



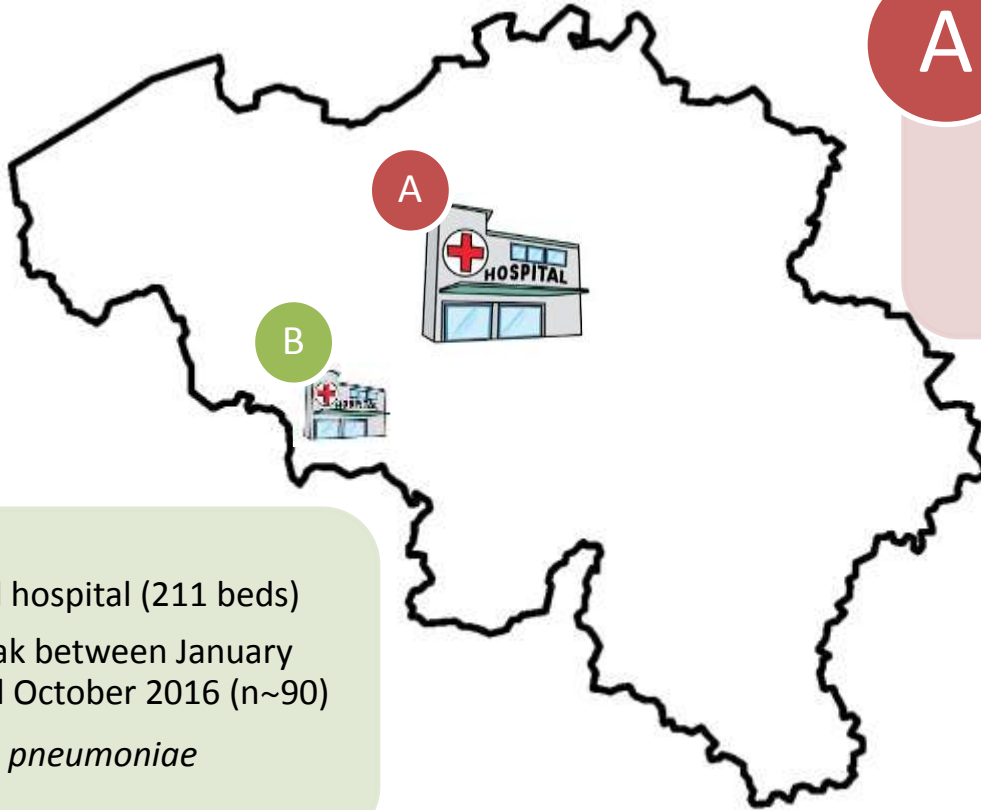
# Investigation by whole genome sequencing of two outbreaks of NDM-producing *Klebsiella pneumoniae* in Belgium

Sandrine Roisin

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Louise Nienhaus, Sandrine Roisin, Caroline Bauraing, Te-Din Huang, Jill Dombrecht,  
Katrien De Bruyne, Sammy Place, Baudouin Byl, Youri Glupczynski, Olivier Denis







