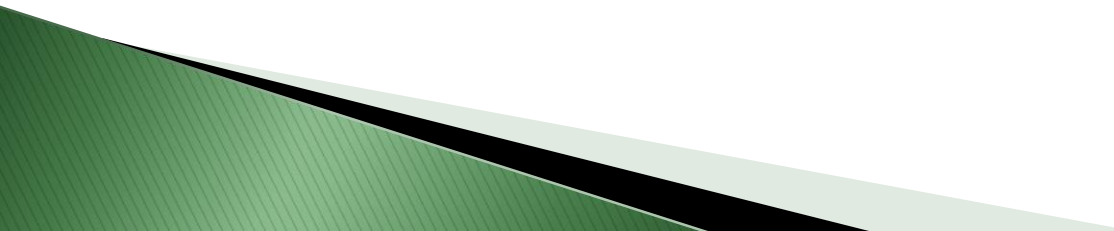


Plasmids and their role in transmission of β -lactam resistance genes in Enterobacteriaceae from divers reservoirs

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Key points:

1. Introduction to plasmid biology
 2. Plasmid encoded resistance to β - lactams (basic theories)
 3. Projects I work with
 4. Characterization of resistance plasmids -overview of methods
 5. Discussion/ Questions
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Definition of a plasmid

- ▶ Plasmid– usually circular, double stranded DNA molecule found in diverse bacteria
- ▶ Plasmids replicate autonomously from bacterial chromosome
- ▶ The minimal region required for replication and maintenance is termed basic replicon
- ▶ In nature replication occurs only in the cytoplasm of bacterium
- ▶ Plasmids tend to have modular compositions

References:

Carattoli,A., 2011. Plasmids in Gram negatives: Molecular typing of resistance plasmids. Int. J. Med. Microbiol..

Plasmids have modular compositions

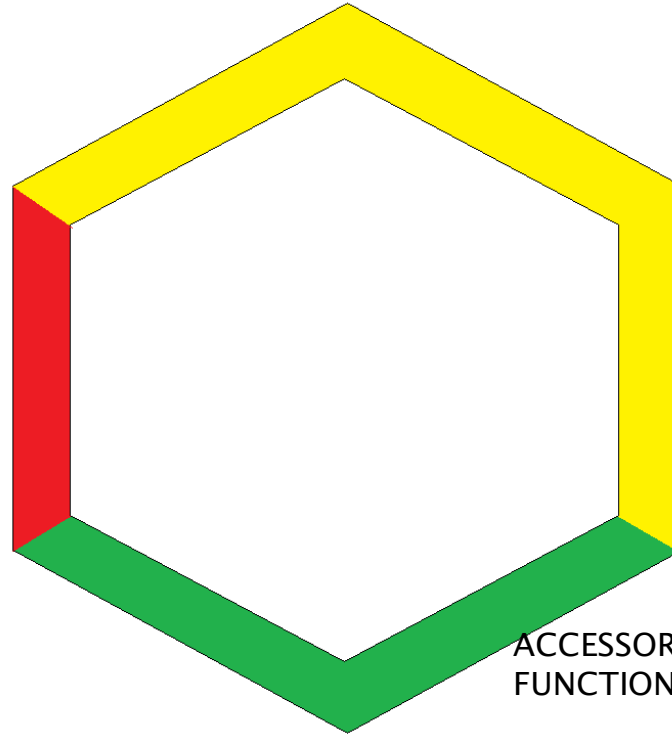
REPLICON

Subunits

- i) initiation of replication
- ii) control of replication

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PARTITIONING / STABLE INHERITANCE MODULE



