

ANTIMICROBIAL SUSCEPTIBILITY TESTING AND SURVEILLANCE: FROM LABORATORY TO CLINIC

Genetic Methods for Detection of Resistance

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OBJECTIVES

1. Identification of resistant organisms

2. Detection of genes:

Resistance genes

Mobile elements (integrons, Plasmids,...)

Promoter regions

Regulatory genes

3. Molecular Typing

PCR assays (Rep-PCR,...)

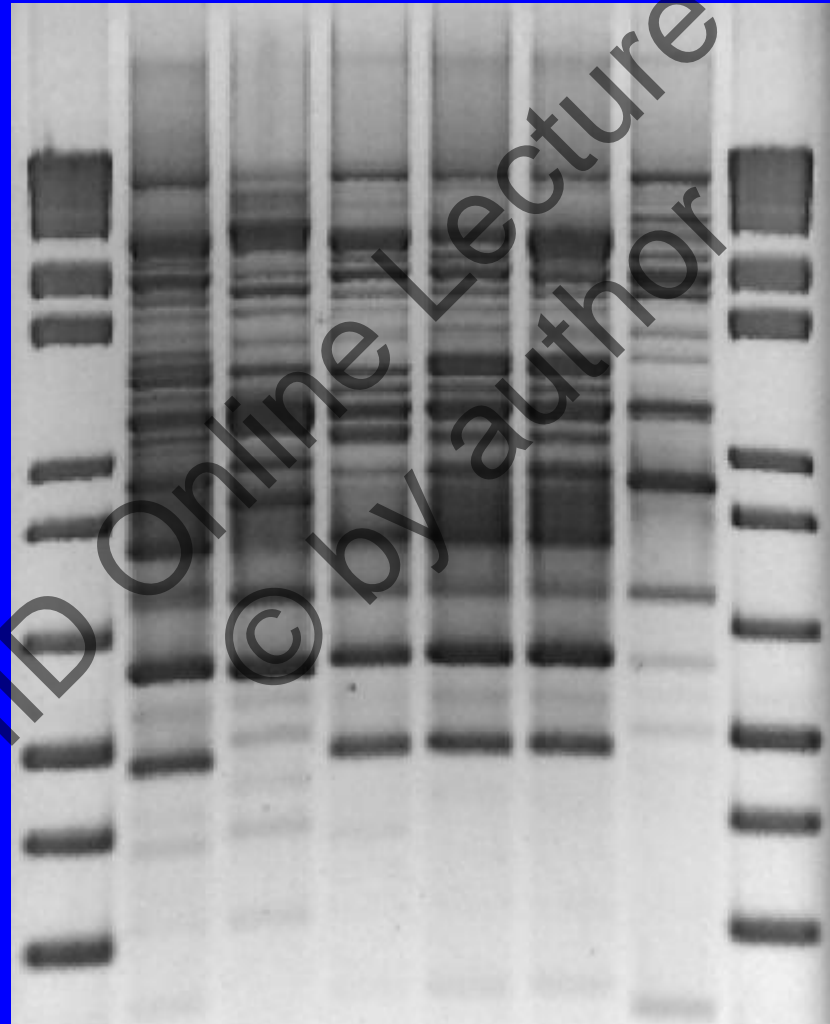
PFGE

MLST

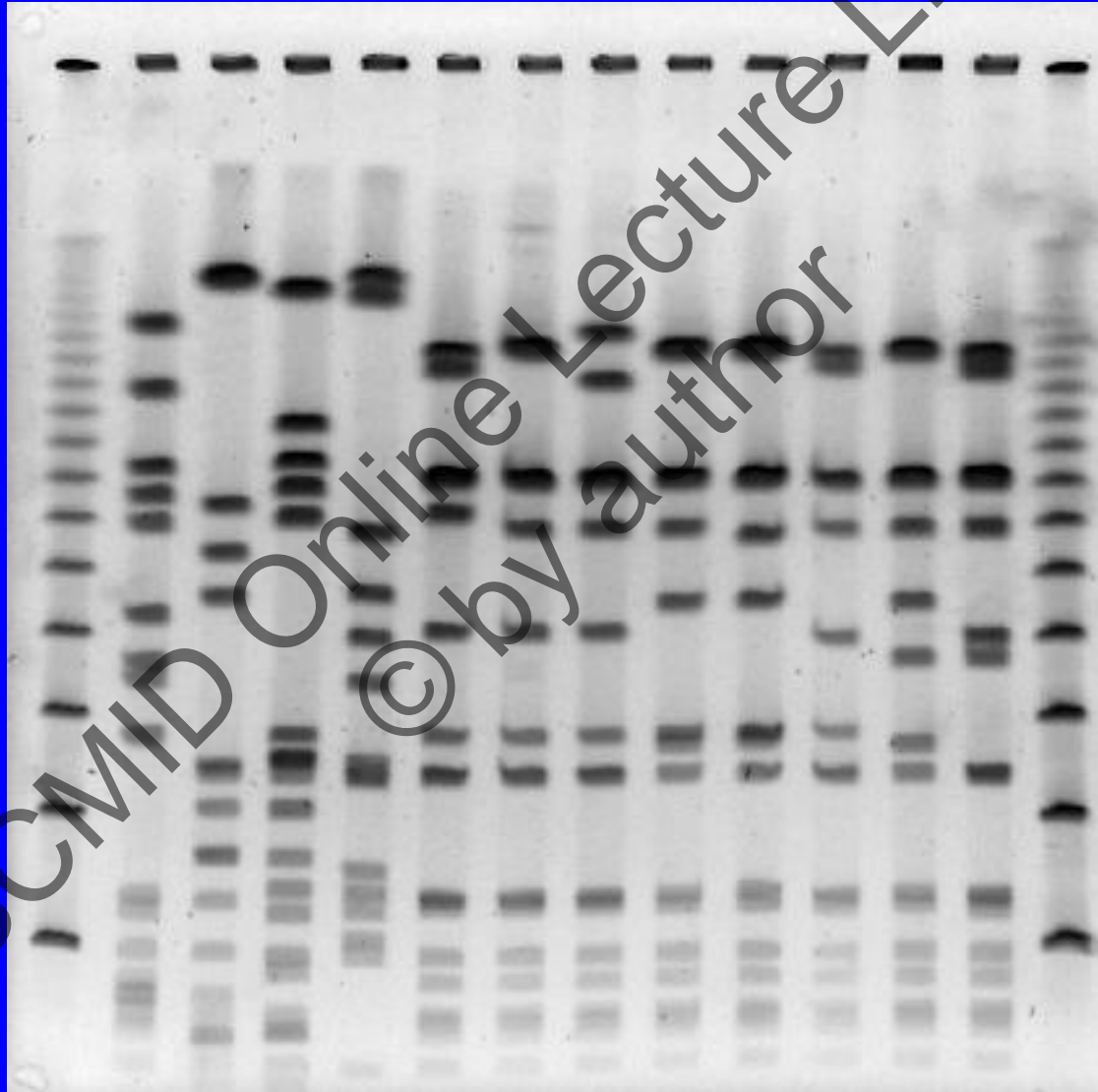
MOLECULAR TYPING

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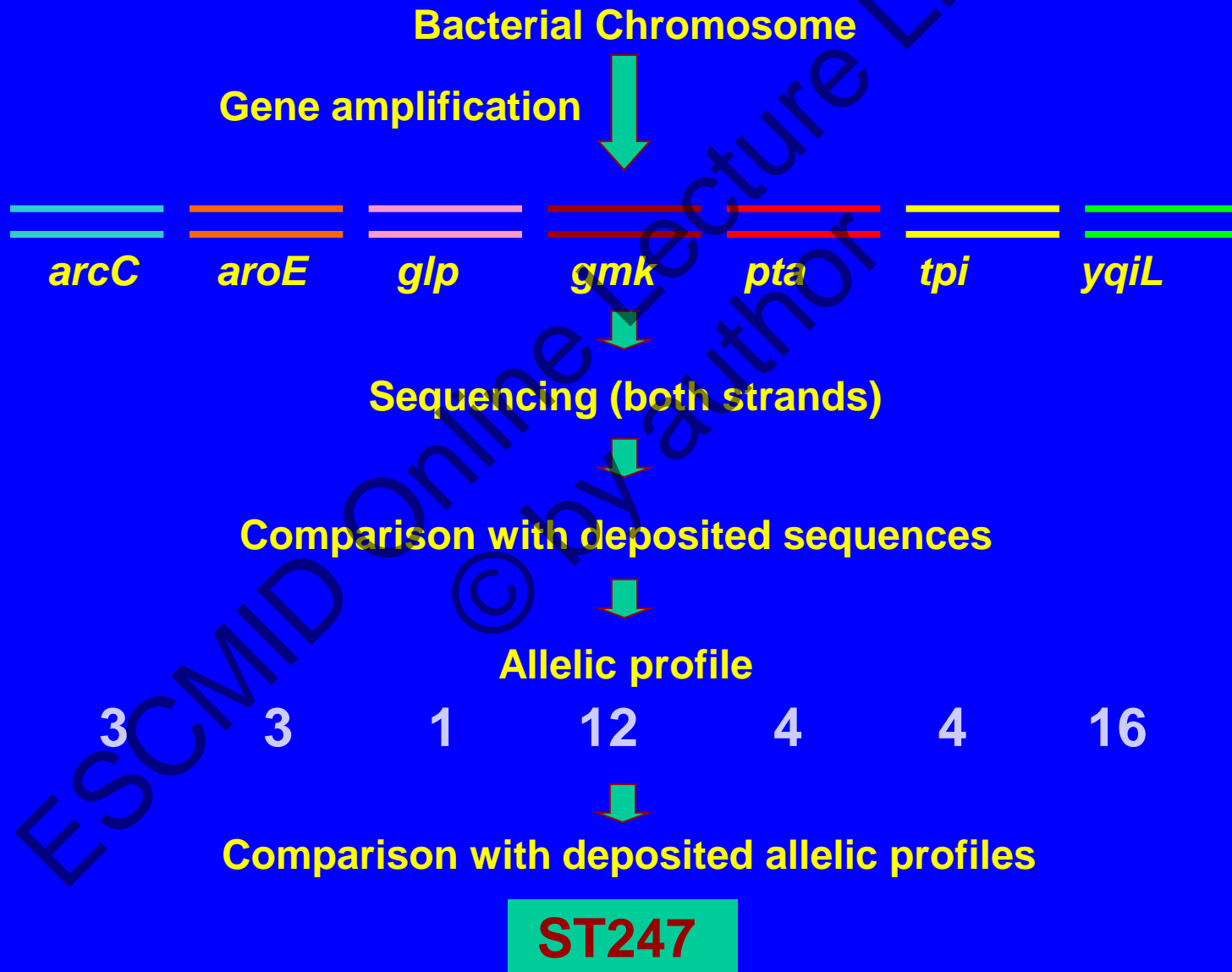
Rep-PCR: Blood isolates of *K. oxytoca*, HUM Valdecilla



PFGE: Met-R *S. aureus*



MLST



1. Identification of resistant organisms

2. Detection of genes:

Resistance genes

Promoter regions

Mobile elements (integrons, plasmids,...)

