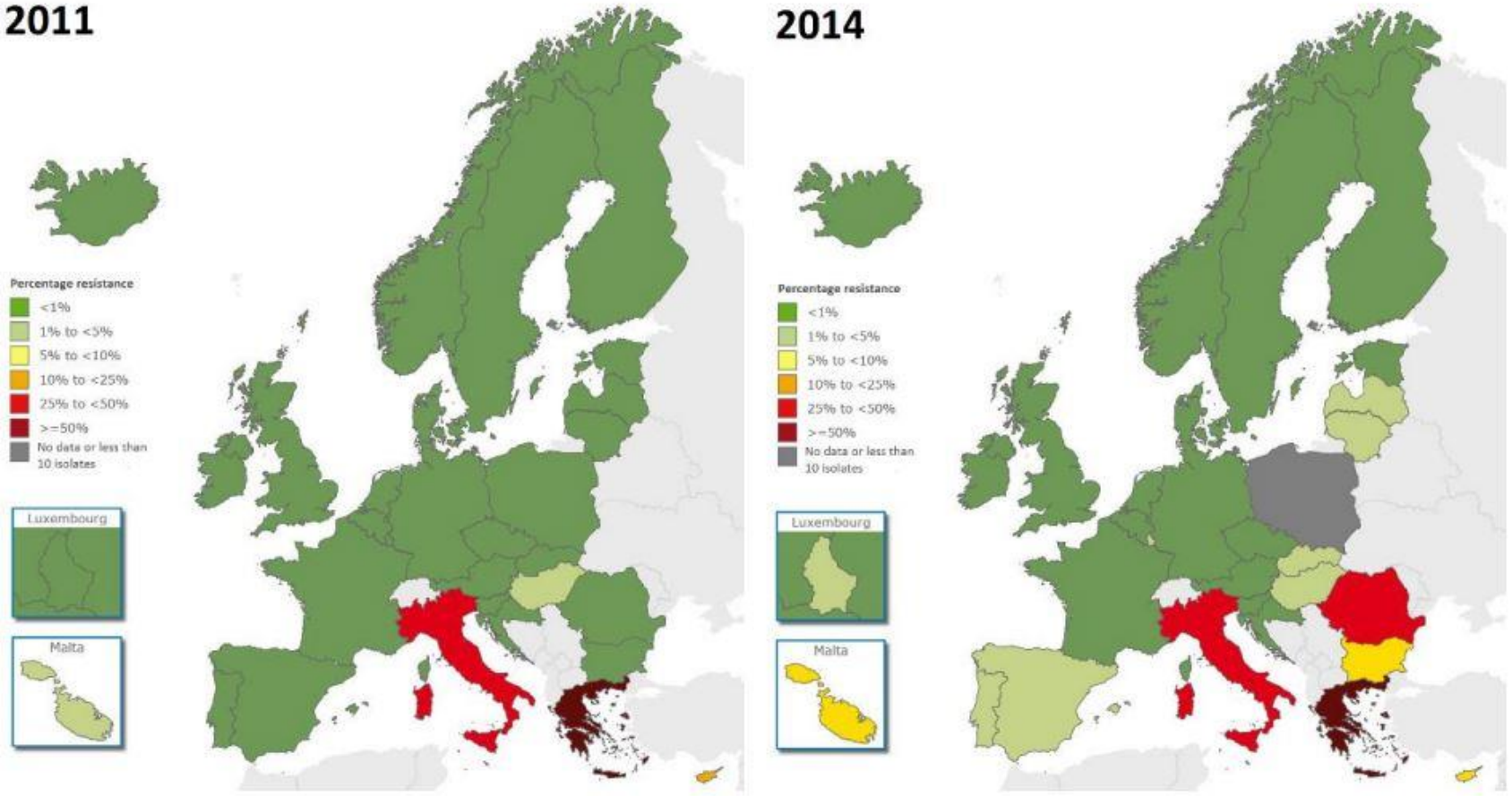
SEVENTH FRAMEWORK PROGRAMME

VAXINFECTIO
Vaccine & Infectious Disease Institute
University of Antwerp

Klebsiella pneumoniae (KPN)

