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# **Dissemination of acquired carbapenemases in Gram-negative bacilli: a story of succesful clones**

Christian G. Giske, MD/PhD

Associate professor / Consultant physician

Clinical microbiology

Karolinska University Hospital

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# Acknowledgements

- Members of my own research group
    - Aina Iversen
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    - Timothy Walsh and Mark Toleman (Cardiff)
    - Neil Woodford and David Livermore (HPA)
  - And finally: Gunnar Kahlmeter
-

## What is a clone – MLST definition (strict)

Isolate	Gene A	Gene B	Gene C	Gene D	Gene E	Gene F	Gene G
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	2
3	1	1	1	1	1	2	1
4	1	1	1	1	2	2	2

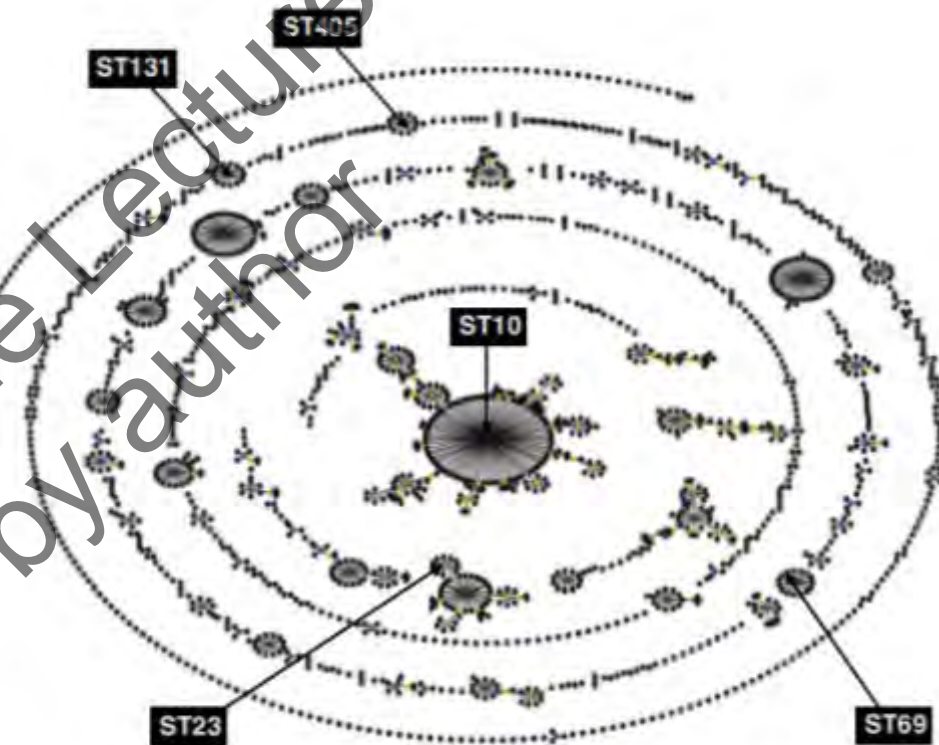
## High-Risk Clones (HiRC)

- Associated with resistance of great clinical importance
- AND
- High ability to spread in health-care institutions
- OR
- High ability to confer invasive disease
- OR
- High ability to colonize individuals for long time periods

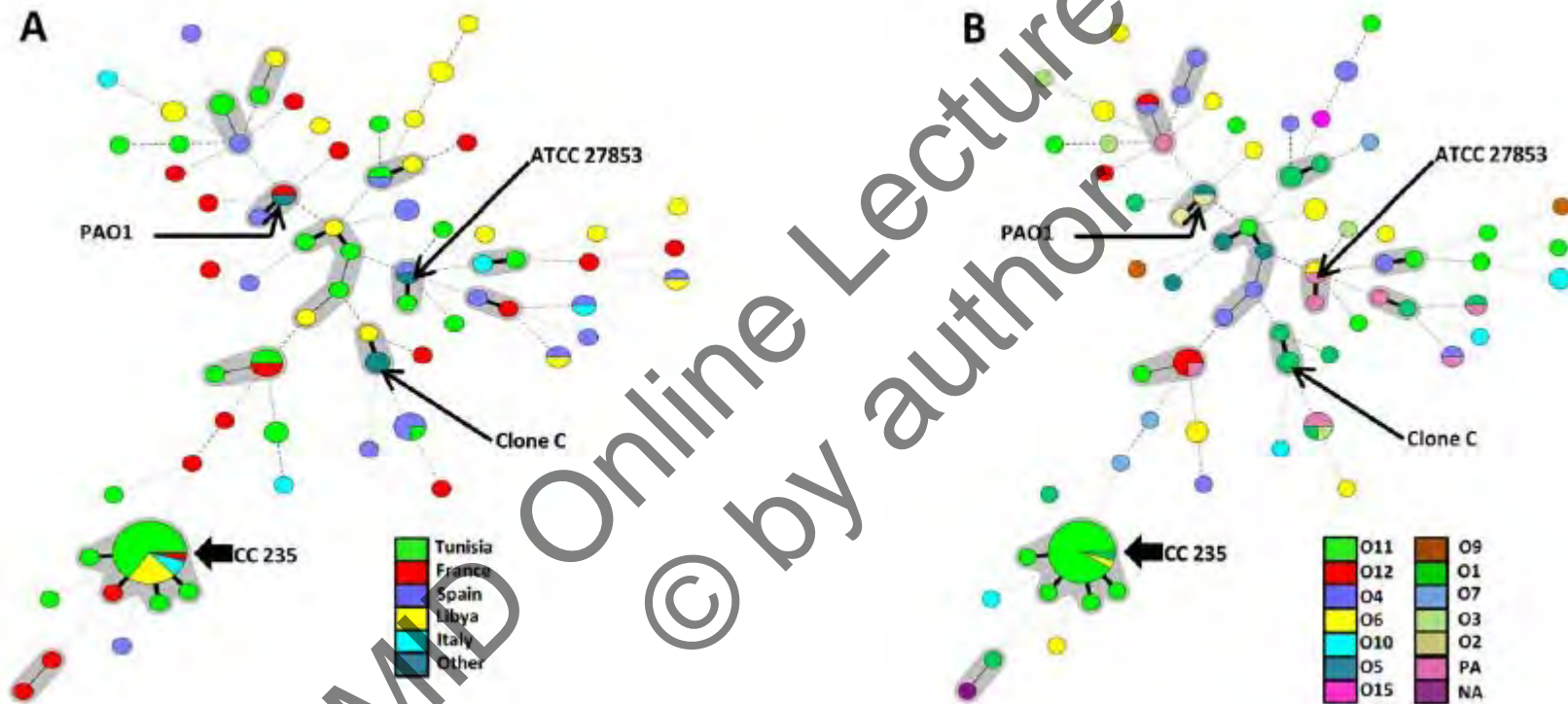
TROCAR project

## *E. coli* O25b ST131

- Uropathogenic clone that has acquired CTX-M
- 30-40% of ESBLs in Sweden belong to this clone
- Pandemic occurrence
- Often associated with community-acquired infections
- Has also been detected in retail meat and companion animals