A

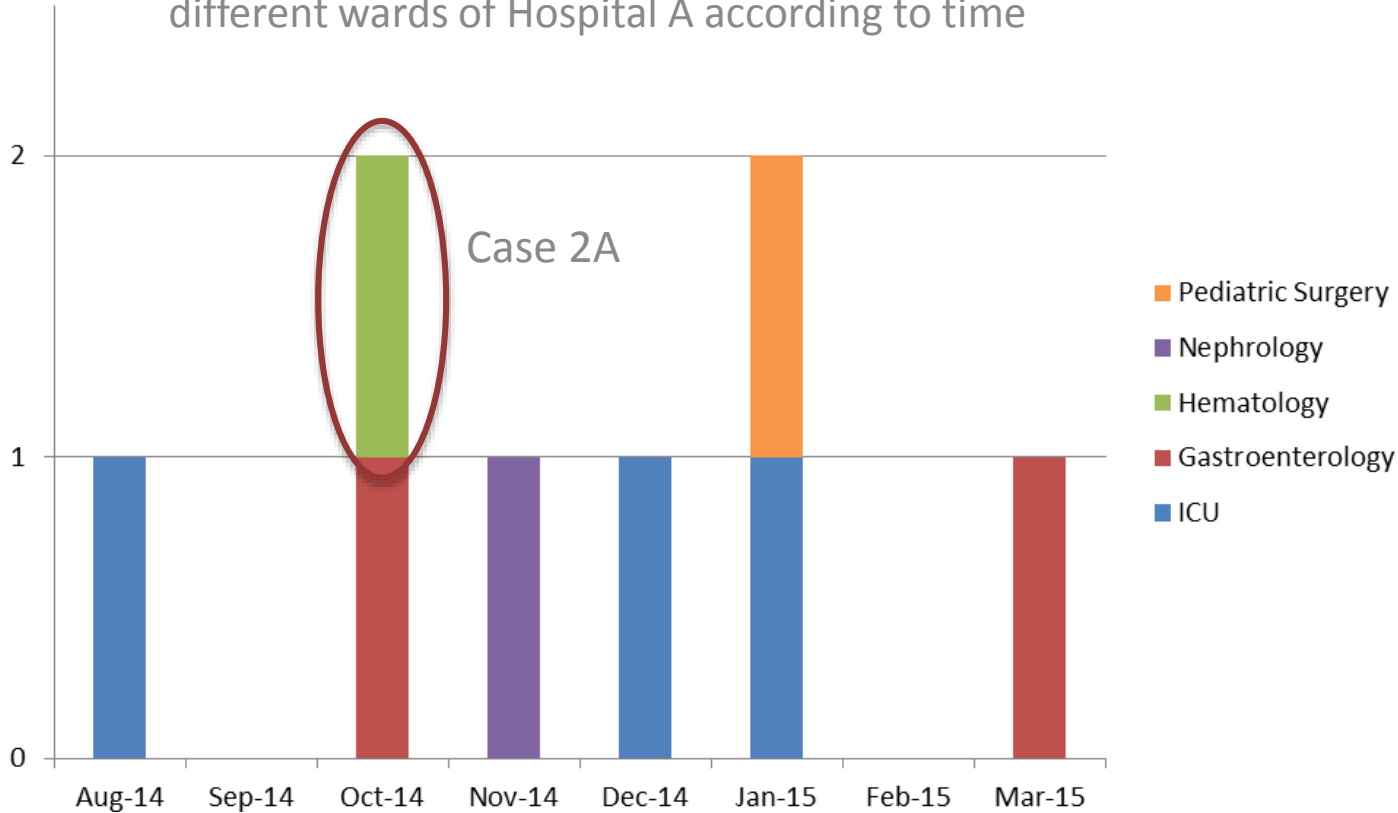
- Teaching hospital (864 beds)
- between August 2014 and March 2015
- NDM *K. pneumoniae* (n=9)

B

- General hospital (211 beds)
- Outbreak between January 2015 and October 2016 (n~90)
- NDM *K. pneumoniae*

# OUTBREAK A: August 2014 to March 2015

Number of NDM-Kp positive patients detected in different wards of Hospital A according to time