Regulatory genes

TECHNICAL APPROACHES

1. Probes

2. PCR assays

- Simple PCR
- Multiplex PCR
- RT-PCR

- **DNA Sequencing**

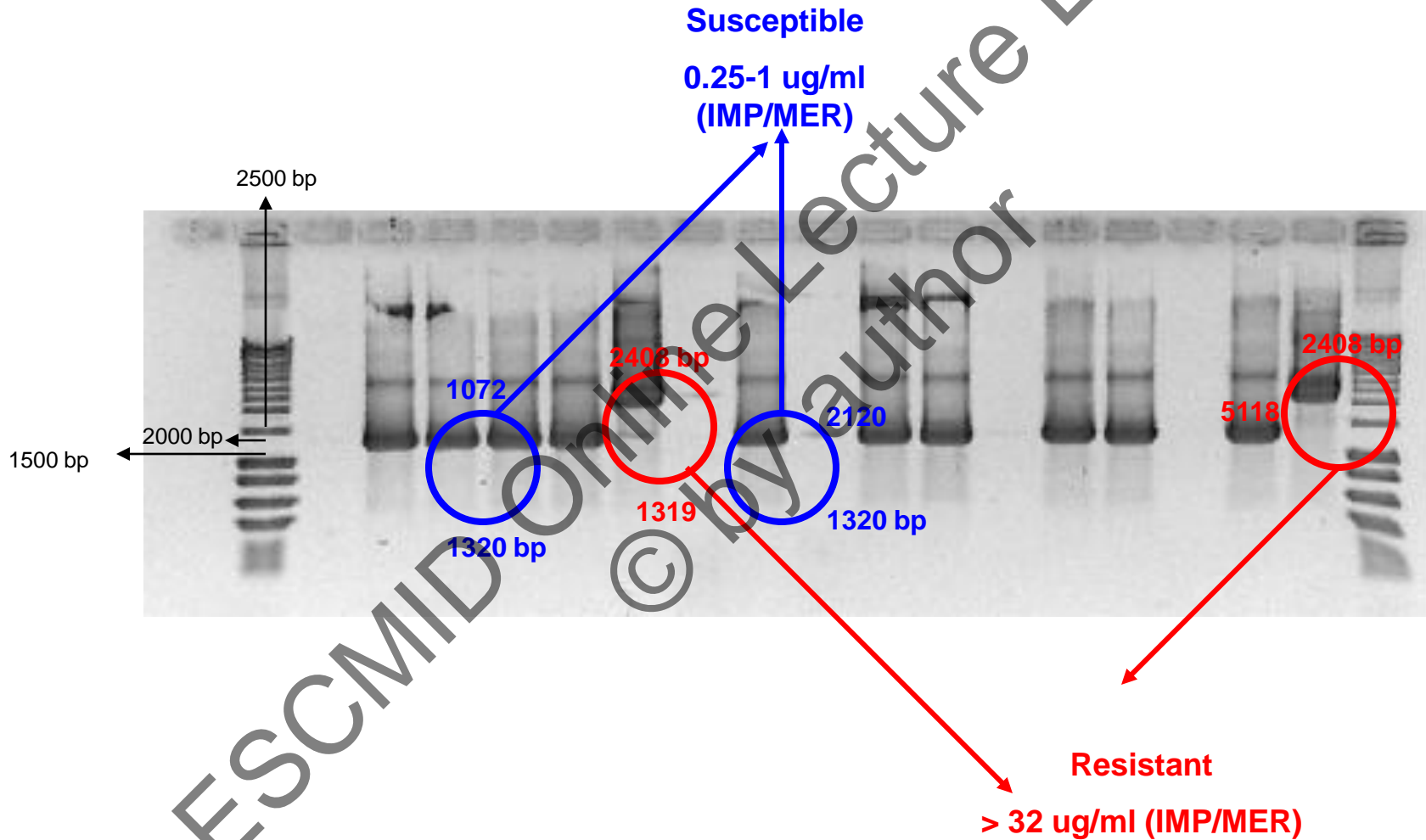
- Conventional sequencing
- Pyrosequencing
- dHPLC
- Mass spectrometry
[In-house vs. Commercial]

3. Arrays

Identification of *A. baumannii* Detection of *bla*_{OXA-51[-like]} genes

| | | | |
|--------|--|----------------------|------------------|
| OXA-66 | Trp ₂₂₂ → Gly (T ₆₆₄ → G) | OXA-79 | UK |
| OXA-66 | Pro ₁₃₀ → Leu (C ₃₈₉ → T) | OXA-80 | UK |
| OXA-66 | Leu ₁₆₇ → Val (C ₄₉₉ → G) | OXA-82 | Turkey, USA |
| OXA-95 | Phe ₇ → Leu (C ₂₁ → A) | OXA-104 ^a | USA |
| | Asp ₁₁₇ → Asn (G ₃₄₉ → A) | | |
| | Lys ₁₄₆ → Asn (G ₄₃₈ → T) | | |
| OXA-70 | His ₁₉₈ → Asp (C ₅₉₂ → G) | OXA-106 | Estonia |
| OXA-69 | Leu ₁₆₇ → Val (C ₄₉₉ → G) | OXA-107 | Poland, Slovenia |
| OXA-95 | Phe ₇ → Leu (C ₂₁ → A) | OXA-108 ^a | Poland |
| | Ala ₂₅ → Thr (G ₇₃ → A) | | |
| | Ala ₁₅₆ → Thr (C ₄₆₆ → A) | | |
| | Leu ₁₆₇ → Val (C ₄₉₉ → G) | | |
| OXA-66 | Pro ₁₃₀ → Gln (C ₃₈₉ → A) | OXA-109 | UK |
| OXA-69 | Ile ₁₂₉ → Leu (A ₃₈₅ → C) | OXA-110 | Poland |
| OXA-51 | Thr ₂₄ → Ser (A ₇₀ → T) | OXA-111 | Belgium |
| | Val ₄₈ → Ala (T ₁₄₃ → C) | | |
| | Lys ₂₀₉ → Met (A ₆₂₆ → T) | | |
| OXA-69 | Ser ₂₄₆ → Pro (T ₇₃₆ → C) | OXA-112 | UK |

Detection of the PBP6b-encoding gene in *A. baumannii* (isolates susceptible or resistant to carbapenems)



Acinetobacter baumannii 1072 (S)

PBP6b

Acinetobacter baumannii 2120 (S)