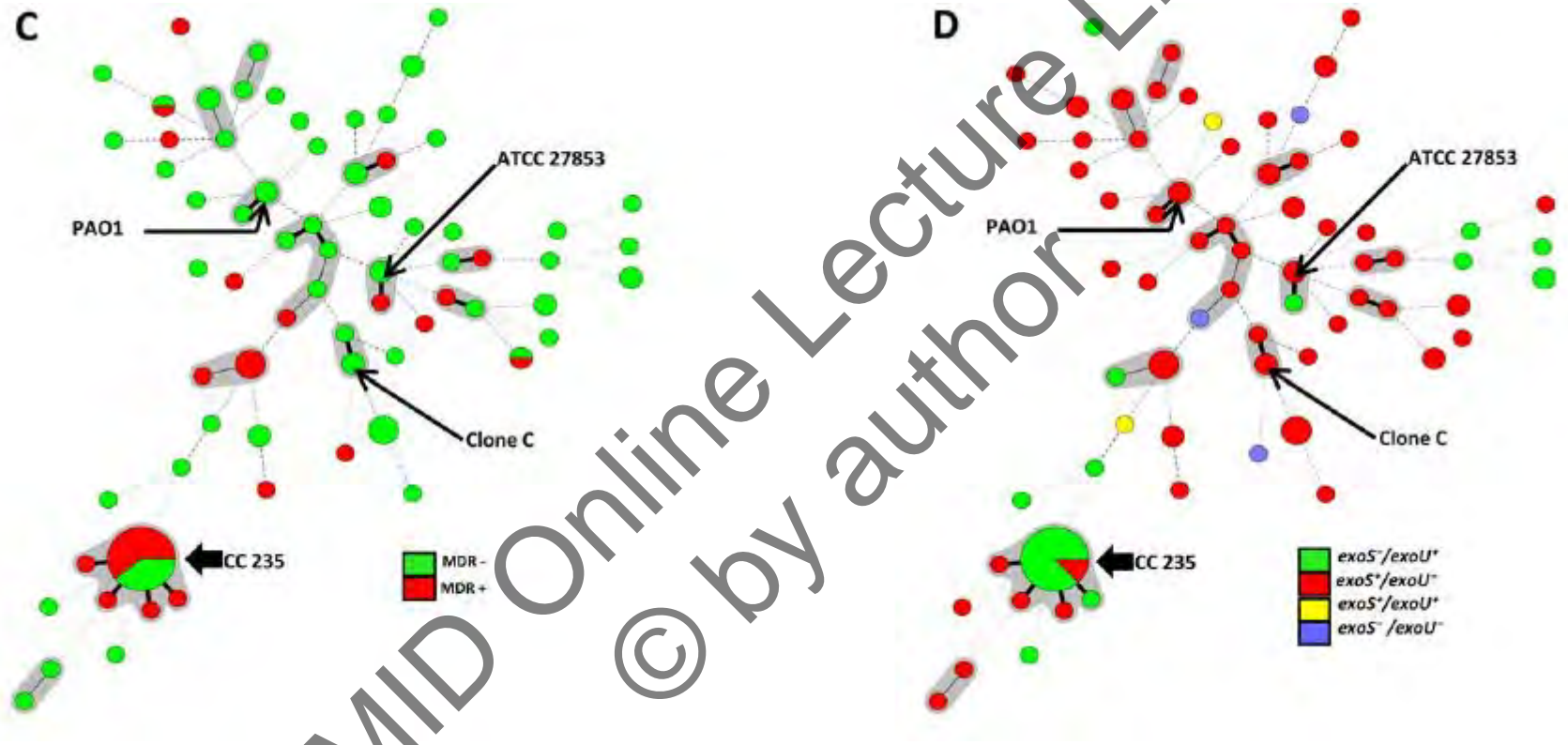


# *P. aeruginosa* CC235 O11



Maatallah M et al. PLoS One 2011

# *P. aeruginosa* CC235 cont'd

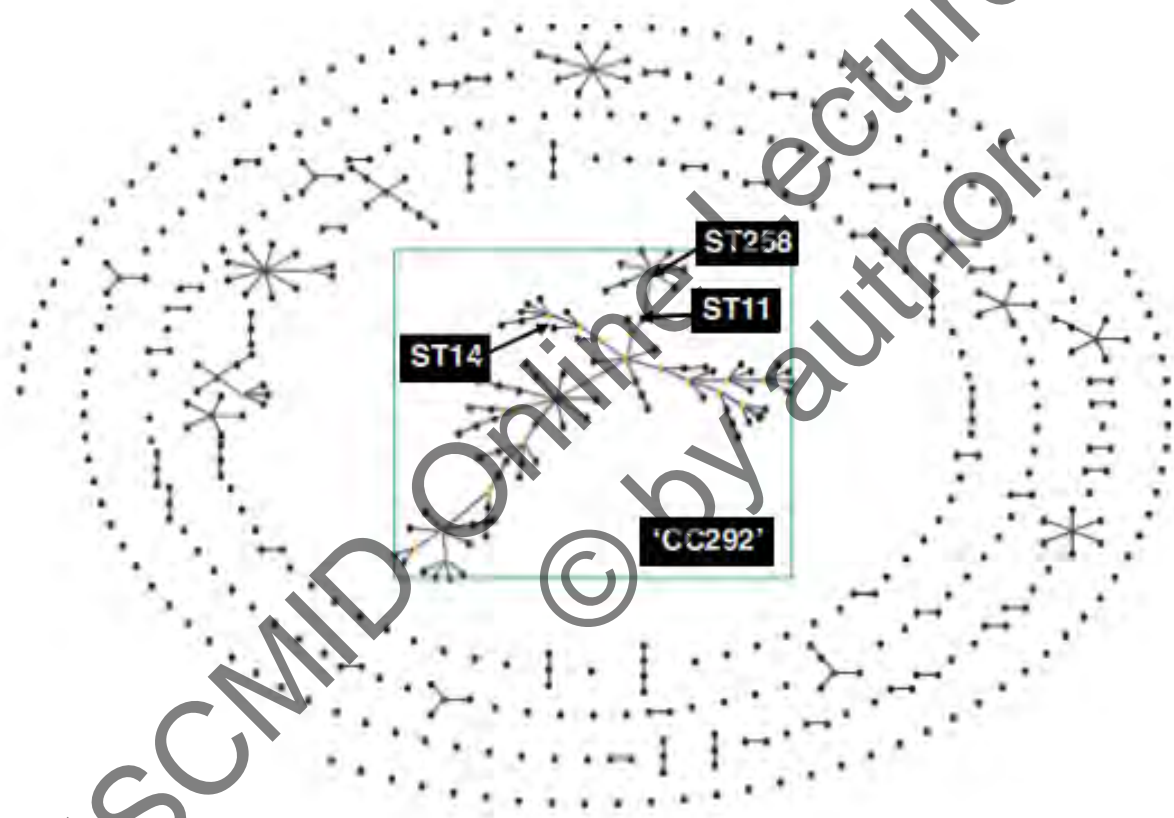


Maatallah M et al. PLoS One 2011

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# Population snapshot *K. pneumoniae*