TRANSFER REGION

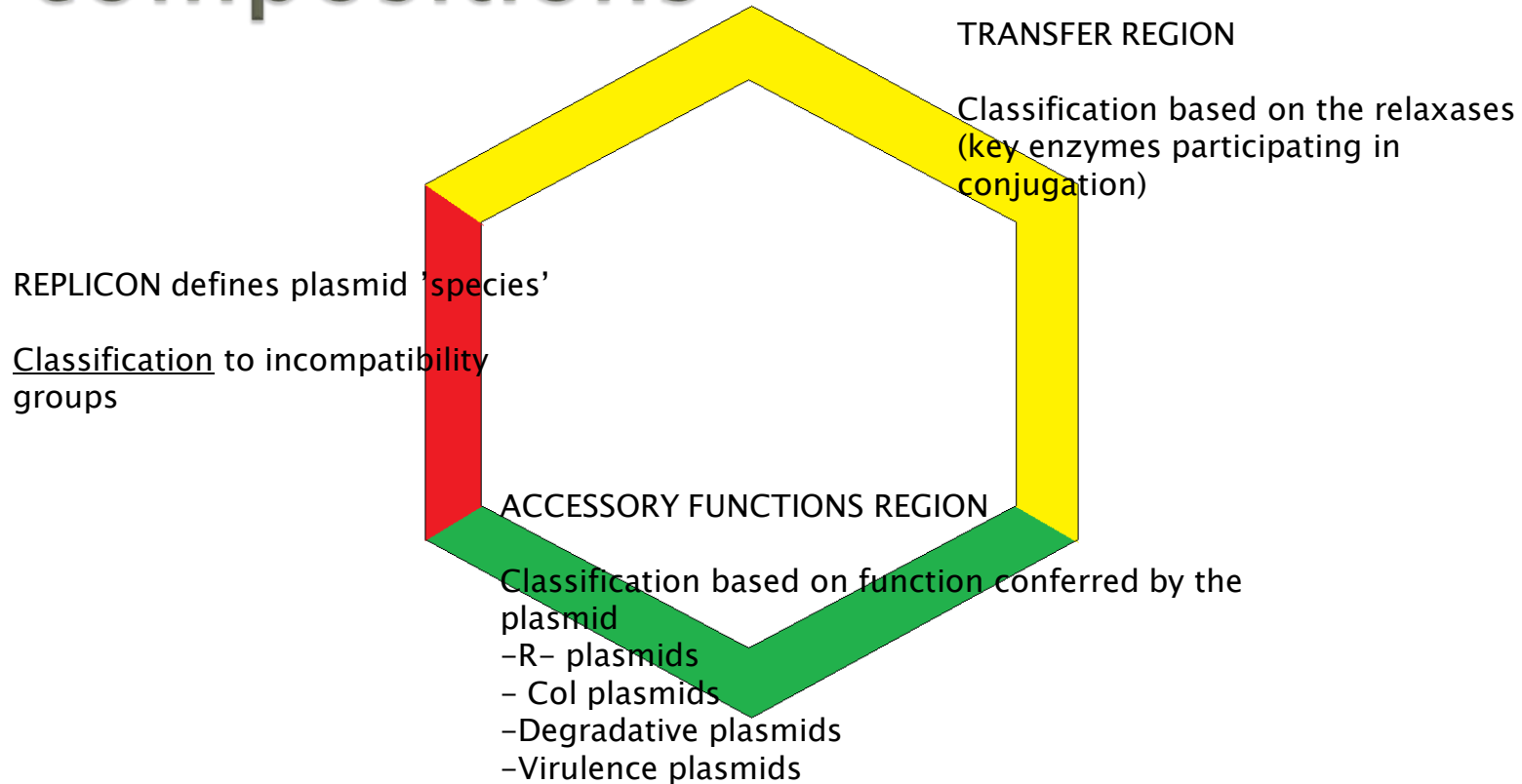
Subunits

- i) Mating pair formation (genes encoding 'the channel')
- ii) Coupling protein unit
- iii) Relaxases (enzymes 'processing' the plasmid DNA in conjugation)

ACCESSORY FUNCTIONS REGION

- i) Resistance genes (antibiotics, heavy metals, disinfectants etc.)
- ii) Colicins (bacteriocins) production
- iii) Degradation of various compounds
- iv) Virulence functions

Plasmids have modular compositions



References:
Carattoli,A., Bertini,A., Villa,L., Falbo,V., Hopkins,K.L., Threlfall,E.J., 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods, 63, 219-228.

Garcillan-Barcia,M.P., Alvarado,A., de la,C.F., 2011. Identification of bacterial plasmids based on mobility and plasmid population biology. FEMS Microbiol. Rev., 35, 936-956.

Replicon defines plasmid 'species'

- If two replicons are similar then they tend to be incompatible (not able to stably co-exist in the same bacterium)
- Different Incompatibility groups \approx different plasmid 'species'
- Currently 27 Inc groups recognized in Enterobacteriaceae by Plasmid Section of the National Collection Type Culture (Colindale, London)
- In practice there are more than 30 Inc groups termed from IncA/C to IncZ and numerous subgroups (IncFI, FII-V, IncX1,-2 etc...)