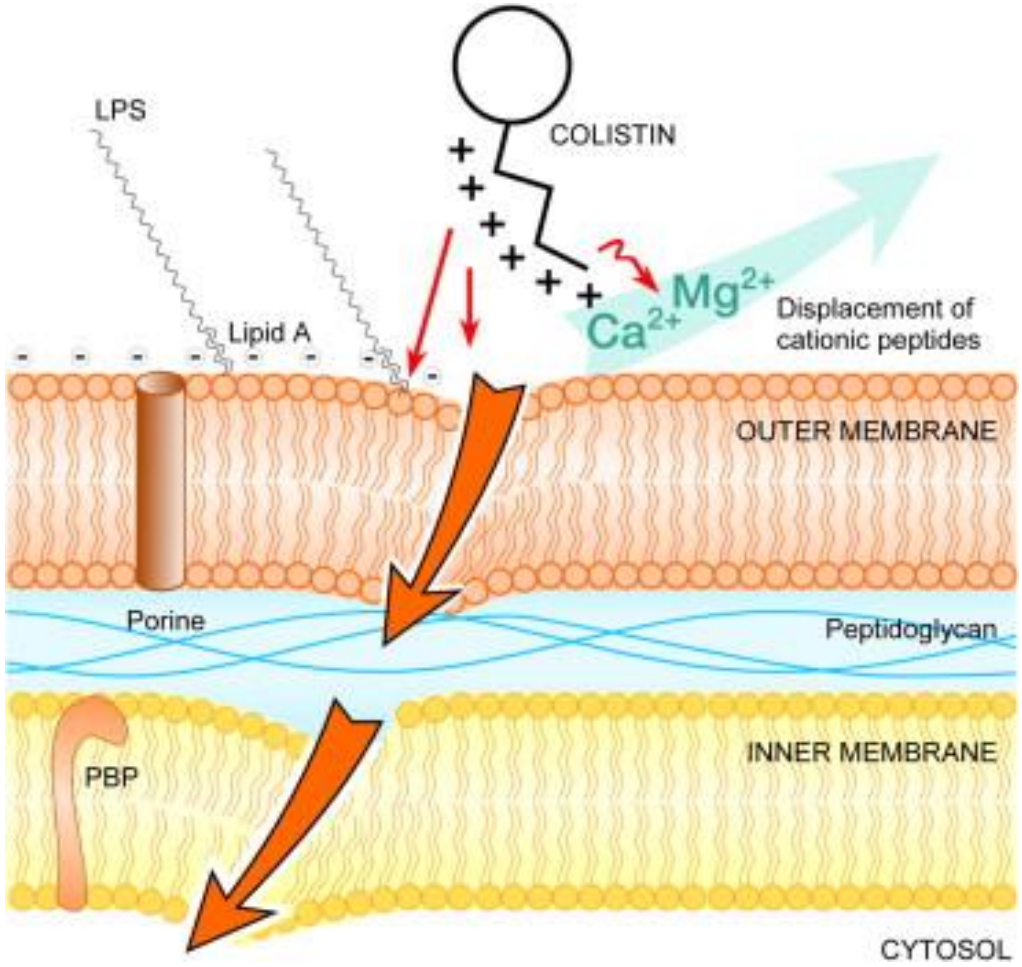
- *K. pneumoniae* causes, urinary tract, respiratory tract and blood stream infections
- Increase of multi-drug resistance in *K. pneumoniae* is a public health concern in Europe and worldwide
- Resistance to carbapenems in *K. pneumoniae* in Europe significantly increased over the last four years
- Countries with already high percentages of carbapenem resistance also report large numbers of isolates with polymyxin resistance → an indication of the further loss of effective treatment options for Gram-negative bacterial infections

K. pneumoniae: percentage of invasive isolates with resistance to carbapenems



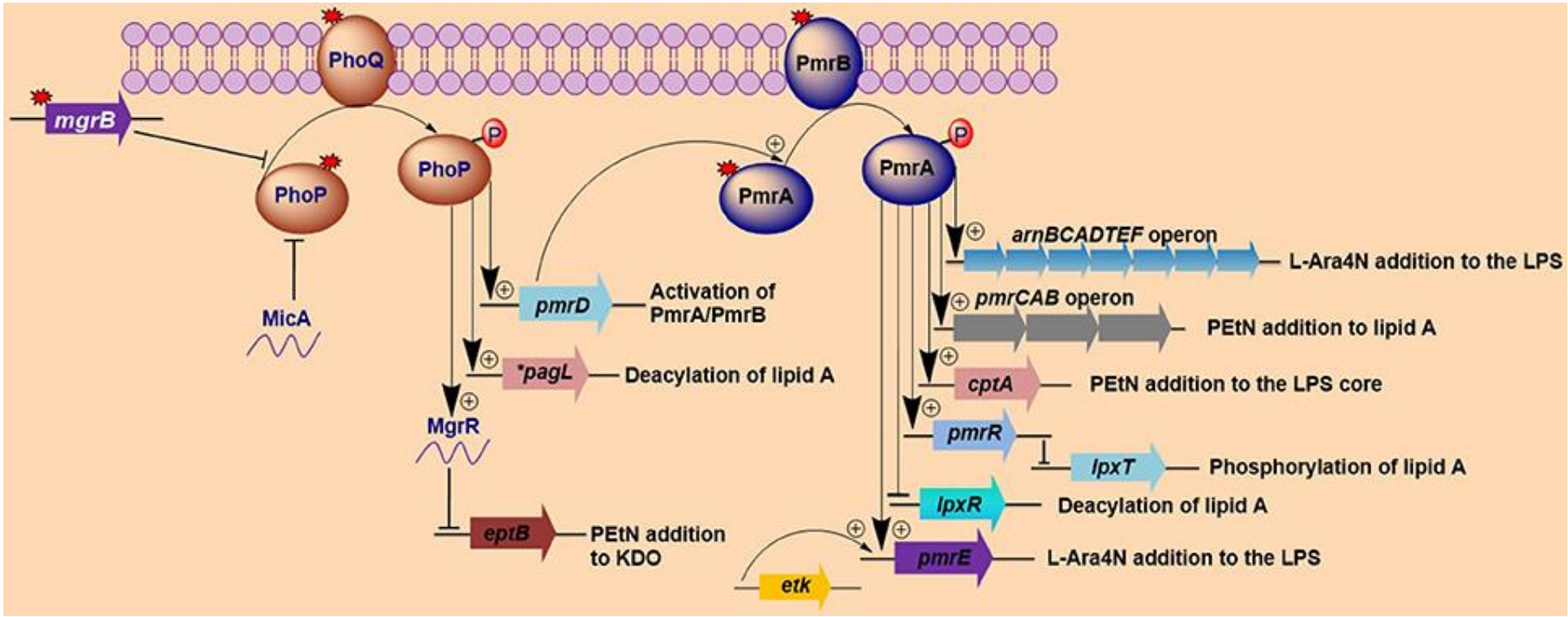
Source: European Centre for Disease Prevention and Control (ECDC) 2015