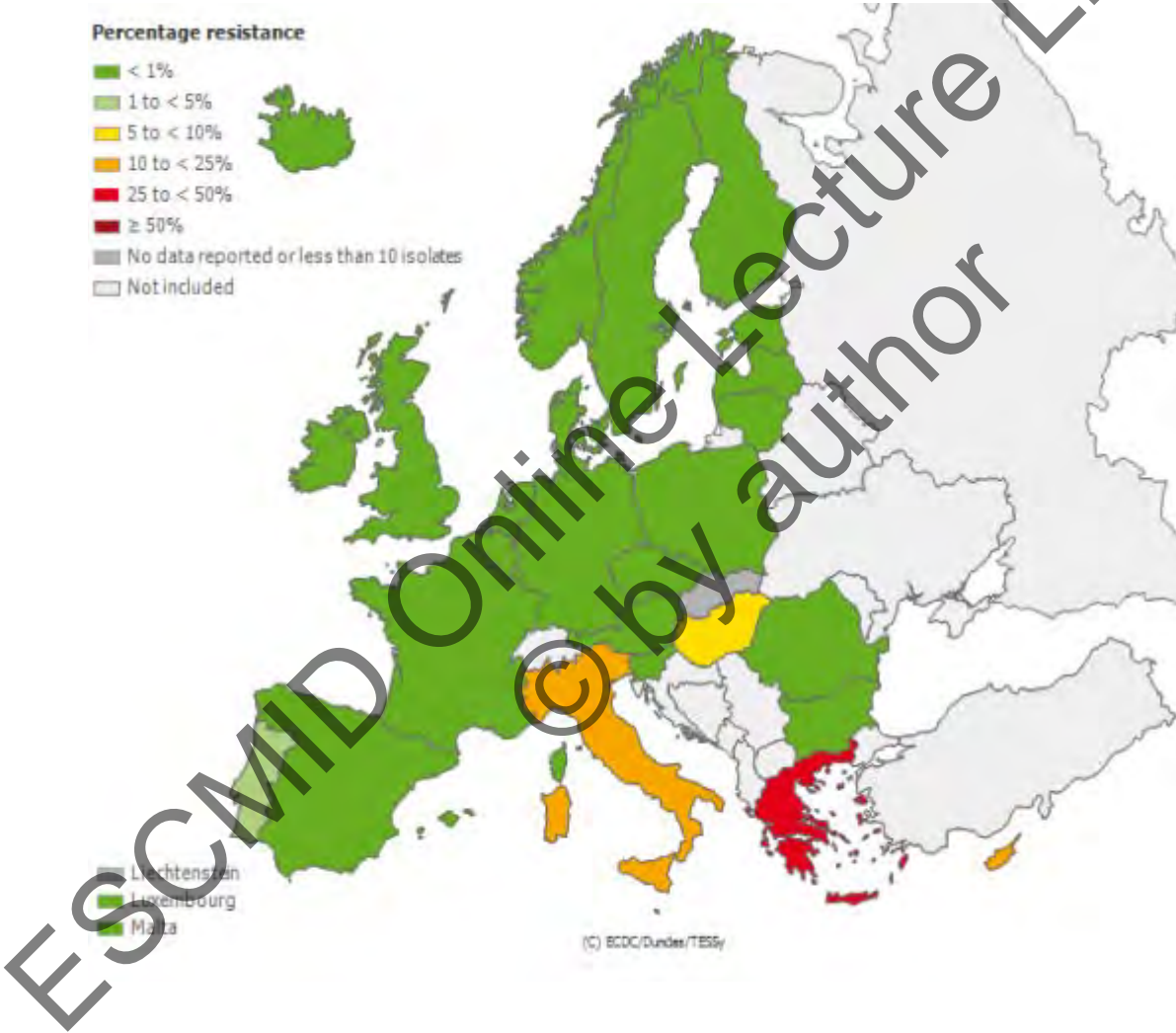
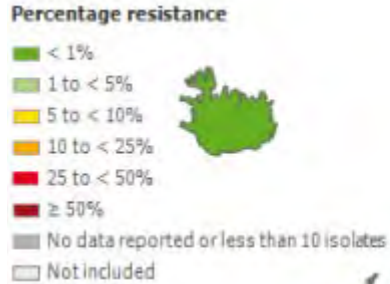
Woodford et al. FEMS Microbiol Lett 2011