▶ Plasmids from different Inc groups differ in their properties

Narrow host range/ usually encode full conjugation machinery	Broad host range/ often mobilizable
<p data-bbox="239 636 938 665">IncF (FIA, FIB, FII and others) large, low copy, conjugative</p> <p data-bbox="558 689 620 715">IncI1</p> <p data-bbox="542 739 635 765">IncL/M</p> <p data-bbox="504 789 674 815">IncHI1, IncHI2</p> <p data-bbox="542 839 635 865">IncB/O</p> <p data-bbox="562 889 616 915">IncX</p> <p data-bbox="562 939 616 965">IncU</p>	<p data-bbox="1288 636 1717 665">IncQ, small, high copy, mobilizable</p> <p data-bbox="1288 689 1717 718">IncA/C large, low copy, conjugative</p> <p data-bbox="1338 739 1667 768">IncN medium, conjugative</p> <p data-bbox="1470 789 1532 815">IncW</p> <p data-bbox="1470 839 1532 865">IncP</p> <p data-bbox="1470 889 1532 915">IncR</p>

Inc groups most frequently detected in Enterobacteriaceae

Resistance to β -lactams

- ▶ β -lactam antibiotics are classified to different families: penicillins, aminopenicillins, cephalosporins, carbapenems and monobactams
- ▶ They are group of most commonly used antimicrobials in treatment of bacterial infections, especially infections caused by G-negatives from family Enterobacteriaceae
- ▶ Increasing occurrence of resistance to β -lactams in G negative bacteria has become a major problem
- ▶ *bla* genes (*bla* for beta lactam resistance) often reside on transferable plasmids!

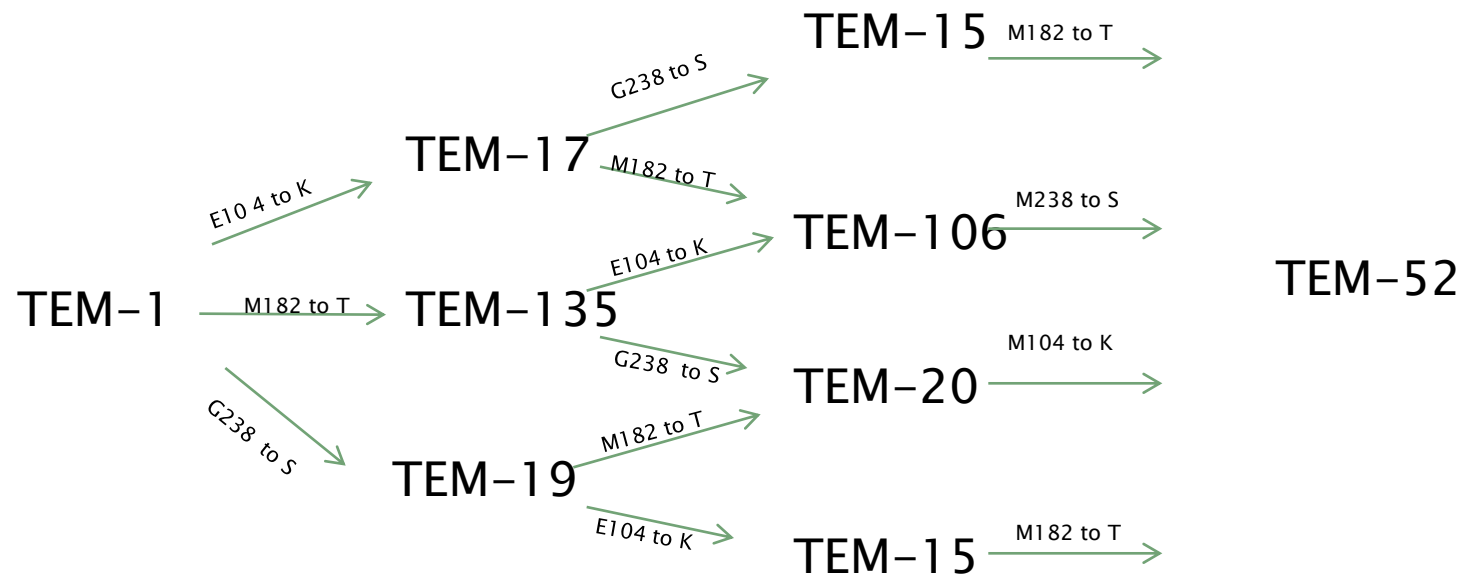
Variety of *bla* genes found on plasmids