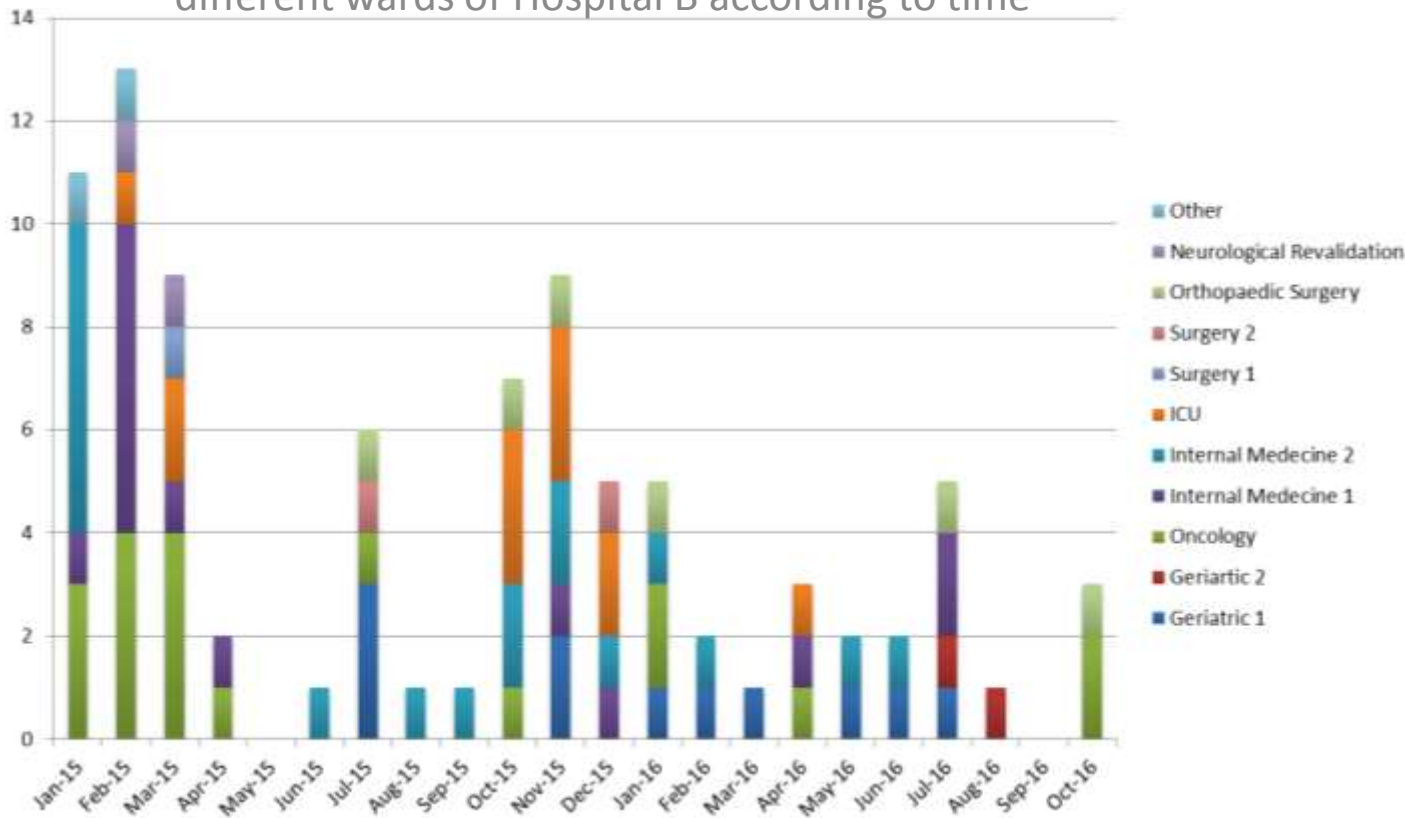


Total:  
9 patients  
“spread through  
whole hospital”

5/9 patients  
were infected

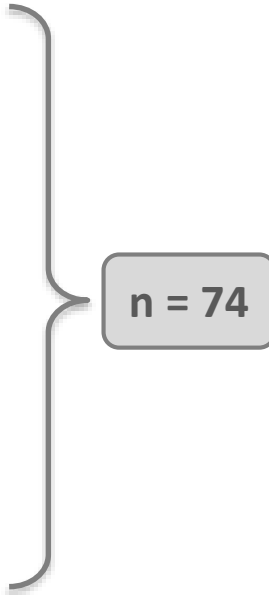
# OUTBREAK B: January 2015 to October 2016