# Carbapenem-resistant *K. pneumoniae*

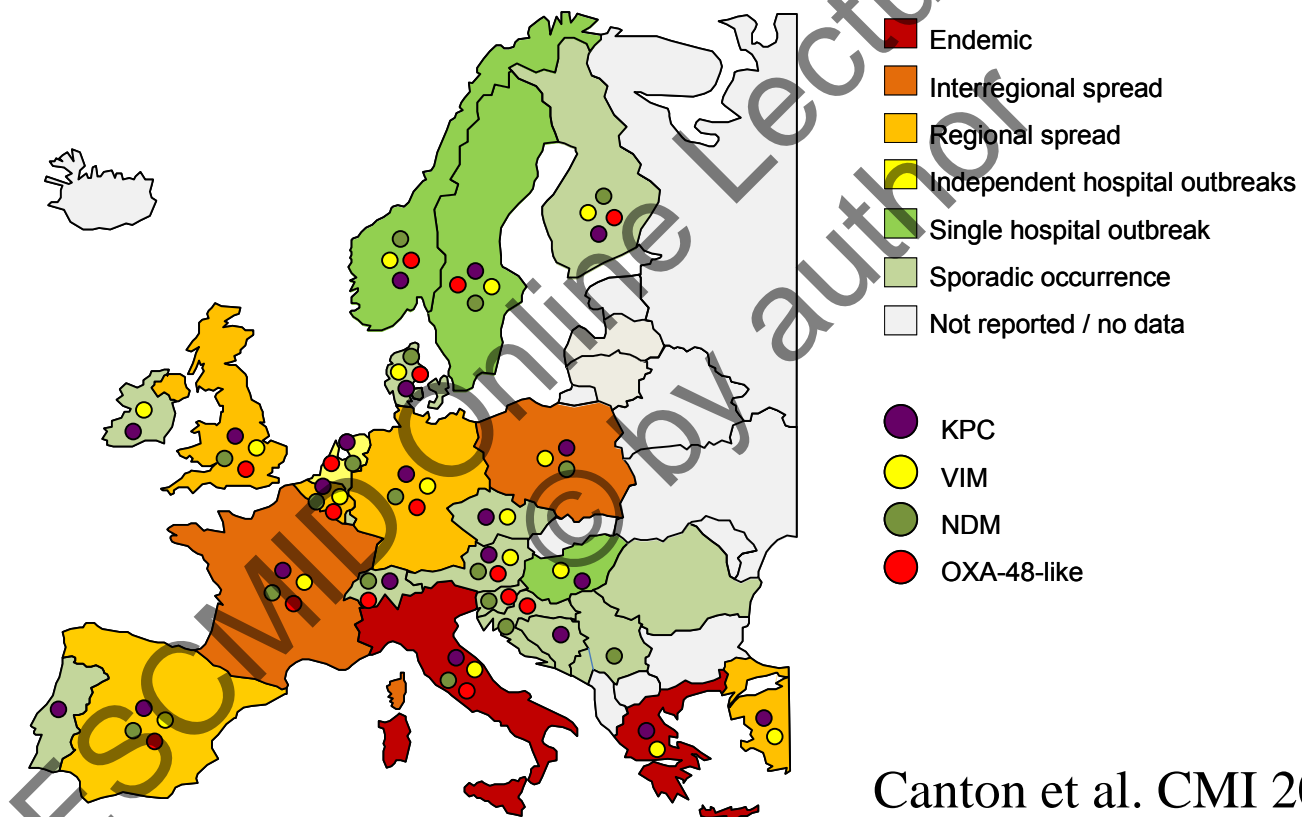


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(C) ECDC/Durder/TESSy

# Dissemination of carbapenemases in Europe



Canton et al. CMI 2012. In press



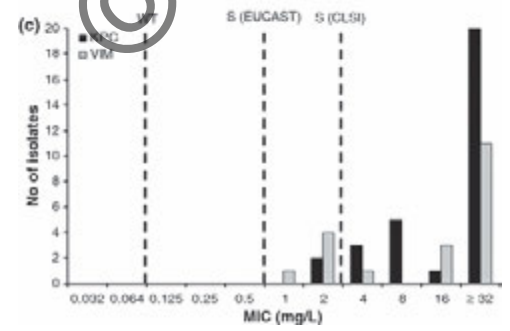
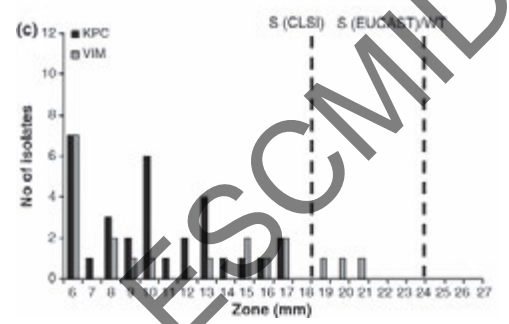
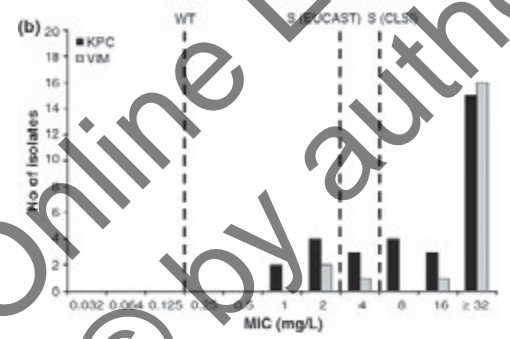
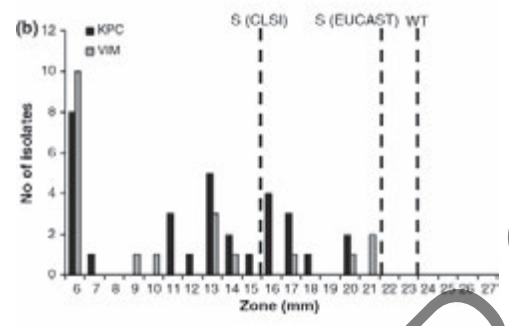
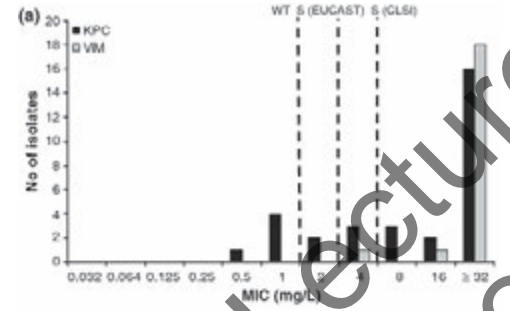
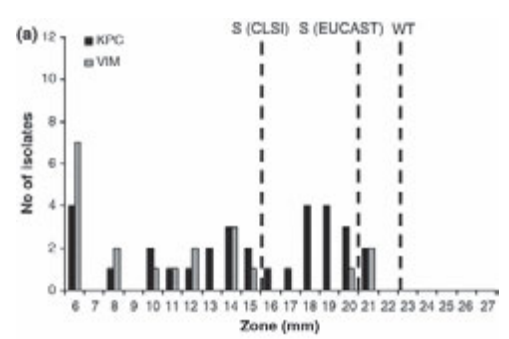
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## Major activities related to CPE

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# Are we identifying the carbapenemases?



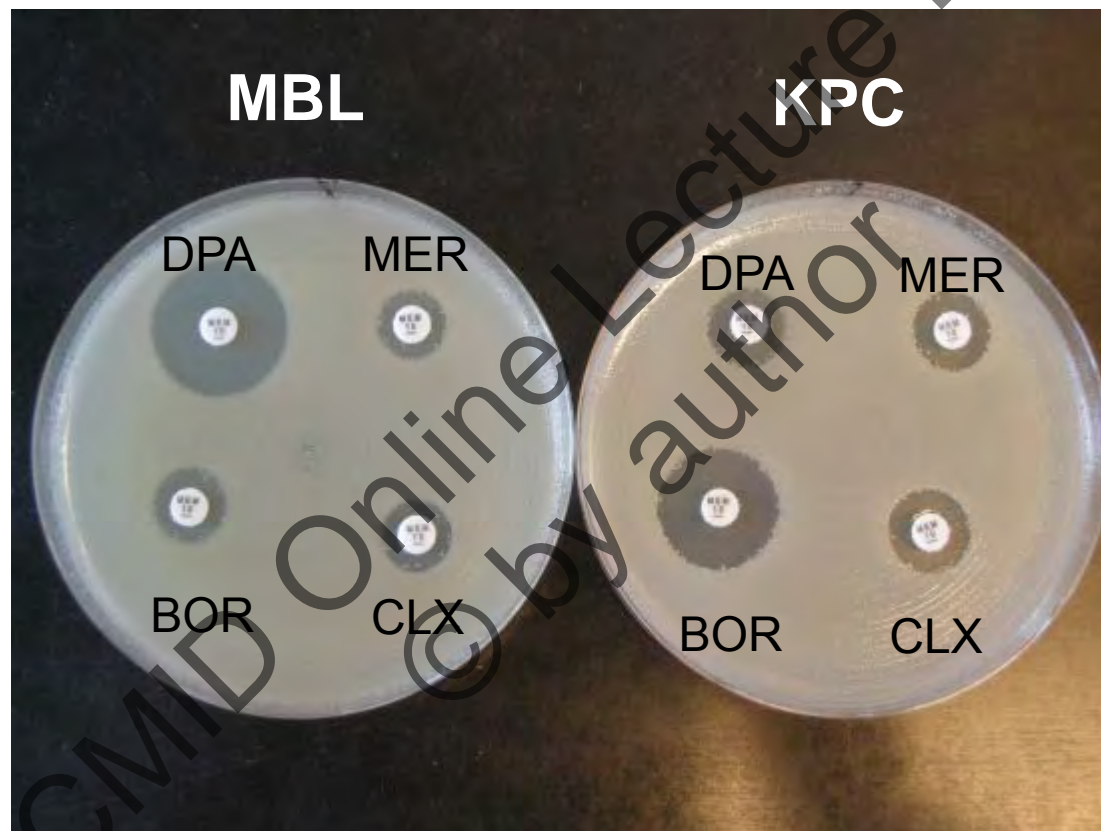
Vading et al. CMI 2011.

## Phenotypic detection of carbapenemases

β-lactamase	Inhibited by		
	EDTA/DPA	Boronic acid	Cloxacillin
MBL	Y	N	N
KPC/class A carbapenemases	N	Y	N
OXA-48 <sup>1</sup>	N	N	N
ESBL	N	N	N
AmpC	N	Y	Y

<sup>1</sup>OXA-48: high-grade resistant to temocillin

## Positive phenotypic tests: KPC and MBL



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# Sensitivities and specificities



Test	$\beta$ -lactamase	Sensitivity	Specificity
APBA	KPC	100%	98%
APBA+CLX	AmpC	80%	100%
DPA	MBL	100%	100%
EDTA	MBL	100%	88%
Modified cloverleaf (Hodge) test	Carbapene- mases	100%	77%