Gene		β -lactamases encoded (e.g. enzymes degrading the β -lactam antibiotics)
<i>bla</i> _{TEM}	→	TEM
<i>bla</i> _{CTX-M}	→	CTX-M
<i>bla</i> _{SHV}	→	SHV
<i>bla</i> _{OXA, VIM, IMP, KPC etc.}	→	OXA, VIM, IMP, KPC etc.
<i>ampC</i> (<i>bla</i> _{CMY} , <i>bla</i> _{DHA})	→	AmpC (CMY, DHA)

TEM type β -lactamases

- ▶ Numerous variants of bla_{TEM} exist that encode corresponding TEM enzymes (these TEM variants are designated with numbers)
- ▶ TEM-1 is the most commonly encountered β -lactamase in G-negative bacteria (resistance to penicillins, aminopenicillins like ampicillin, amoxycillin)
- ▶ Mutations in the $bla_{TEM 1}$ gene may result in enzyme products with Extended Spectrum β -lactamases (ESBLs) properties

TEM type β -lactamases



This figure originates from: Hasman, H., Bergenholtz, R. D., Jørgensen, M. J., Jensen, L. B. & Aarestrup, F. M. 'Global diversity of plasmids carrying the *TEM-52* gene', International Plasmid Biology Conference 2008, Poster presentation.

Why are plasmids harbouring the *bla*_{TEM} genes interesting to study

Why are plasmids harbouring resistance genes interesting to study

- ▶ Because plasmids are transporters for the resistance genes between bacteria and between reservoirs
- ▶ Are there specific plasmid 'species' that are responsible for transmission of the *bla*_{TEM} genes (or other resistance genes) in enteric bacteria ?
- ▶ Specifying thus limiting the number of targets [for example diverse replicons] increases the chances to find the anti-target approach →
- ▶ Knowing on which platforms the resistance genes are transmitted and on which platforms the evolution of the resistance genes occurs we should be able to model the spread of plasmids and associated resistance genes
predict and prevent the further spread based on such models

Diversity of plasmids harbouring *bla*_{TEM-1} and *bla*_{TEM-52} genes

- ▶ Characterization of *bla*_{TEM-1} plasmids from *Escherichia coli* from poultry, cattle, humans (healthy and clinical isolates), pigs
- ▶ Characterization of *bla*_{TEM-52} plasmids (e.g. encoding ESBLs) from *Escherichia coli* and *Salmonella enterica* (isolates from poultry, poultry meet, human infections, broiler meet, beef)
- ▶ Bielak, E., Bergenholtz, R.D., Jorgensen, M.S., Sorensen, S.J., Hansen, L.H., Hasman, H., 2011. Investigation of diversity of plasmids carrying the *bla*_{TEM-52} gene. J. Antimicrob. Chemother., 66, 2465–2474.

Replicons associated with *bla*_{TEM-1} and *bla*_{TEM-52}

- ▶ IncI1, IncX1 (poultry)
- ▶ IncFII, IncFIA and IncFIB (all reservoirs)
- ▶ IncN
- ▶ IncB/O
- ▶ IncL/M; IncA/C; IncR

- ▶ Very similar IncI1 and IncX1 plasmids found both in human and animal reservoirs!

Plasmid characterization- methods

1.

Select wild type strains (WTs) with features of interest (fx harbouring *bla*_{TEM} gene) [Susceptibility test on WTs, sequencing of genes of interest, PFGE or other strain typing methods to verify the relatedness of WTs]

2.

Purify plasmids from WTs

3.

Transform plasmids mix into plasmid-free recipient – selection against the given marker on the plasmid of interest (fx ampicillin to select plasmid encoding TEM-1 enzyme)

4.

Verify that only the individual plasmid with the feature of interest transferred to the recipient (fx PCR to confirm the presence of the *bla*_{TEM} gene or other examined feature; S1-PFGE to see the number of plasmids in the strains)

5.