Action of colistin (CL) on Gram negative bacterial cell membrane



Martis et al., 2014

Activation of lipopolysaccharide-modifying genes involved in polymyxin resistance in Gram-negative bacteria



Olaitan et al., 2014

Known CL resistance mechanisms in *K. pneumoniae*

Genes	Modifications implicated to Colistin resistance	Mechanism	References
mgrB	L24H, C28Y, G37S,	Inactivation of phop/phoQ negative feedback regulator	Cannatelli et al. 2013, Wright MS et al. 2015, Poirel L et al. 2015
	IS element interruption (IS1F, IS4, IS5, ISKpn13, ISKpn14, ISEcp1) loss of gene and fragments		Poirel L et al. 2015, Zowawi MH et al. 2015
phoP	G385S	Activation of LPS-modifying operon by mutations	Olaitan et al.2014
	L26Q		
phoQ	L96P		
	L348Q		
	S174N		Choi & Ko, 2014
	D434N		Wright MS et al. 2015
pmrA	G53C		Olaitan et al. 2014
pmrB	L82R		Cannatelli et al. 2014
	T157P		Jayol et al. 2014
	S85R		Olaitan et al. 2014
	T140P		
	ΔR14		Choi and Ko, 2014
	ΔY209		
	T157P		
	S208N		
pmrC		Modification of the lipidA	Kim et al. 2006
crrAB	L94M	Induces expression of the glycosyltransferase-like protein which transfers an as-yet-unidentified sugar to lipid A	Wright MS et al. 2015
	Q10L		

Modified & updated from Olaitan et al. , 2014

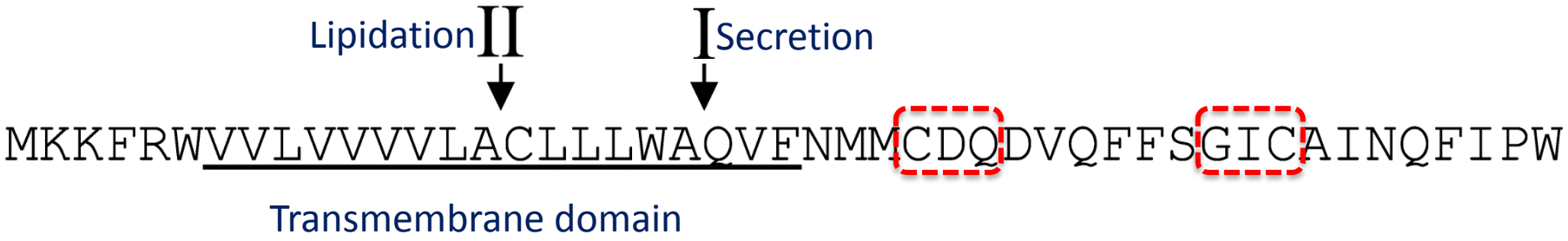
Known CL resistance mechanisms in *K. pneumoniae* (cont.)

Genes	Modifications implicated to Colistin resistance	Mechanism	References
arnBCADTEF operon and pmrE	NA	Modification of the lipidA or Kdo	Helander et al. 1996
acrAB and kpnEF	NA	Efflux pump	Padilla et al. 2010, Srinivasan and Rajamohan 2013
NA	NA	Trapping of polymyxin by capsule	Campos et al. 2004
lpxM	NA	Increased aceylation of lipid A enhancing its modification with aminoarabinose	Velkov et al 2013
mcr-1	NA	Modification of lipid A, resulting in reduction of polymyxin affinity through plasmid mediated gene	Liu et al. 2015