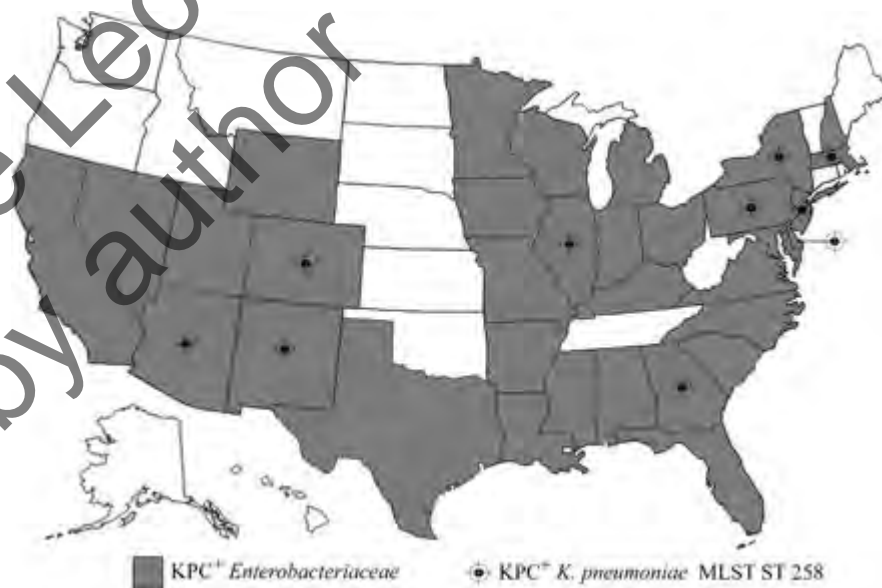
Giske et al. CMI 2011



## *K. pneumoniae* ST258



- Clone associated with the spread of the carbapenemase KPC
- Initially described in USA, then in most countries where KPC has been detected
- Responsible for 70% of the cases of KPC in the US and most cases in Israel
- Also capable of causing invasive disease