PBP6b

Acinetobacter baumannii AB 307

PBP6b

Acinetobacter baumannii RUM 134

PBP6b

Acinetobacter baumannii 1319 y 5118 (R)

PBP6b::*ISAba125-like*

aaacttgaagtcgaca

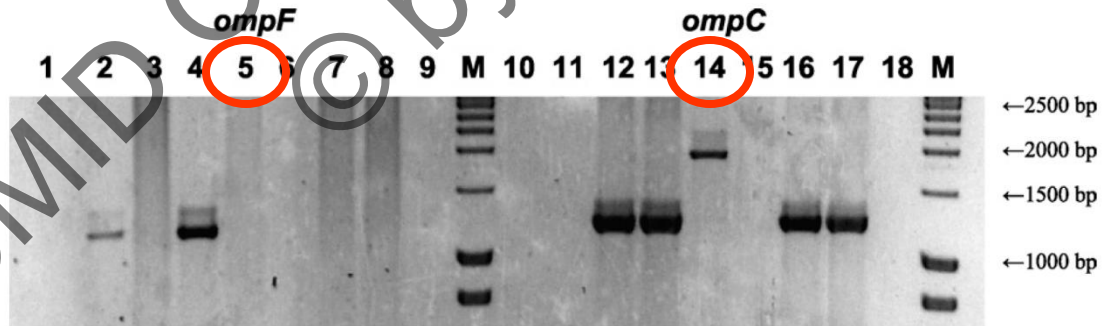
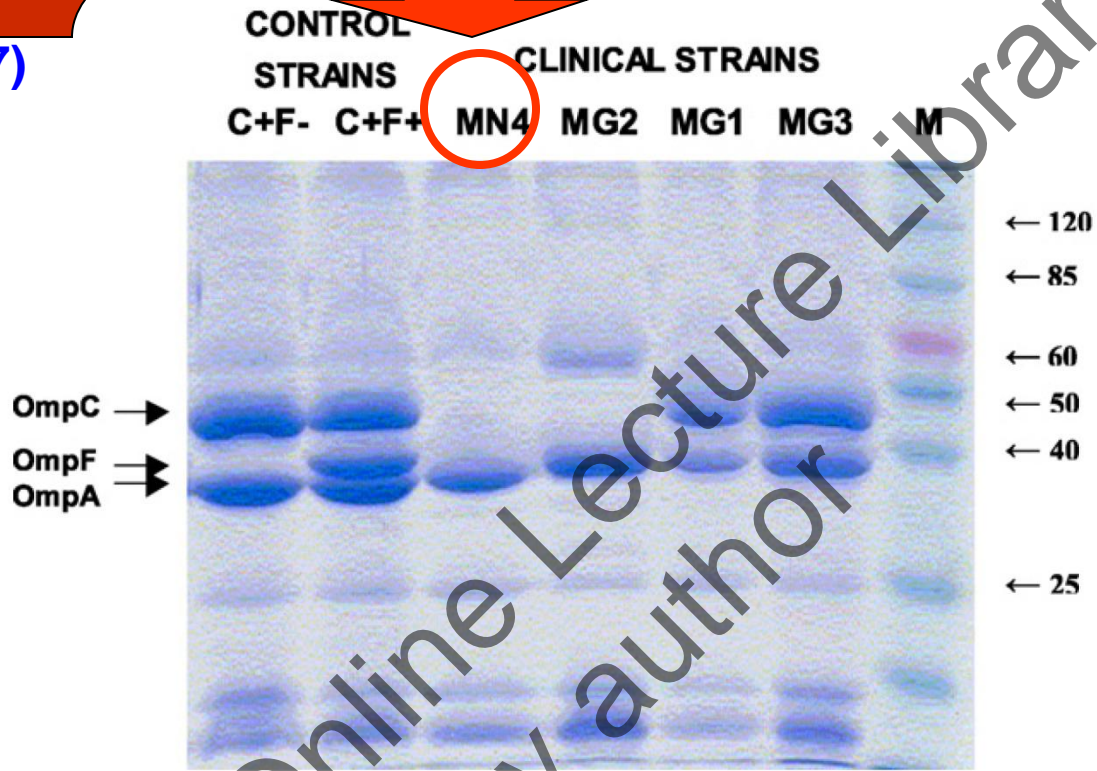
IR-L

tgtcgcacctcatggtt

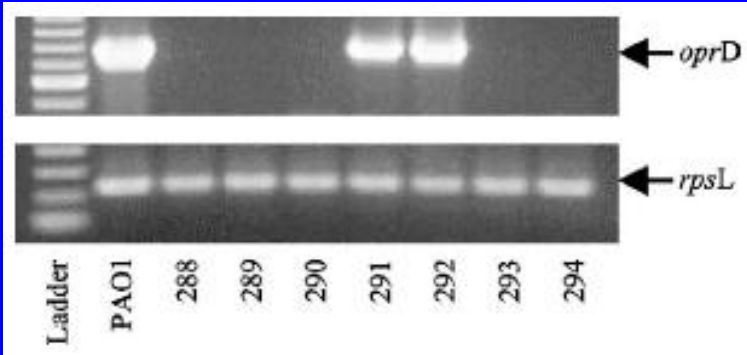
TR-R

MN4 (CTX-M-67)