Modified & updated from Olaitan et al. , 2014

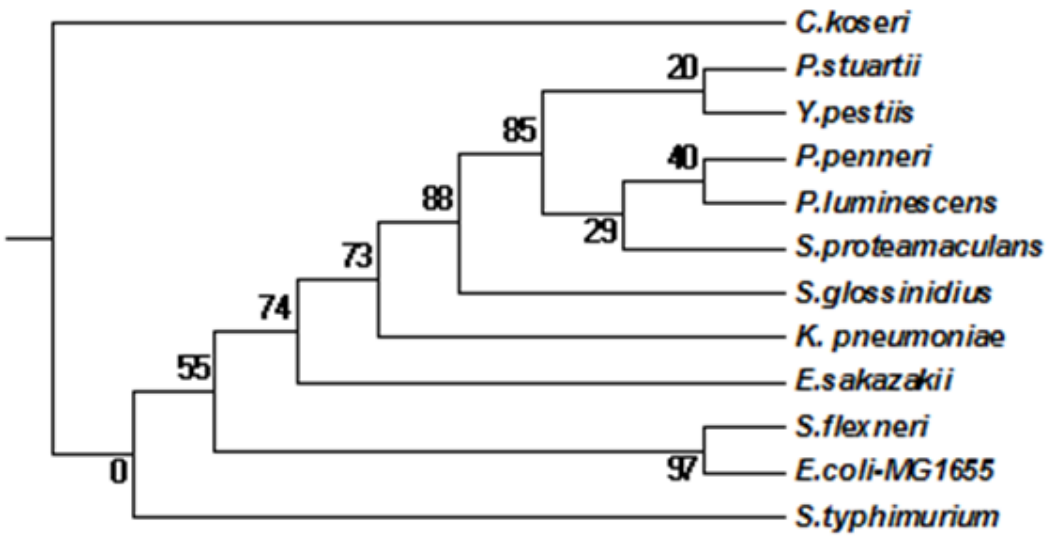
The transmembrane protein *mgrB*

- Negative feedback regulator of PhoQ/PhoP signalling system
- Short transmembrane protein (47 aa long) and conserved among Enterobactericea
- *mgrB = yobG*, hypothetical protein, membrane protein, putative inner membrane protein
- Automated annotations fail to annotate this gene