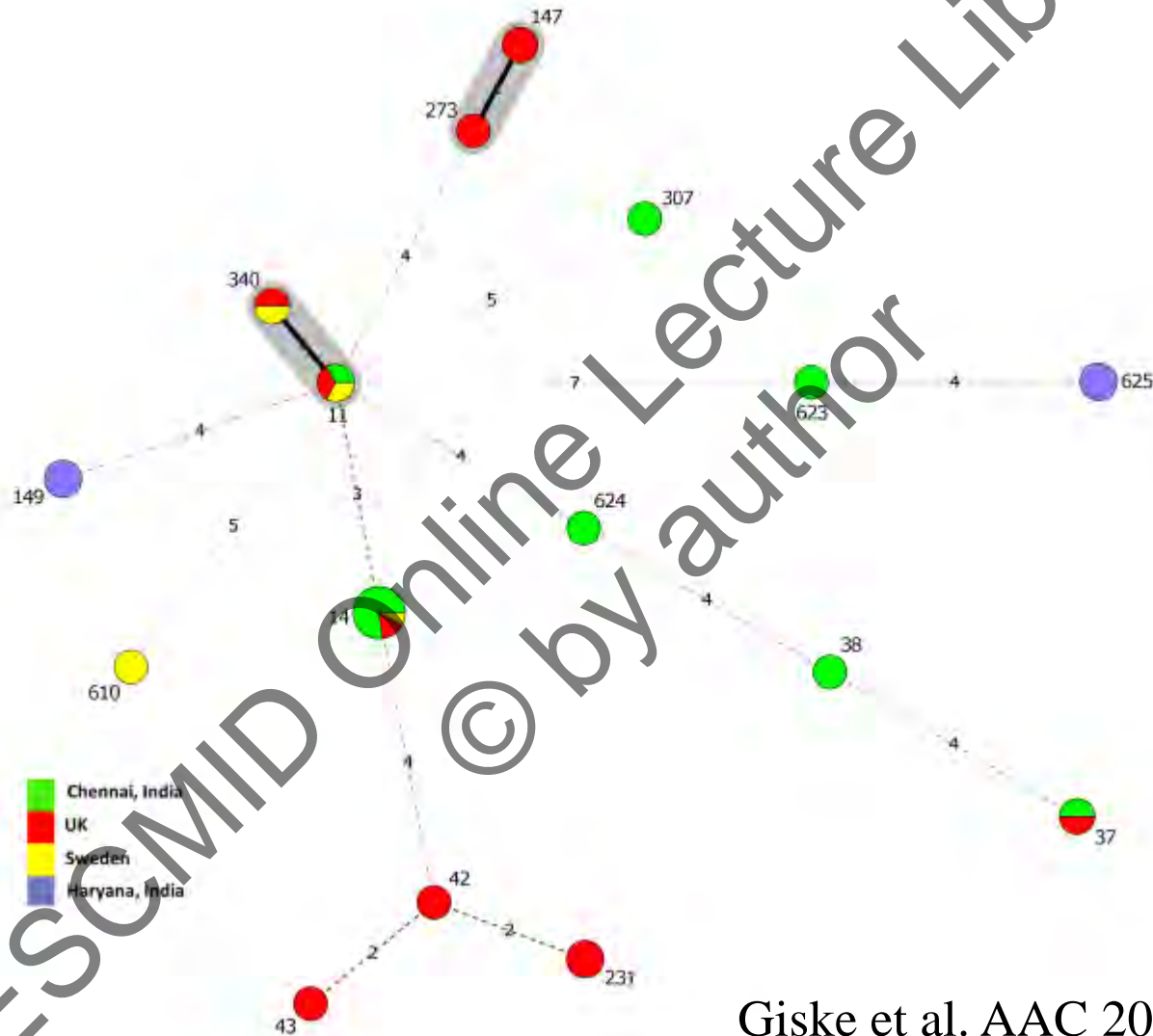


Kitchel et al. AAC 2009

# NDM-1: STs vs replicon types



# NDM: ST vs country

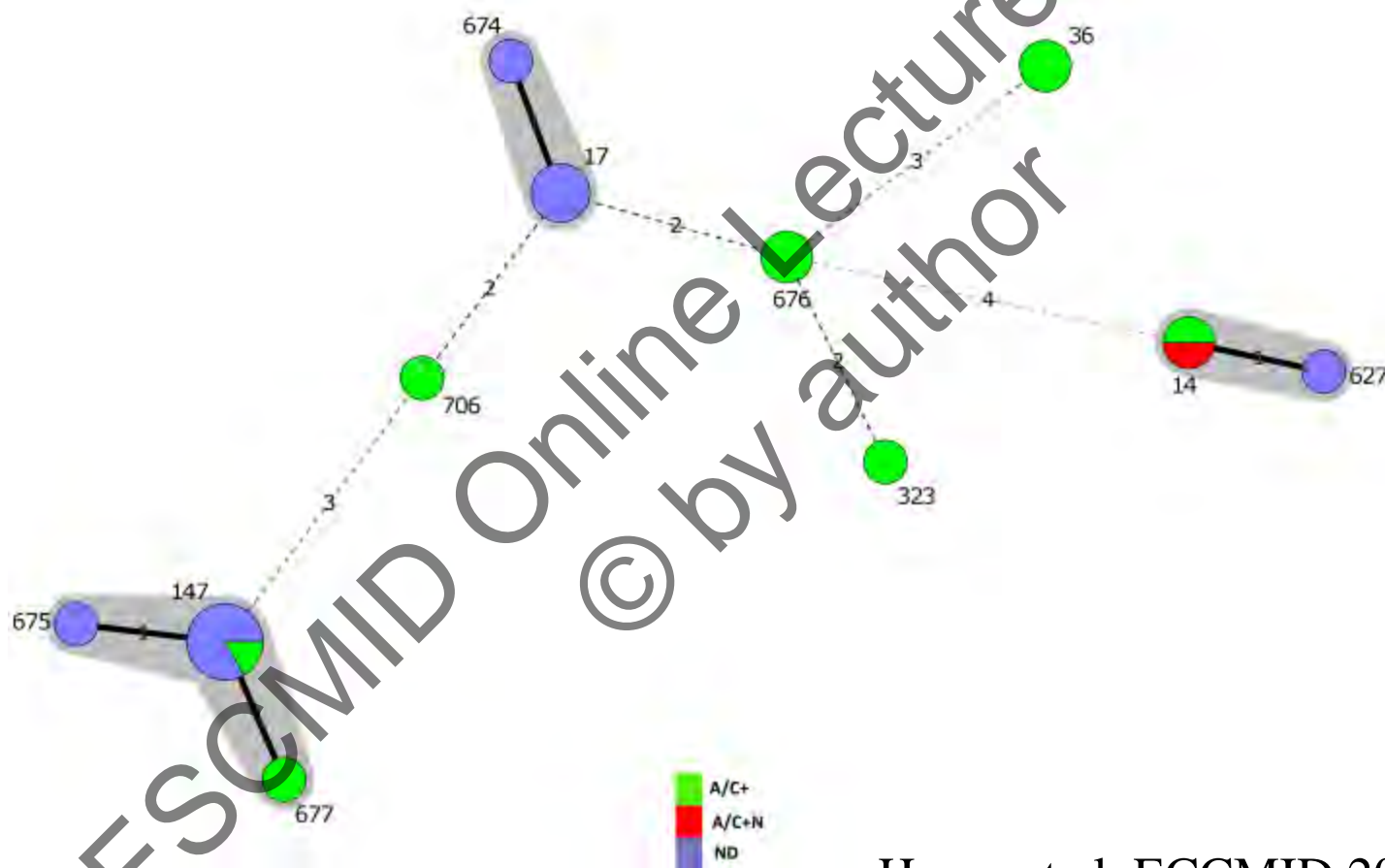


Giske et al. AAC 2012 In press

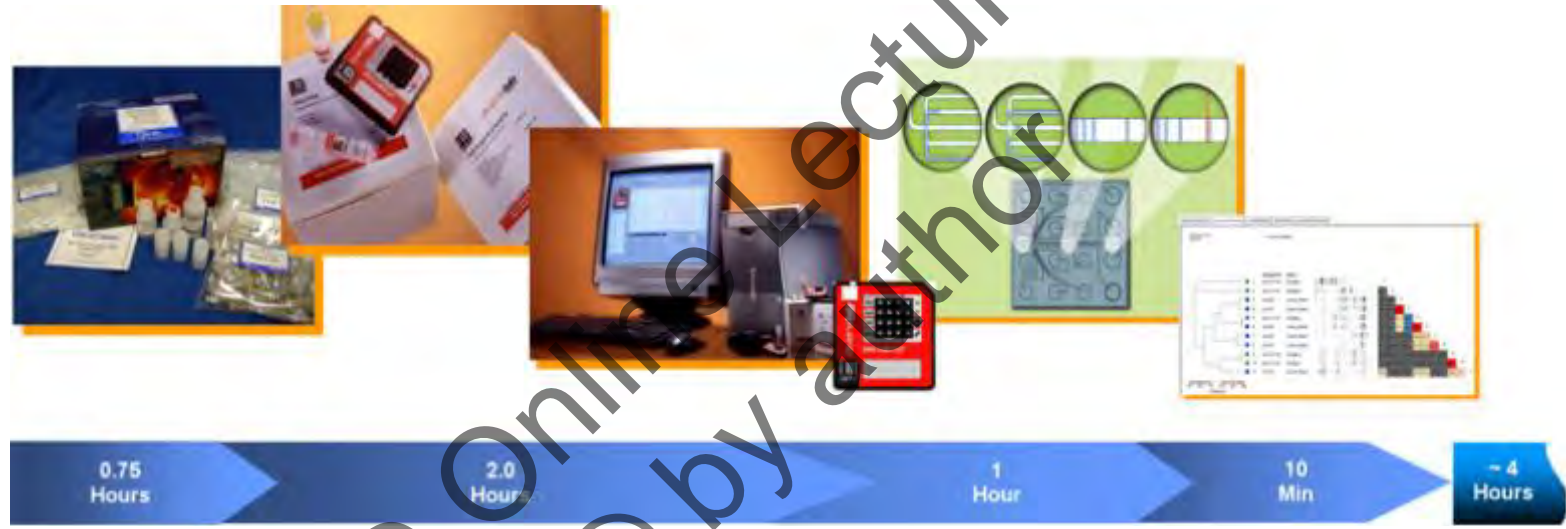
## Serotypes and virulence factors

- Capsular serotype K2 detected in all ST14 isolates
- K1, K5, K20, K54, K57 (associated with invasive disease) not detected
- Virulence gene screening did not generate any positive isolates among NDM-1- and VIM-producers
  - *allS* (promoting growth in iron-deficient media)
  - *rmpA* (regulating extra-capsular polysaccharide synthesis)
  - *wcaG* (increasing escaping capacity of phagocytosis by synthesizing fucose)

# VIM-producing *K. pneumoniae*



# Automated rep-PCR



## Extraction

From clinical isolates

## Rep-PCR amplification

With suitable  
fingerprinting kits

## Detection

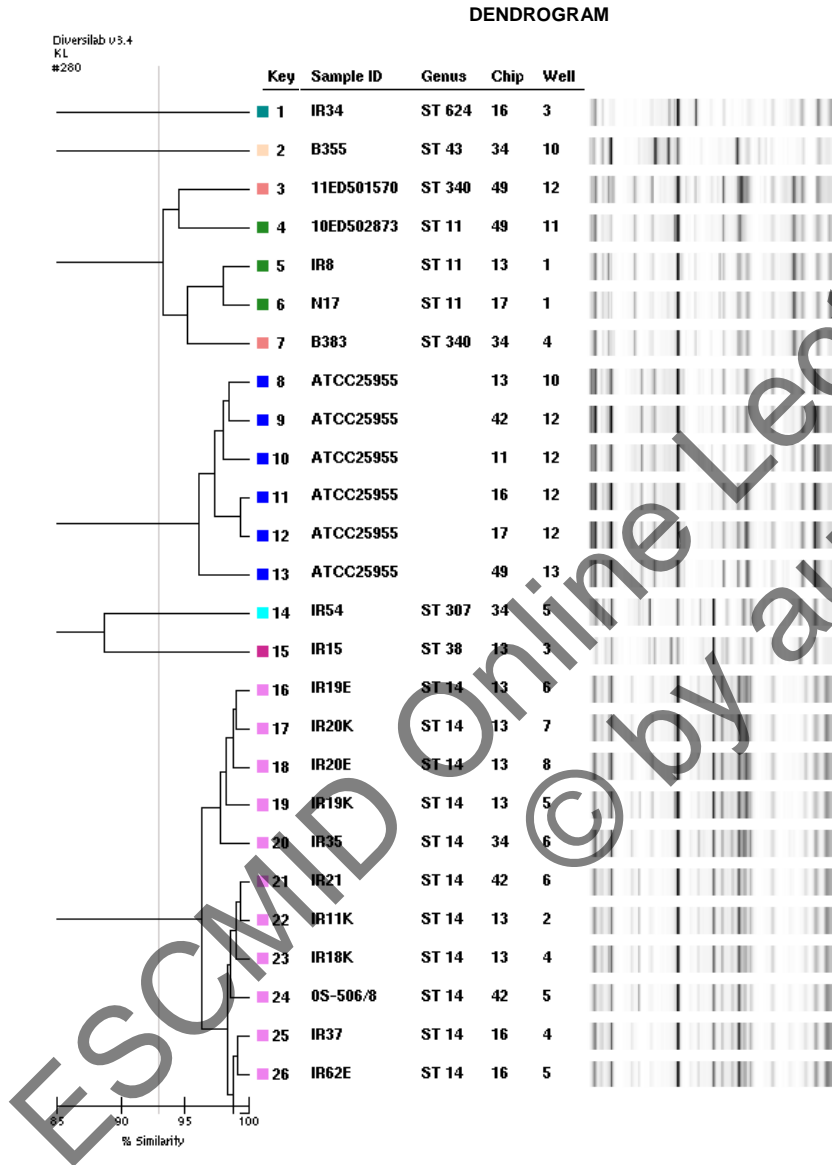
Eelectrophoresis  
(Agilent  
Bioanalyzer)