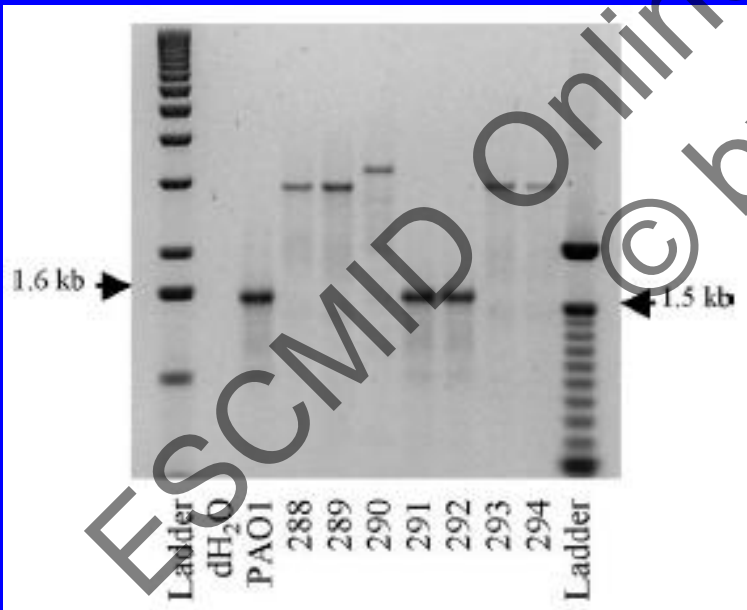
IMP 32 mg/l
MPM 8 mg/l



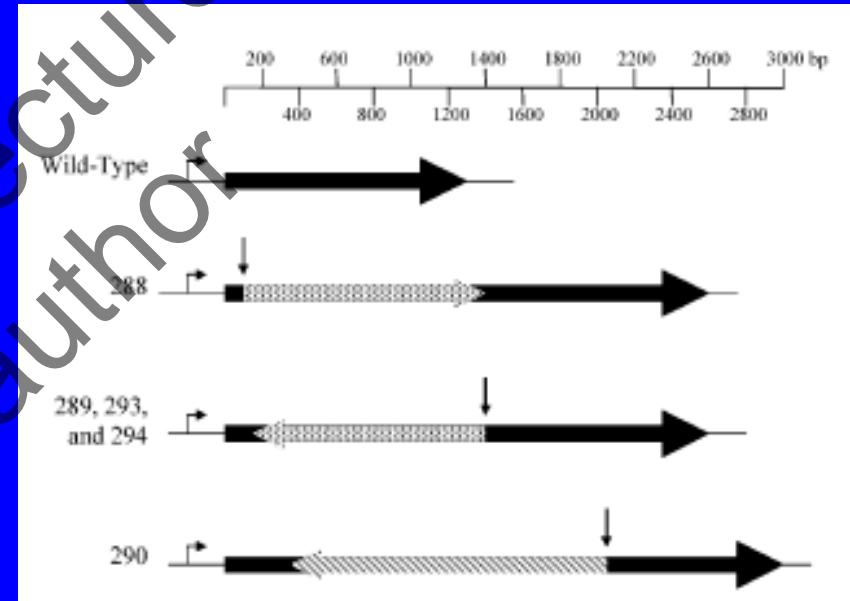
Loss of OprD porin in *P. aeruginosa*



RNA expression of *oprD*

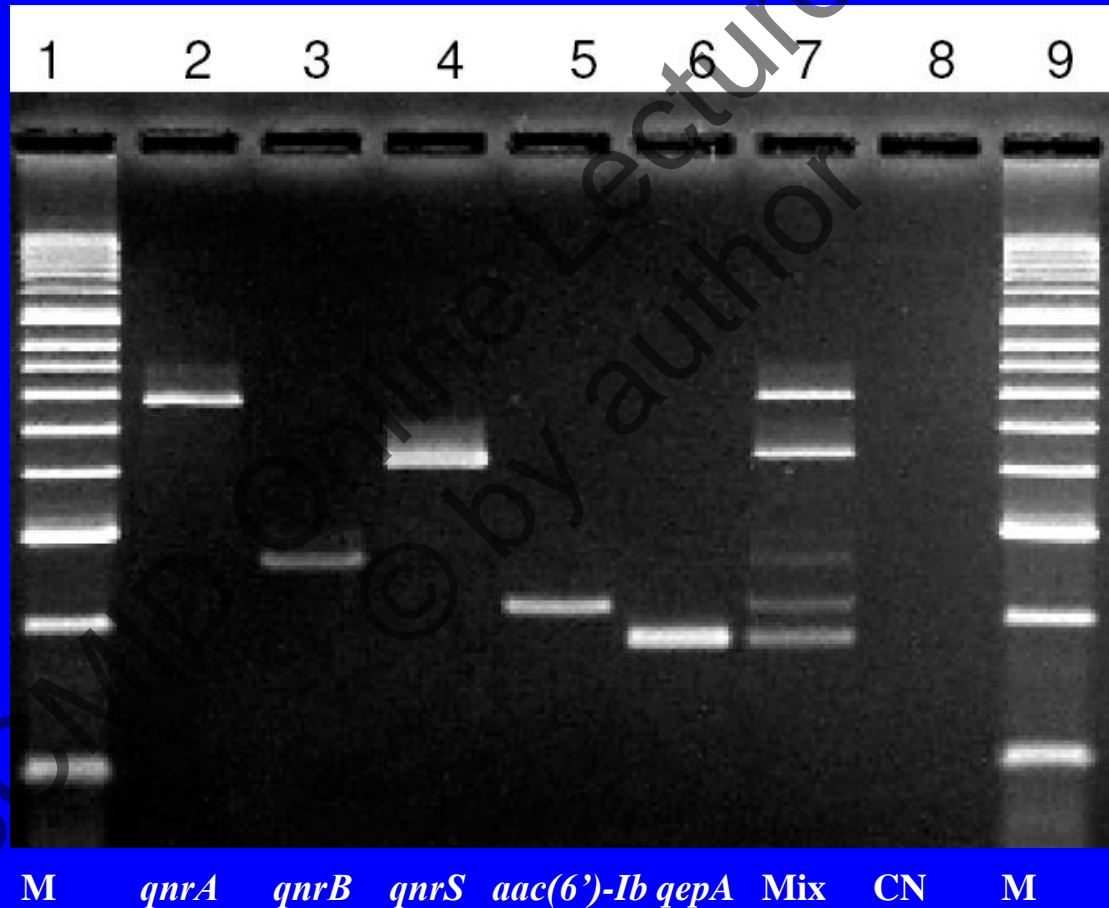


PCR amplification of *oprD*

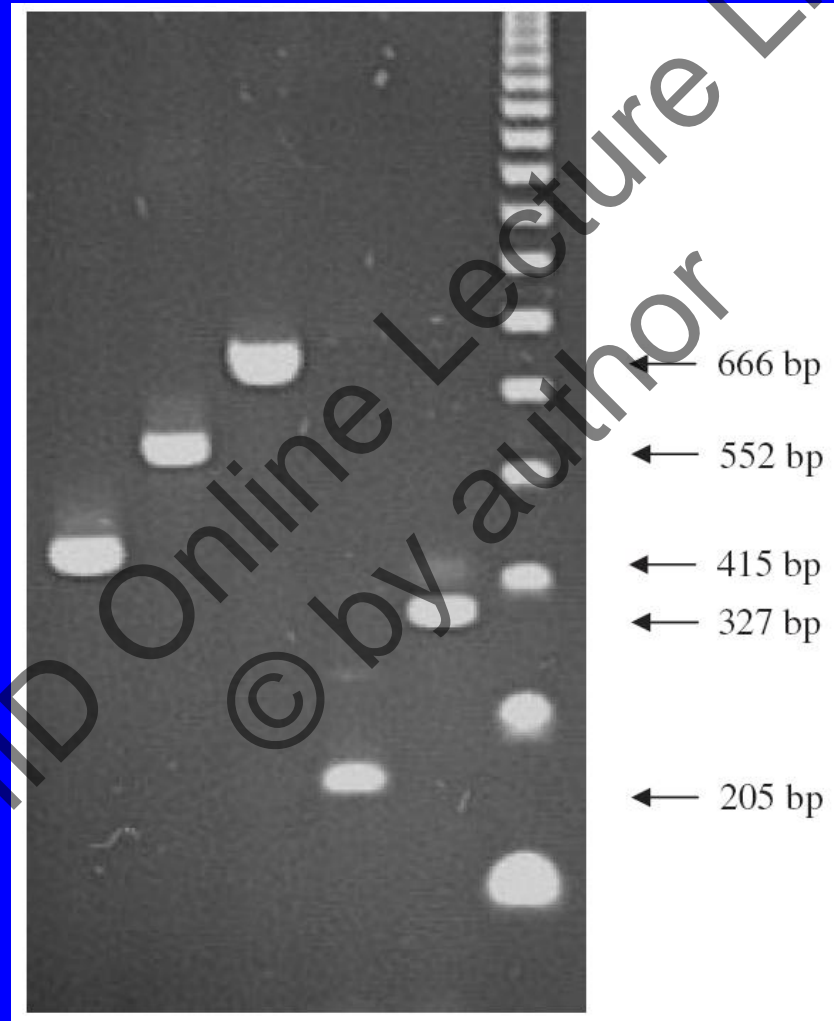


IS within *oprD*

Multiplex PCR: Detection of PMQR genes



Multiplex PCR: Detection of *bla*_{CTX-M} genes



Gr.1 Gr.2 Gr.8 Gr.9 Gr.25 SM