Lippa AM., 2009

mgrB conservation among Enterobacteriaceae



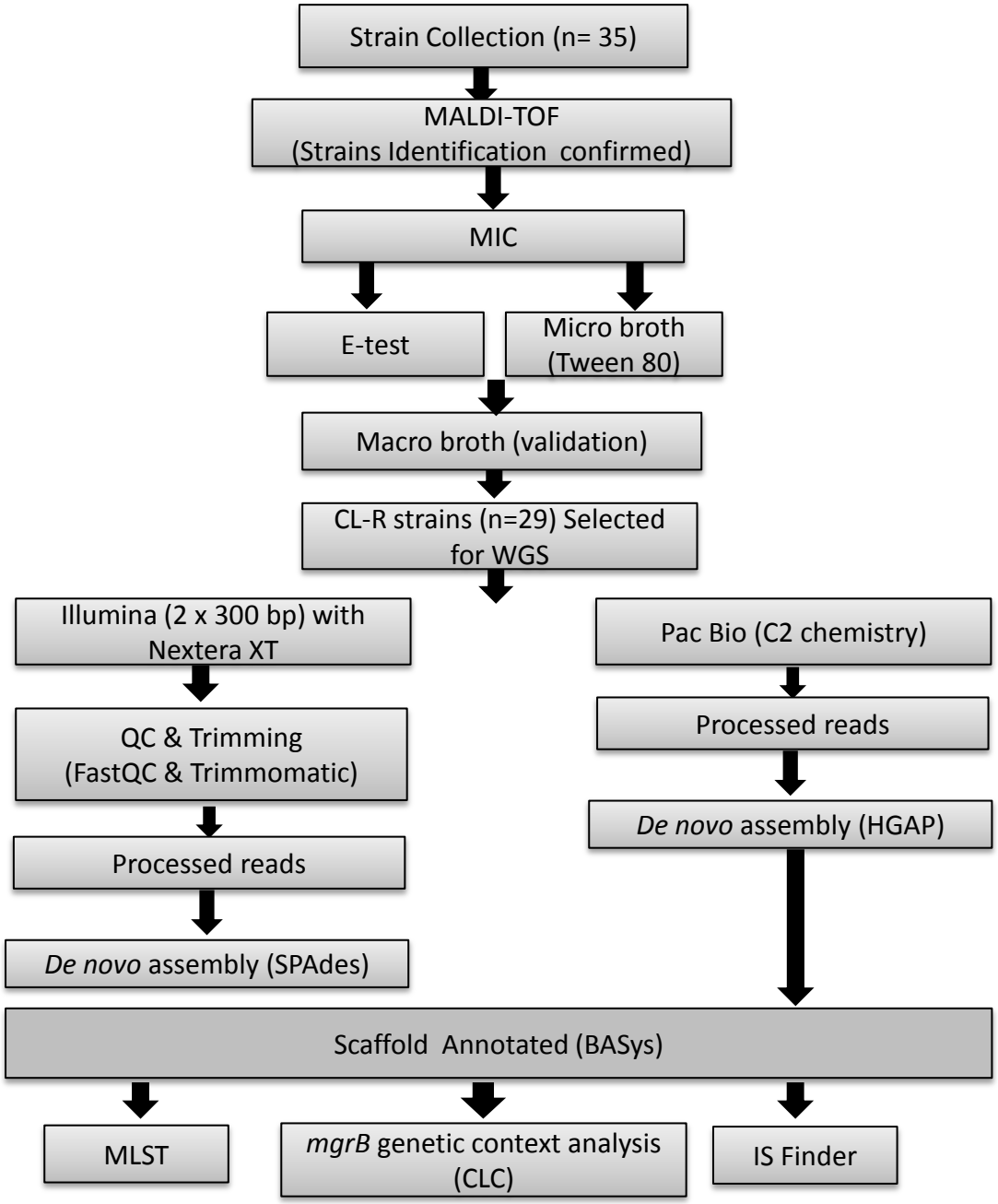
WGS

Sequencing

Quality control

Assembly & annotation

Analysis

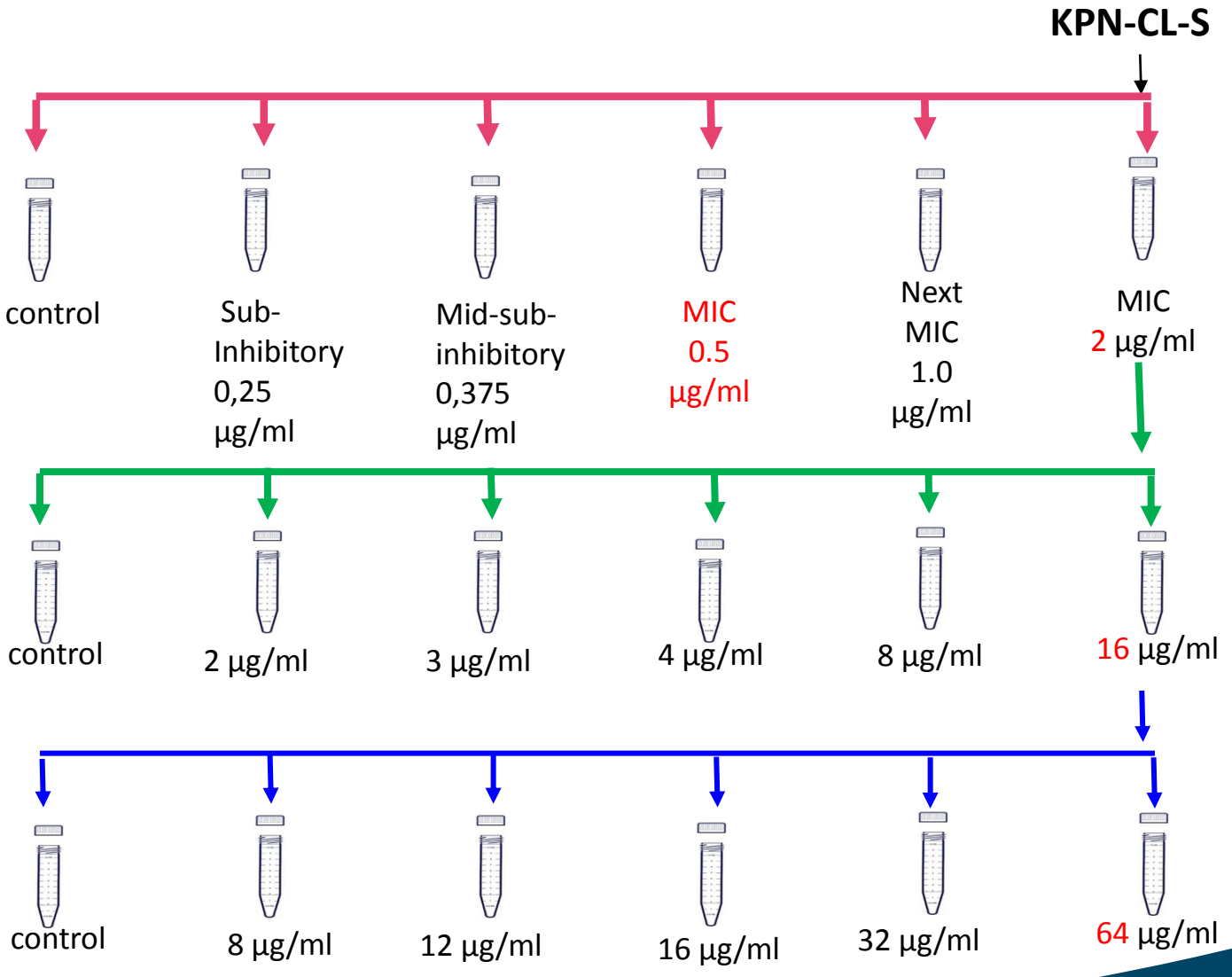


In-vitro generations of CL-R strain from a clinical CL-S KPN

Passage I:
Inoculum used from MIC of sensitive strain

Passage II:
Inoculum used from MIC 2 µg/ml

Passage III:
Inoculum from MIC 16 µg/ml



Xavier et al., unpublished data

Comparison of MICs to colistin by E-test, micro- and macro-broth dilution

Strain	MIC (E-test)	MIC (micro broth)	MIC (macro broth)
1	0.75	32	32
2	2	>128	32
3	2	64	32
4	3	32	16
5	3	32	16
6	4	32	32
7	4	64	ND
8	4	64	ND
9	4	64	ND
10	4	64	ND
11	4	64	ND
12	4	64	ND
13	6	32	ND
14	6	32	ND
15	6	128	ND