## Analysis

Web-based  
software

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# DiversiLab NDM-1-producers



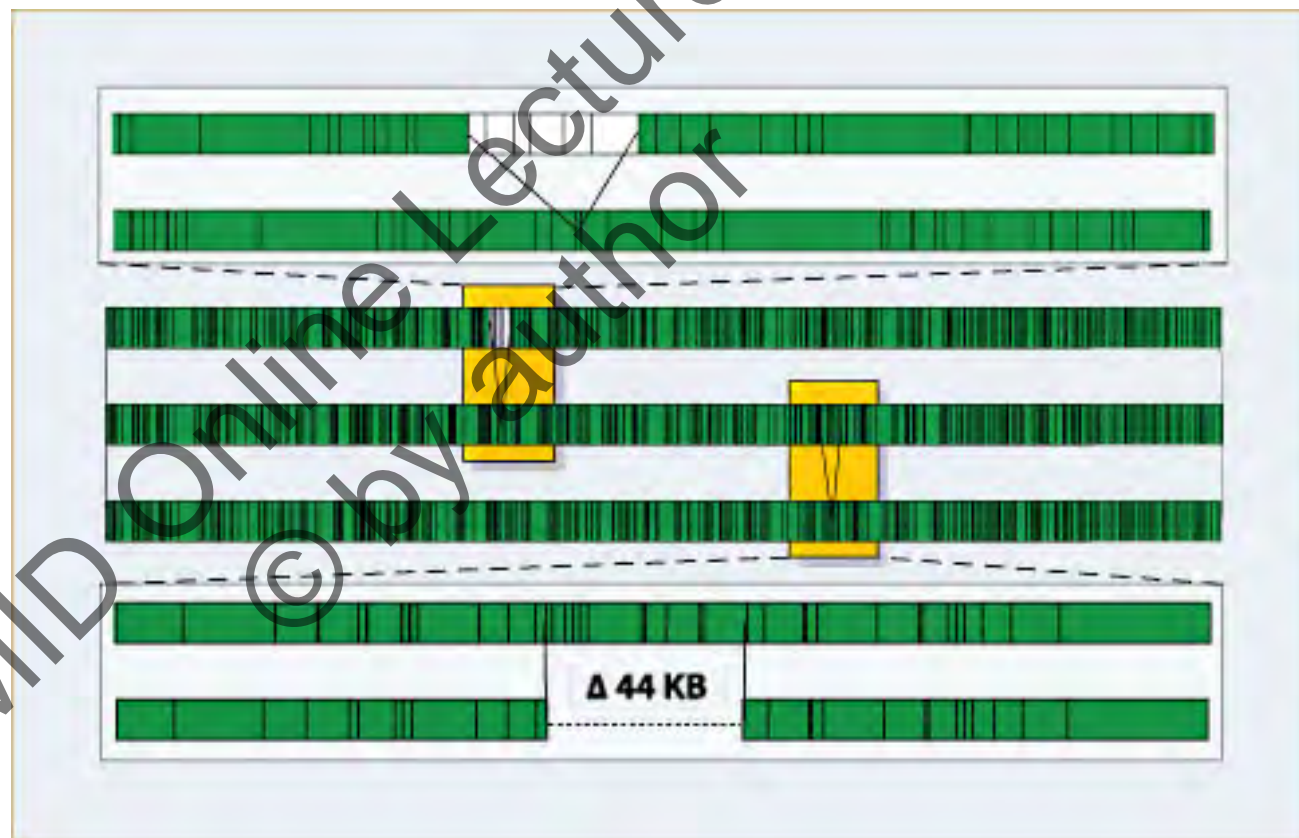
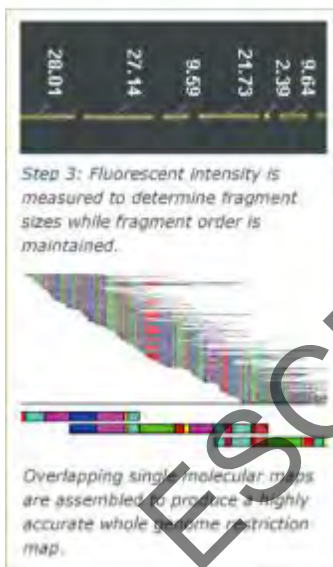
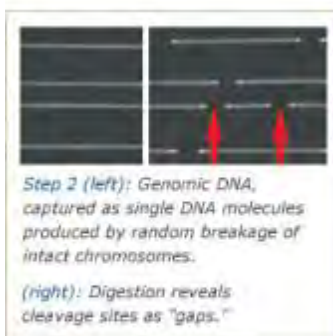
Giske et al. AAC 2012 In press



# DiversiLab and identification of HiRC



# Why are some strains successful?





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**Some other topics dealt with in the group**

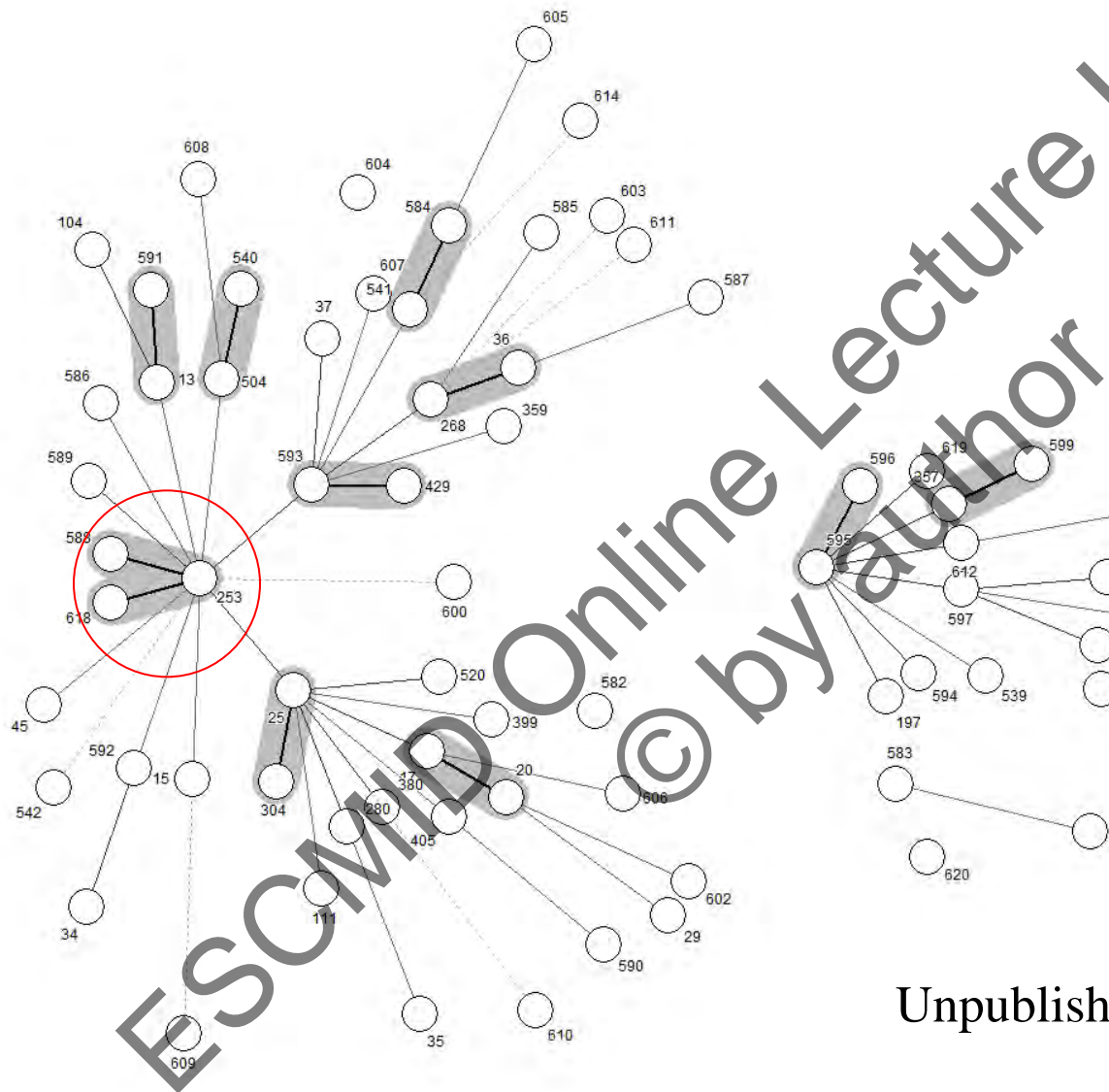
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# Clonality of invasive *K. pneumoniae*