Number of NDM-Kp positive patients detected in different wards of Hospital B according to time



Total:  
90 patients  
“spread through  
whole hospital”  
Same AST  
52 confirmed by PCR

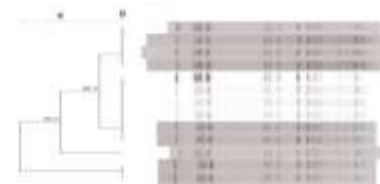
18/90 patients  
were infected



- Antimicrobial Susceptibility Testing (AST)<sup>1</sup>



- Pulsed-Field Gel electrophoresis (PFGE)<sup>2</sup>



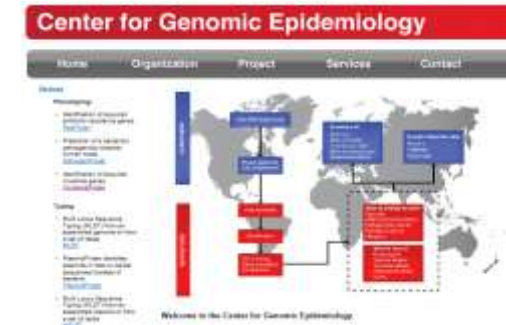
- Whole genome sequencing (WGS) on Illumina MiSeq<sup>®</sup> platform



1) EUCAST 2015

2) Laurent C, et al. Infect Control Hosp Epidemiol. 2008; 29:517-24

- De novo assembly
  - SPAdes (Bionumerics v7.6)
- Multi-locus sequence type (MLST)
  - Center for Genomic Epidemiology (CGE)
- Resistome
  - ResFinder of CGE
- Whole genome MLST (wgMLST)
  - Bionumerics v7.6 (Applied Maths)
- SNP analysis on the outbreak isolates (wgSNP)
  - Bionumerics v7.6