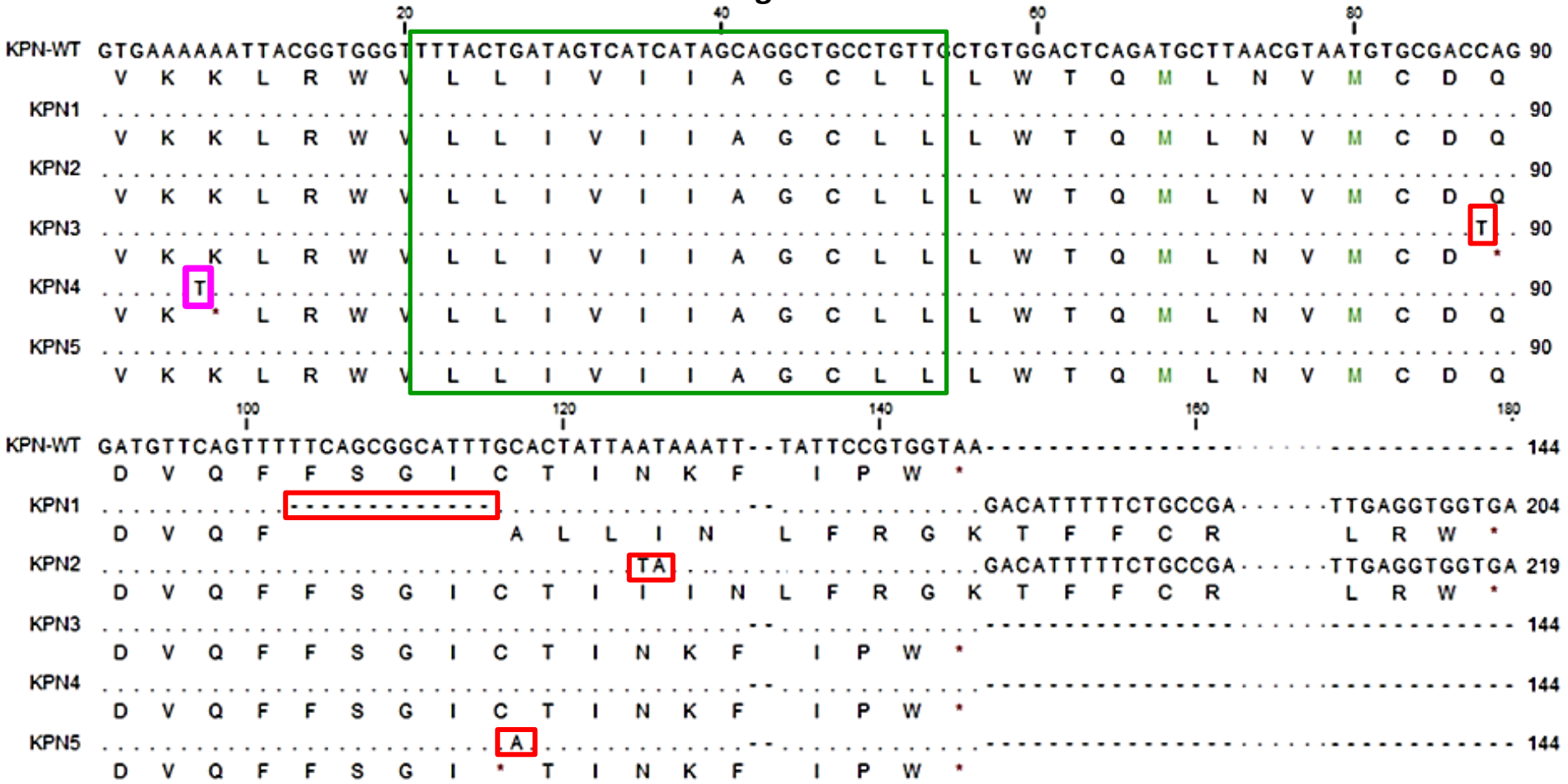
Strain	MIC (E- test)	MIC (micro broth)	MIC (macro broth)
16	8	64	ND
17	8	64	ND
18	8	128	ND
19	12	128	ND
20	12	128	ND
21	12	256	ND
22	16	64	ND
23	16	128	ND
24	24	128	ND
25	24	128	ND
26	24	256	ND
27	24	256	ND
28	32	>128	ND
29	32	32	ND