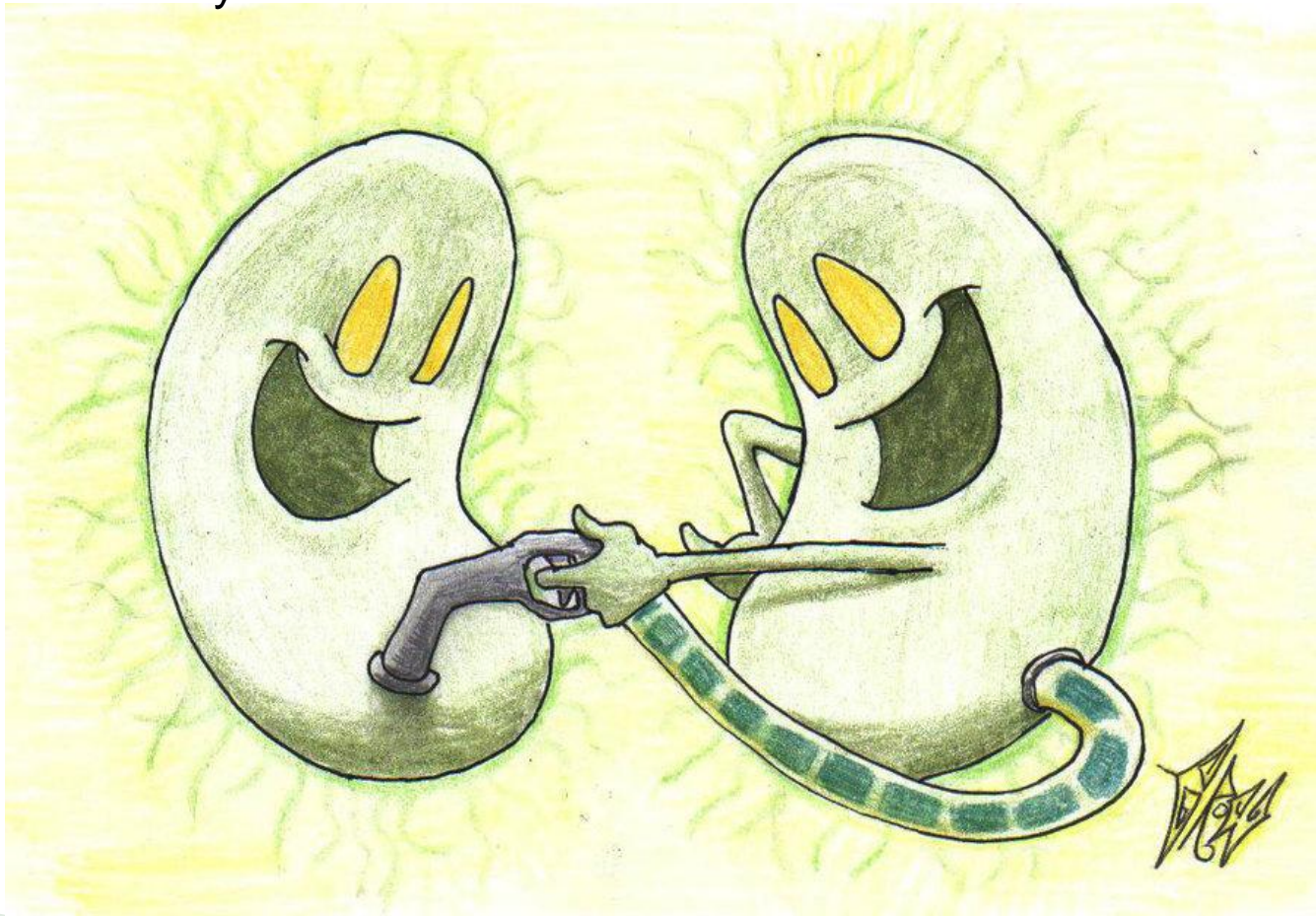
Characterize the individual plasmids in the transformants by:
Replicon typing
Size determination (S1-PFGE)
Plasmid purification from transformants and comparison by RFLP
Susceptibility testing on the transformants to assign the resistance conferred by the plasmid etc.

6.

ANALYSIS OF THE RESULTS

Questions?

Thank you for attention



Bacterial conjugation; <http://edwardcrow.deviantart.com>