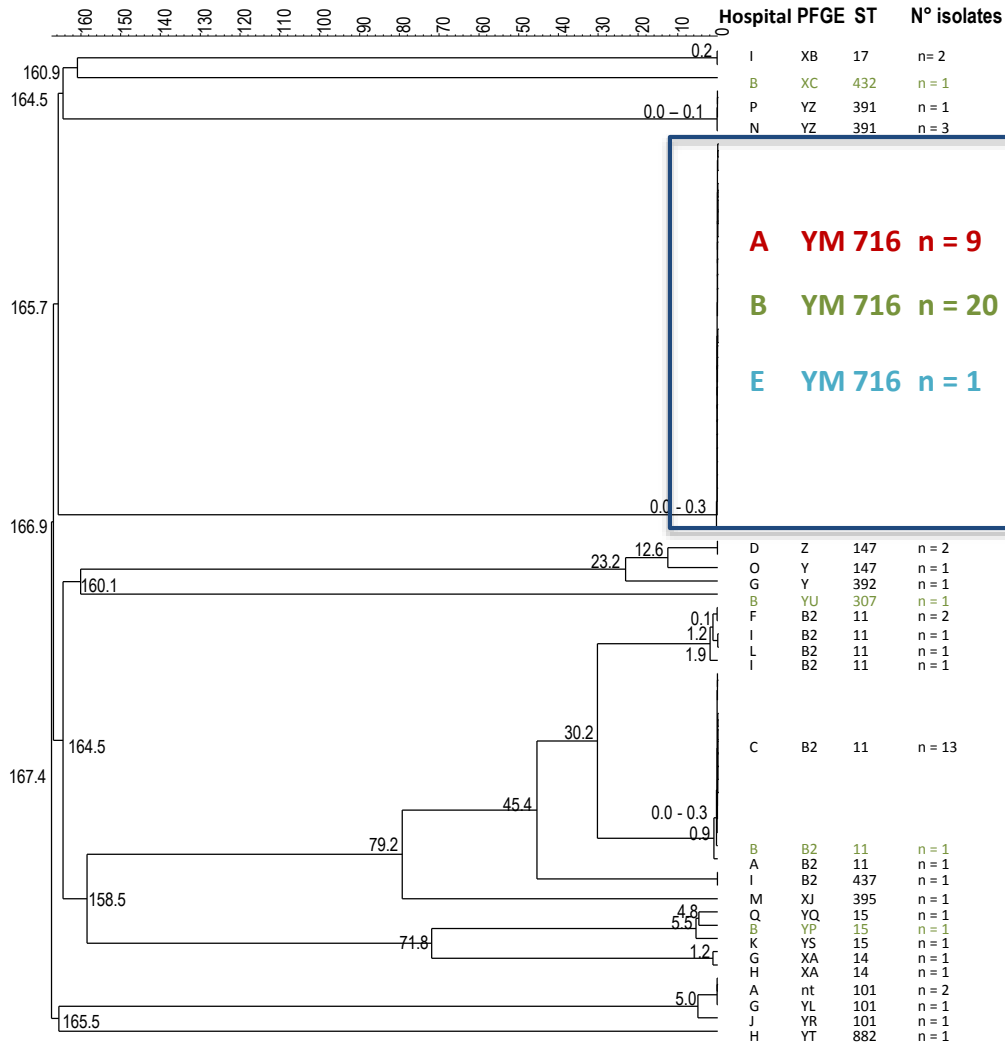






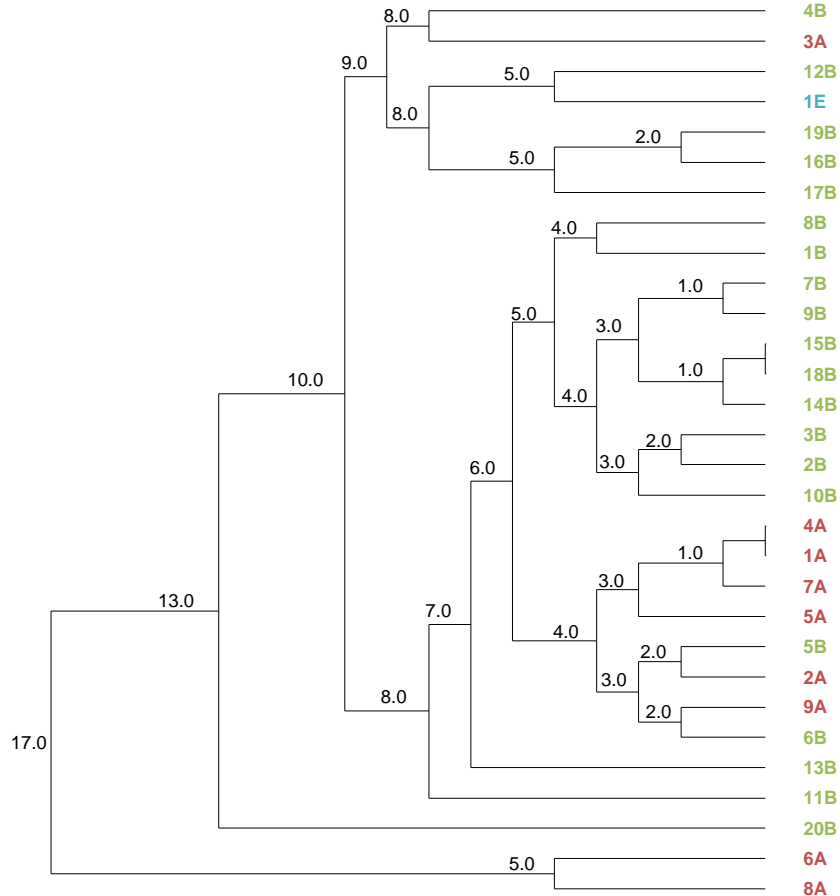


Similar results

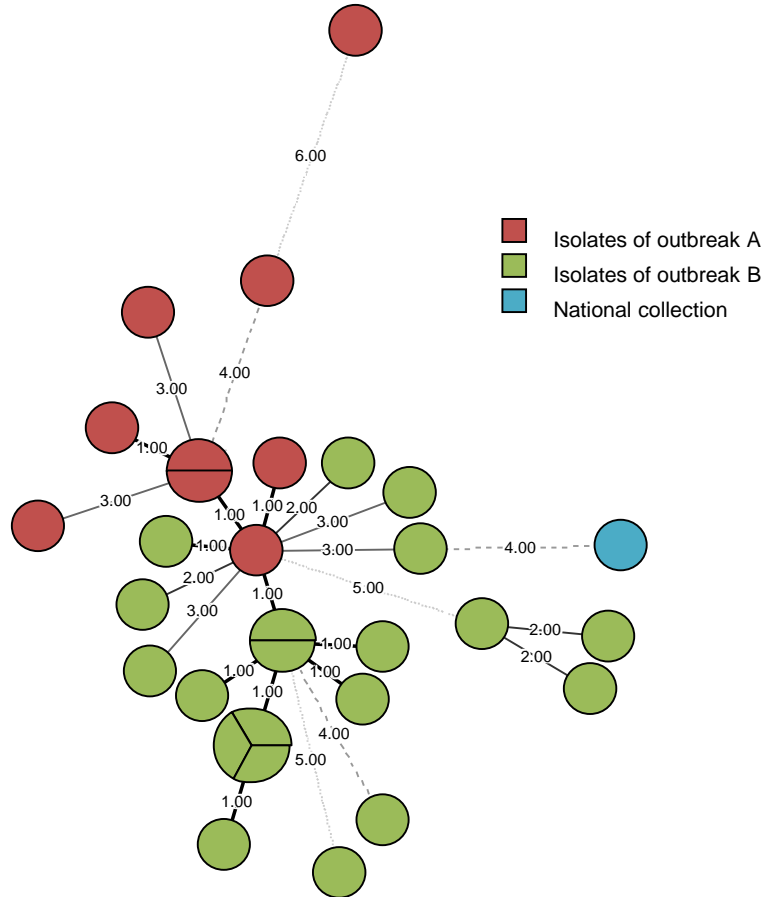


- n = 74
  - Outbreak A
  - Outbreak B
  - National collection
- Comparison of 4015 alleles

Scaling factor: x20



- 30 isolates
  - ST716
  - YM-PFGE-type
- 5386 alleles analysed
  - Max 17 alleles of difference



- o  $n = 30$ 
  - Outbreak A ( $n = 9$ )
  - Outbreak B ( $n = 20$ )
  - National collection ( $n=1$ )
- o 90% of similarity between outbreak strains
- o Max 6 SNPs of difference between 2 isolates

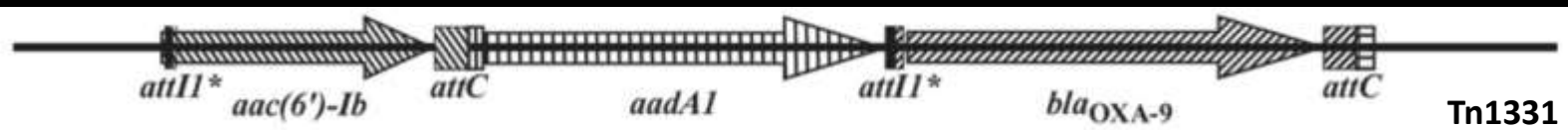
# RESISTOME: OUTBREAK A & B NDM-Kp ST716