P. aeruginosa clinical isolates

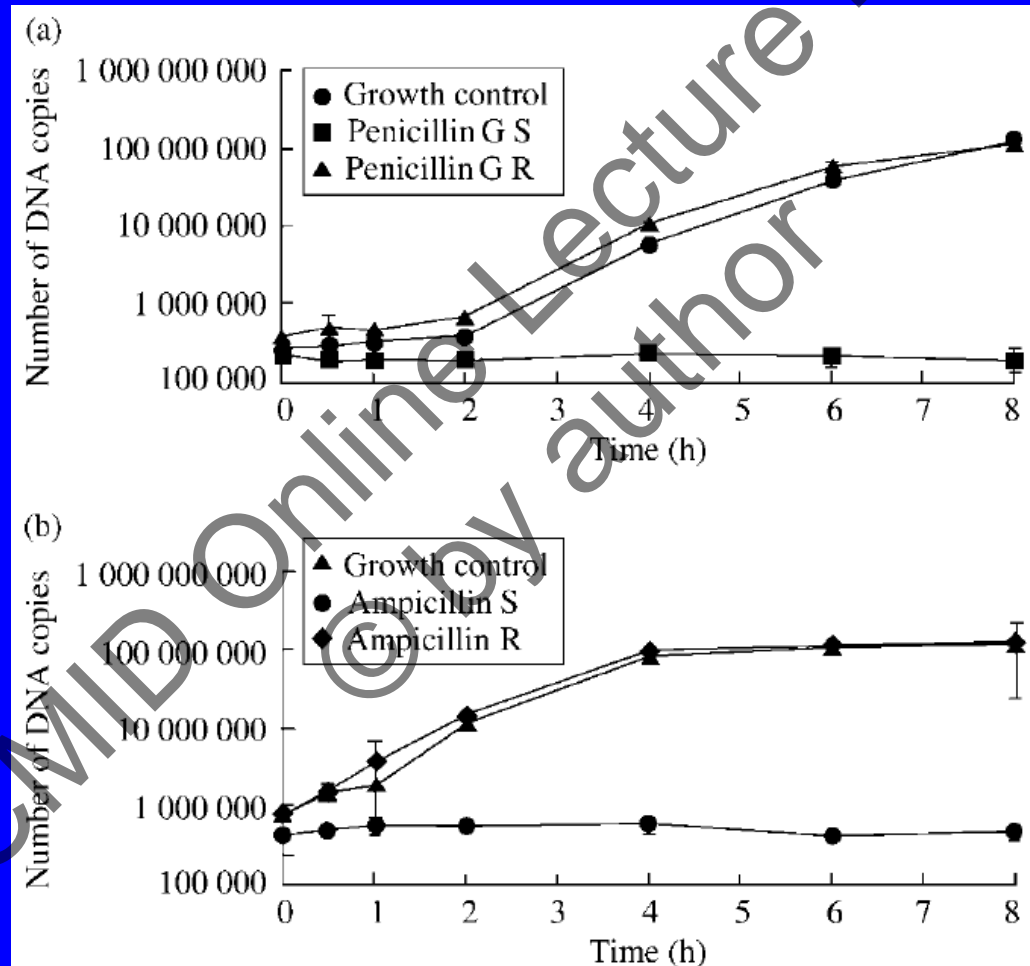
MICs of FEP>CAZ

| Cepas | Pat. | Sex | Age | Sample | Microd. | Etest | Microd. | Etest |
|----------|------|-----|-----|--------|---------|-------|---------|-------|
| | | | | | CAZ | CAZ | FEP | FEP |
| HUMV-8 | DSG | M | 70 | EH | 8 | 6 | 32 | 64 |
| HUMV-26 | JPB | M | 72 | esputo | 8 | 16 | 16 | 24 |
| HUMV-38 | PAG | F | 76 | EH | 2 | 4 | 4 | 32 |