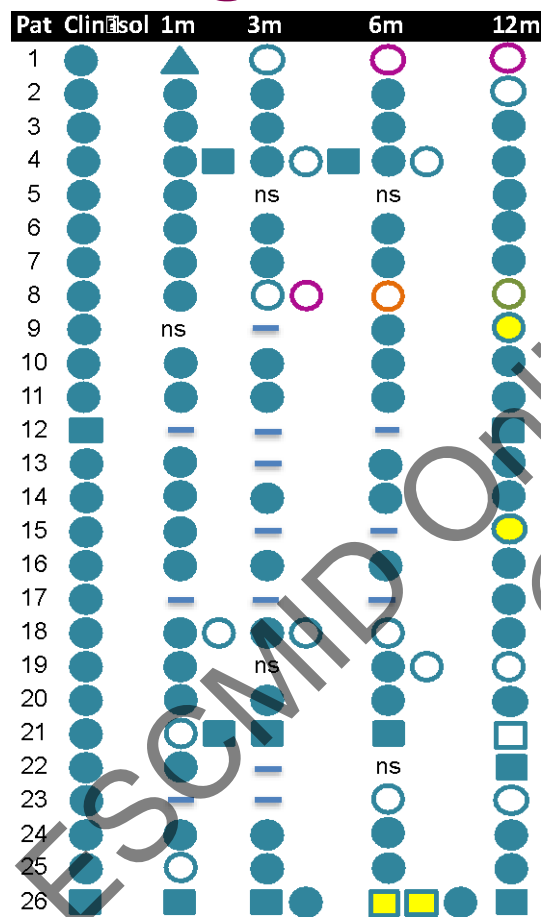


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Unpublished data

# Duration of fecal carriage of ESBL-producing Enterobacteriaceae

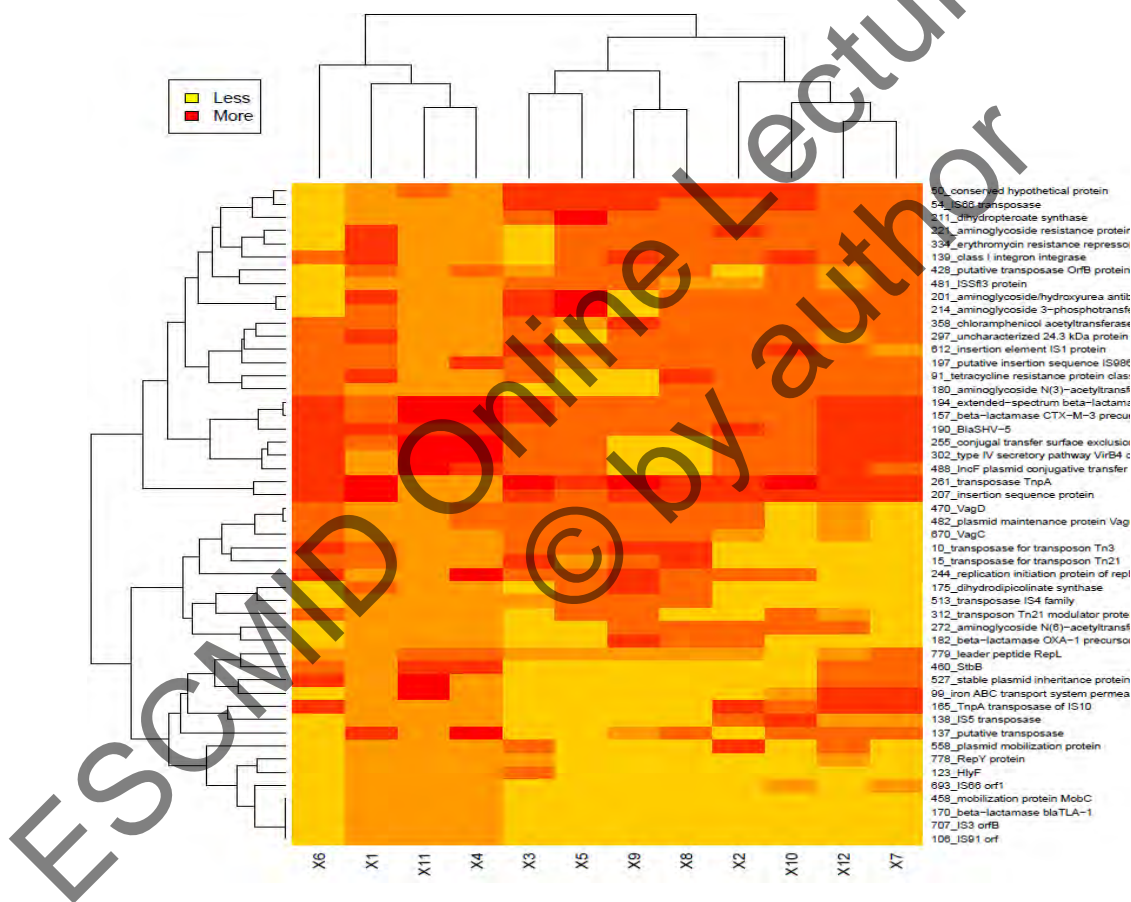


Factor	Carriers (%)	Non-carriers (%)	p
Blood stream infection	23	3	0.04
Carbapenem treatment	19	3	0.07
Phylogroup B2	52	21	0.04
ST131	39	17	0.13



- Control
- 6, clinical isolate
- 6, 1m
- 6, 3m
- 6, 6m
- 6, 12m
- Control
- 30, clinical isolate
- 30, 1m
- 30, 3m
- 30, 6m
- 5, clinical isolate
- 5, 1m
- 5, 12m
- control

# Next generation sequencing of ESBL-plasmids (Swedish Institute for Disease Control)



Brolund et al.



## Concluding remarks

- Successful clones play an important role in the dissemination of carbapenemase-producing *K. pneumoniae*
  - Some of the key CCs are ST11-ST258, ST14-ST15 and ST147-ST273
  - ST14 is coupled to capsular serotype K2, but generally isolates are devoid of important virulence factors (those studied...)
  - It seems possible to use automated rep-PCR as a proxy for MLST for rapid identification of successful clones
  - Further studies are needed to address the question of why some isolates are successful
-