Black : presence  
White: absence

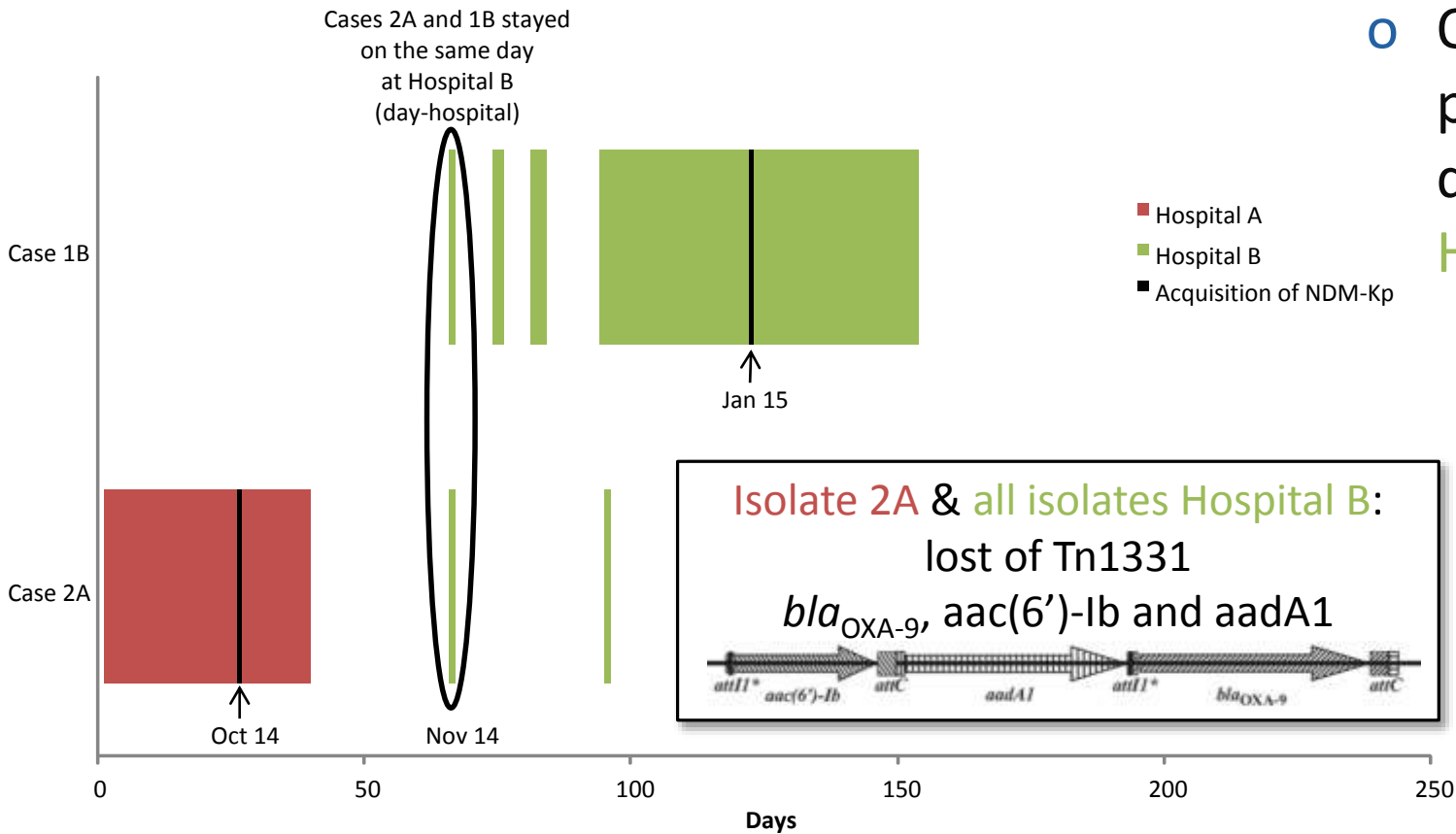
	Beta-lactamase						Aminoglycoside						Fluoroquinolone					Others					Amikacin		
	<i>bla</i> <sub>SHV-27</sub>	<i>bla</i> <sub>NDM-1</sub>	<i>bla</i> <sub>TEM-1B</sub>	<i>bla</i> <sub>CTX-M-15</sub>	<i>bla</i> <sub>OXA-9</sub>	<i>bla</i> <sub>OXA-1</sub>	<i>aph(3')-VIa</i>	<i>strA</i>	<i>strB</i>	<i>aac(3)-IIa</i>	<i>aac(6')IIb-cr</i>	<i>aac(6')-Ib</i>	<i>aadA1</i>	<i>oqxA</i>	<i>oqxB</i>	<i>qnrB66</i>	<i>qnrS1</i>	<i>aac(6')Ib-cr</i>	<i>fosA</i>	<i>catB3</i>	<i>sul2</i>	<i>tet(A)</i>	<i>dfrA14</i>	mg/l	
Hospital A	1A																							12	
	2A																							3	
	3A																							24	
	4A																							16	
	5A																							16	
	6A																							16	
	7A																							16	
	8A																								24
	9A																								16
Hospital B	1B																							8	
	2B																							4	
	3B																							6	
	4B																							2	
	5B																							3	
	6B																							3	
	7B																							4	
	8B																								3
	9B																								1.5
	10B																								3
	11B																								3
	12B																								3
	13B																								2
	14B																								4
	15B																								3
	16B																								4
17B																								3	
18B																								3	
19B																								3	
20B																								3	
NC 1E																								2	