ND: Not Done

Comparison of colistin MIC results

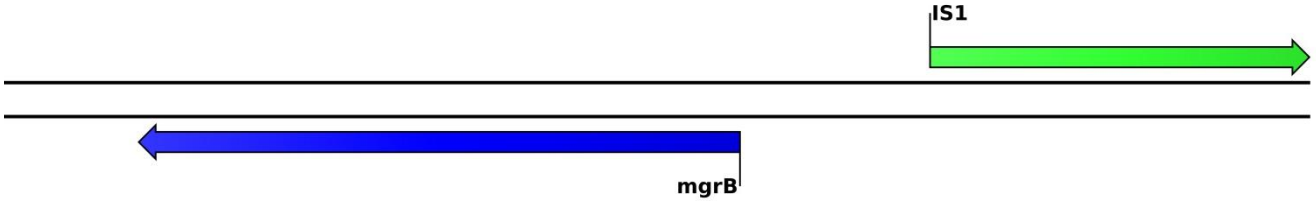
- Of the 29/35 strains screened and 29 strains confirmed as CL-R by broth micro dilution (addition with tween 80)
- However, there were 4 to 100 fold differences between MIC values of the E-test and broth micro dilution
- Interestingly, 3 strains were identified as sensitive by E-test and confirmed as resistant by broth micro and macro dilution