| HUMV-39 | VIA | M | 77 | EH | 2 | 1.5 | 4 | 6 |
| HUMV-57 | FEM | M | 82 | orina | 4 | 6 | 16 | 12 |
| HUMV-72 | ICF | M | 6 | f.f | 1 | 6 | 16 | 12 |
| HUMV-89 | LCC | M | 77 | orina | 4 | 2 | 8 | 12 |
| HUMV-110 | IFC | F | 70 | EH | 8 | 16 | 16 | >256 |
| HUMV-111 | ISG | F | 46 | EH | 16 | 32 | 64 | >256 |

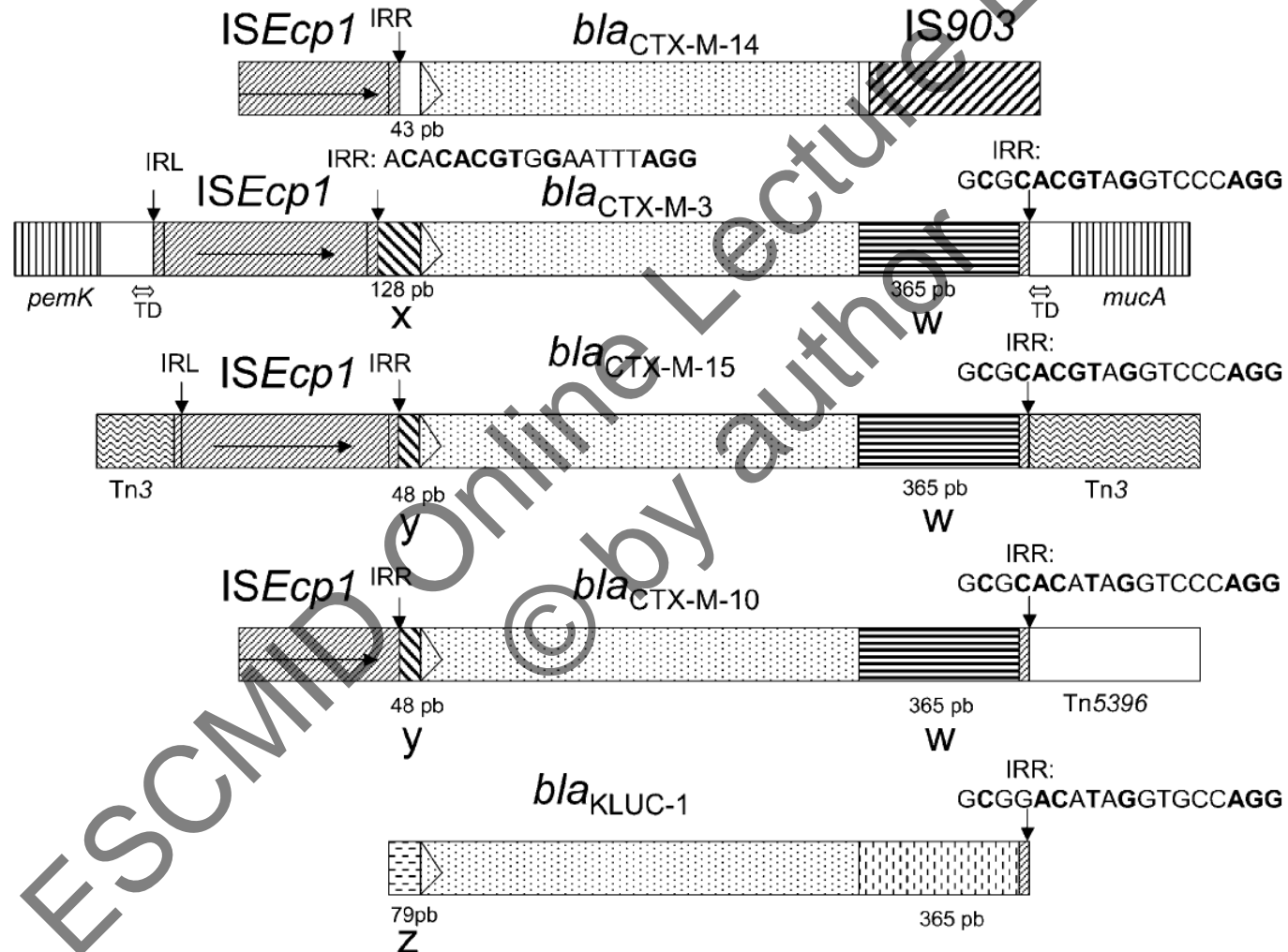
RT-PCR detection of efflux pump genes in *P. aeruginosa*