Antimicrobial resistance phenotype – agreement with antimicrobial resistance gene

# RESISTOME: OUTBREAK A & B NDM-Kp ST716

	Beta-lactamase					Aminoglycoside					Fluoroquinolone					Others					Amikacin			
	<i>bla</i> <sub>SHV-27</sub>	<i>bla</i> <sub>NDM-1</sub>	<i>bla</i> <sub>TEM-1B</sub>	<i>bla</i> <sub>CTX-M-15</sub>	<i>bla</i> <sub>OXA-9</sub>	<i>bla</i> <sub>OXA-1</sub>	<i>aph(3')-VIa</i>	<i>strA</i>	<i>strB</i>	<i>aac(3)-IIa</i>	<i>aac(6')Ib-cr</i>	<i>aac(6')-Ib</i>	<i>aadA1</i>	<i>oqxA</i>	<i>oqxB</i>	<i>qnrB66</i>	<i>qnrS1</i>	<i>aac(6')Ib-cr</i>	<i>fosA</i>	<i>catB3</i>	<i>sul2</i>	<i>tet(A)</i>	<i>dfrA14</i>	mg/l
																								
<b>Tn1331</b>																								
Hospital A	1A																							12
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	4A																							16
	5A																							16
	6A																							16
	7A																							16
	8A																							24
	9A																							16
Hospital B	1B																							8
	2B																							4
	3B																							6
	4B																							2
	5B																							3
	6B																							3
	7B																							4
	8B																							3
	9B																							1.5
	10B																							3
	11B																							3
	12B																							3
	13B																							2
	14B																							4
	15B																							3
	16B																							4
	17B																							3
	18B																							3
	19B																							3
	20B																							3
NC 1E																								2

Lost 3 resistance determinants including blaOXA-9 gene → transposon 1331



- Overlap of two patients at the day hospital of **Hospital B**
  - 2<sup>nd</sup> Case **Hospital A**
  - 1<sup>st</sup> Case **Hospital B**

- NDM-Kp ST716 is a new epidemic clone causing a large outbreak in two Belgian hospitals
- WGS is highly discriminant for molecular typing of NDM-Kp outbreaks
- WGS suggests the transmission pathway of a nosocomial pathogen between a teaching and a general hospital





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Fonds Erasme

POUR LA RECHERCHE MÉDICALE

Research grant  
Amélie Heinrichs



Katrien De Bruyne  
Jill Dombrecht  
Kathleen Vranckx