Alignment of *mgrB* variants



mgrB interruption by IS elements

KPN6



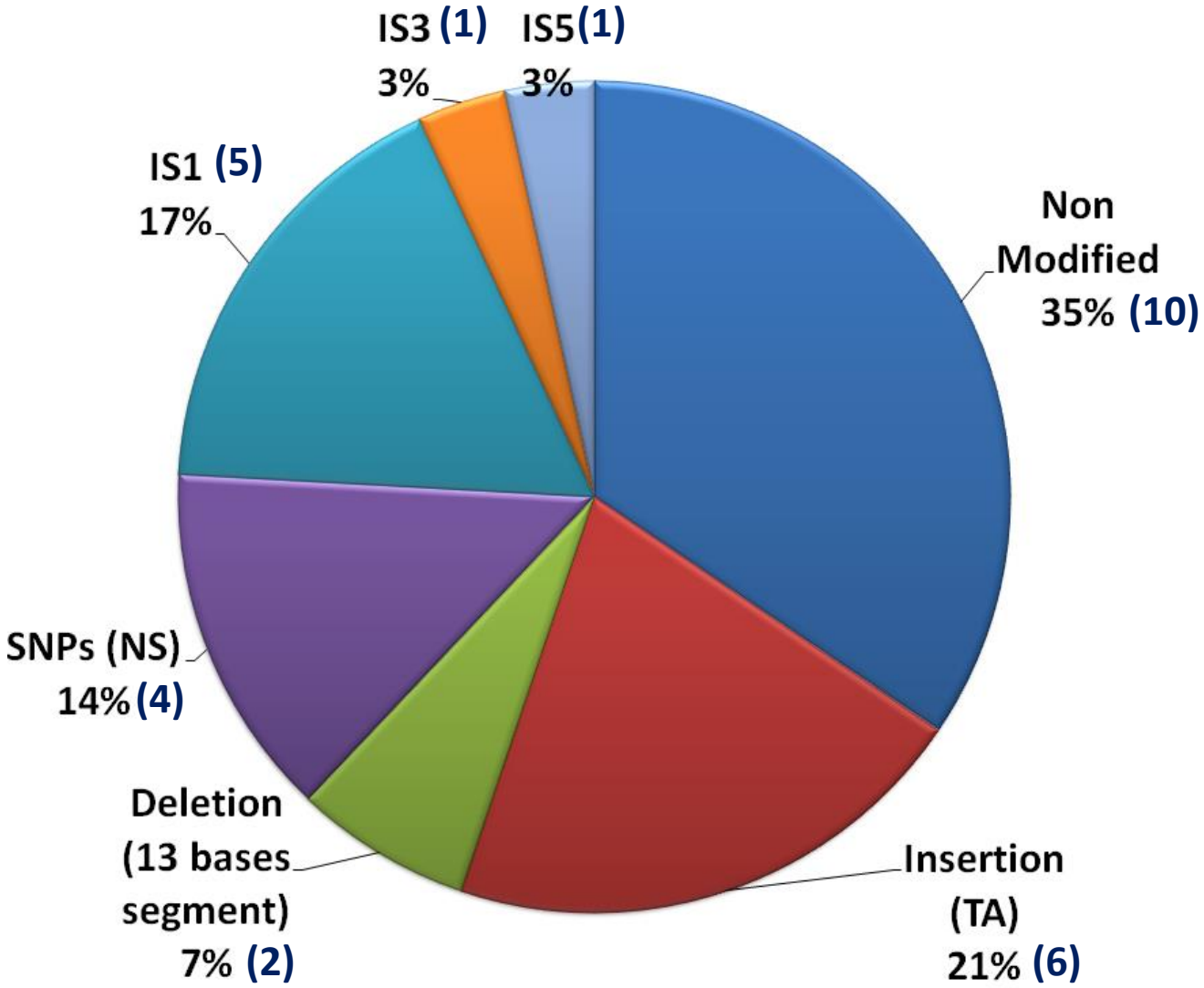
KPN7

KPN8

mgrB variants identified in present study

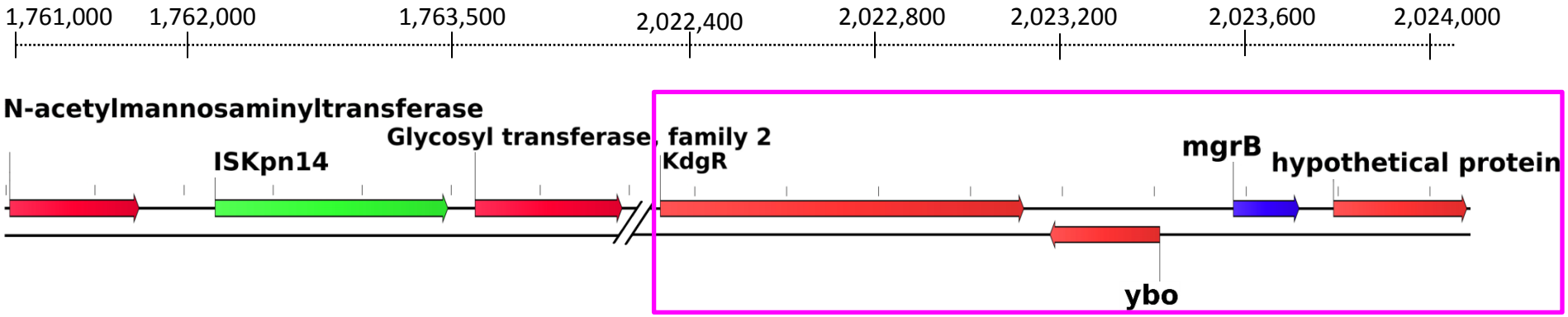
No of strains (n)	MLST	MIC	Modification in *mgrB*	Changes in the gene
n=10	ST323, ST101,ST307, ST512	8-64	No modification	No
n=2	Novel ST, ST101	64	7^{th} position → A to T → 3K*	Truncation
n=1	ST709	32	117^{th} position → C to A → C39*	Truncation
n=1	ST101	256	88^{th} position → C to T → Q30*	Truncation
n=6	ST512	256	124^{th} position → TA insertion	Elongation
n=2	ST101	64	Deletion of gene from 103^{rd} to 115^{th} (13 bases)	Elongation
n=1	Novel ST	64	ISEc16 interruption at 48^{th} position	Truncation
n=2	ST101	64	IS1X2 interruption close to promoter region	No
n=2	ST101	64	ISIR interruption close to promoter region	No
n=1	ST409	128	IS1R interruption at 108^{th} position	Truncation
n=1	ST11	128	ISKpn26 interruption at 75^{th} position	Truncation

Diversity of *mgrB* disruptions among studied strains

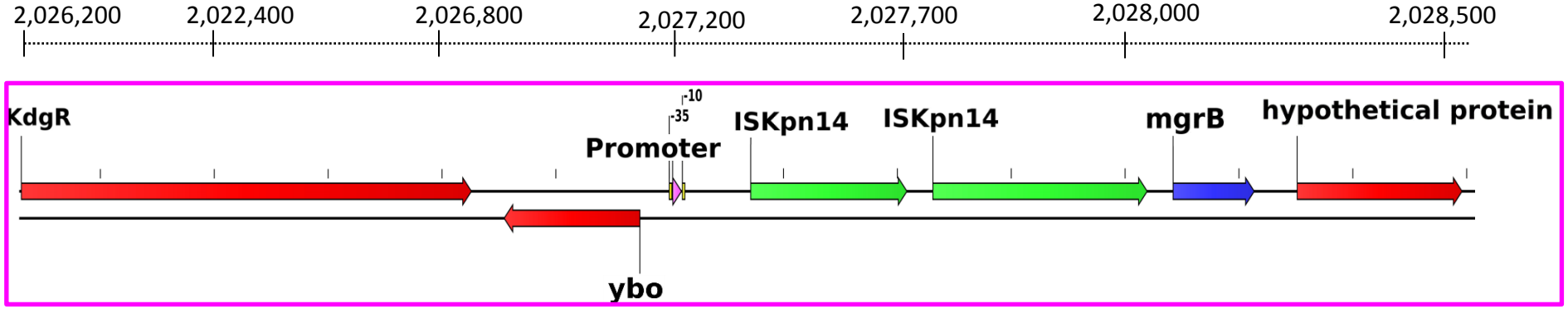


ISKpn14 transposes into *mgrB* promoter under CL pressure

In vivo KPN Sensitive (MIC 2)



In vitro (n=4) (MIC 64)



ISKpn14: IS1 family Tn that transposed (and duplicated) into intergenic region between promoter and start codon of *mgrB*

Xavier et al., unpublished data

Conclusions

- 10 different *mgrB* variants were identified of which four are novel variants
- Variations were not clonally specific
  - Eight different MLST types among them 2 novel STs
- Colistin pressure selects for tranposition events
- Strains with non-modified *mgrB* also did not show any other modifications in known CL-R genes such as *pmrB*, *pmrA*
- *mcr-1* gene was absent in our strains

Acknowledgements

Funding

Universiteit Antwerpen

DOCPRO-BOF 2012-27450 (Research Grants)

R-GNOSIS

SEVENTH FRAMEWORK PROGRAMME