| | CAZ | FEP | Expression related to PAO1 | | | Level of expression | | |
|----------|-----|-----|----------------------------|-------------|-------------|---------------------|-------------|-------------|
| | | | <i>mexB</i> | <i>mexD</i> | <i>mexY</i> | <i>mexB</i> | <i>mexD</i> | <i>mexY</i> |
| HUMV_8 | 8 | 32 | | 1,97 | 24 | | 2,89 | 13,5 |
| | | | | 3,8 | 3 | | | |
| HUMV_26 | 8 | 16 | 2,33 | 6,4 | 9 | 2,21 | 4,05 | 35,63 |
| | | | 2,09 | 4,26 | 22,9 | | | |
| | | | | 1,5 | 48 | | | |
| HUMV_38 | 2 | 4 | 4,29 | 0,7 | 59,9 | 4,97 | 1 | 95,3 |
| | | | 5,64 | 1,3 | 130,7 | | | |
| HUMV_39 | 2 | 4 | 1,39 | 7,7 | 40,1 | 1,24 | 18,1 | 93,35 |
| | | | 1,08 | 28,5 | 146,6 | | | |
| HUMV_57 | 4 | 16 | 0,03 | 151 | 18,4 | 0,42 | 150,4 | 18,32 |
| | | | 0,81 | 151 | 4,76 | | | |
| | | | | 150 | 31,8 | | | |
| HUMV_72 | 1 | 16 | 0,36 | 2 | 74,8 | 0,68 | 2,2 | 139,95 |
| | | | 1 | 2,4 | 205,1 | | | |
| HUMV_89 | 4 | 8 | 0,87 | 2,4 | 45,7 | 0,92 | 1,95 | 54,85 |
| | | | 0,96 | 1,5 | 64 | | | |
| HUMV_110 | 8 | 16 | 24,88 | 2,9 | 99,1 | 15,15 | 2,4 | 186,8 |
| | | | 5,41 | 1,9 | 274,5 | | | |
| HUMV_111 | 16 | 64 | 1,85 | 0,3 | 105,4 | | 1,7 | 162,7 |
| | | | | 3,1 | 220 | | | |

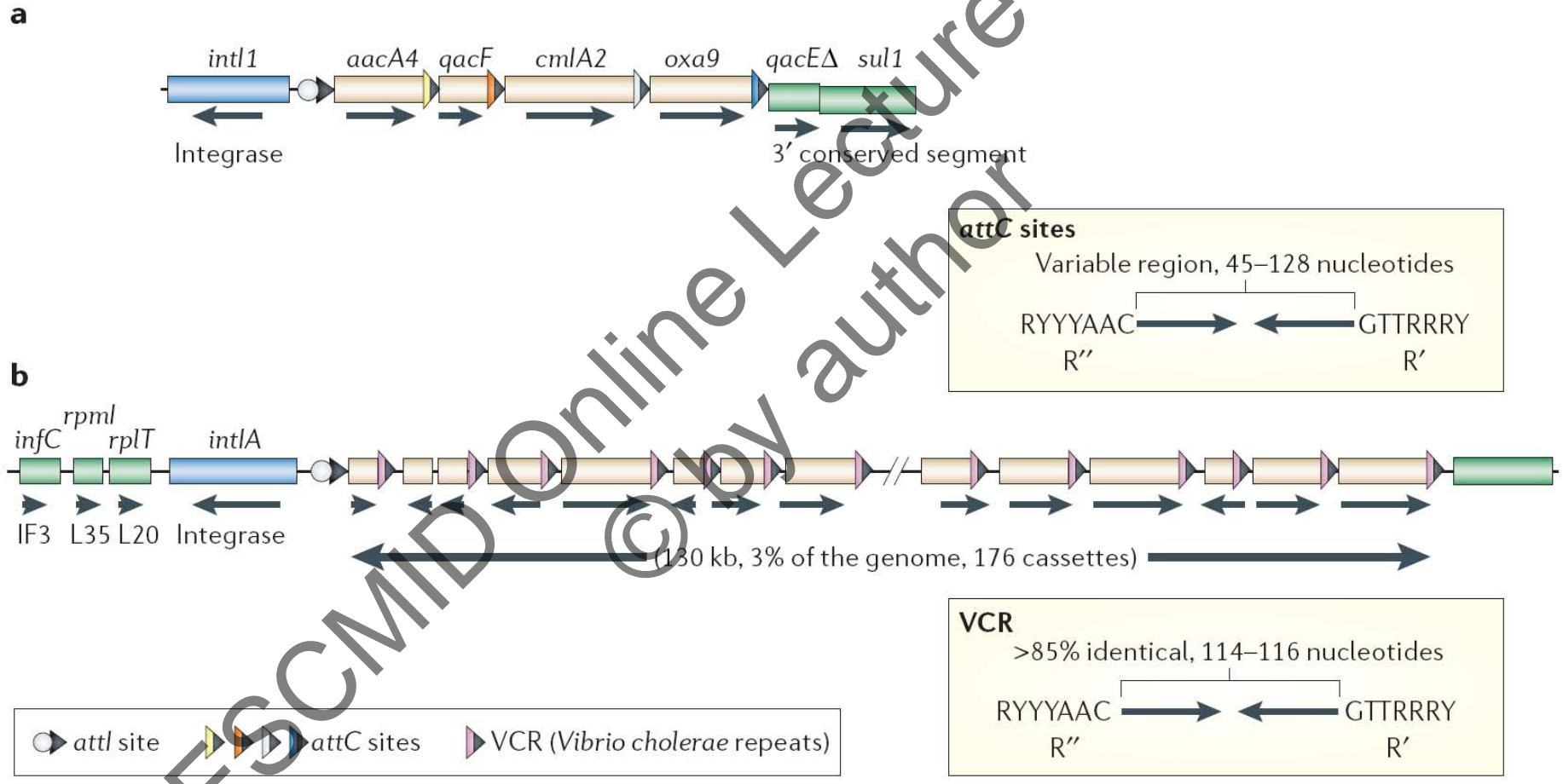
Universal susceptibility testing by RT-PCR



Surrounding DNA sequences for the *bla*_{CTX-M} genes

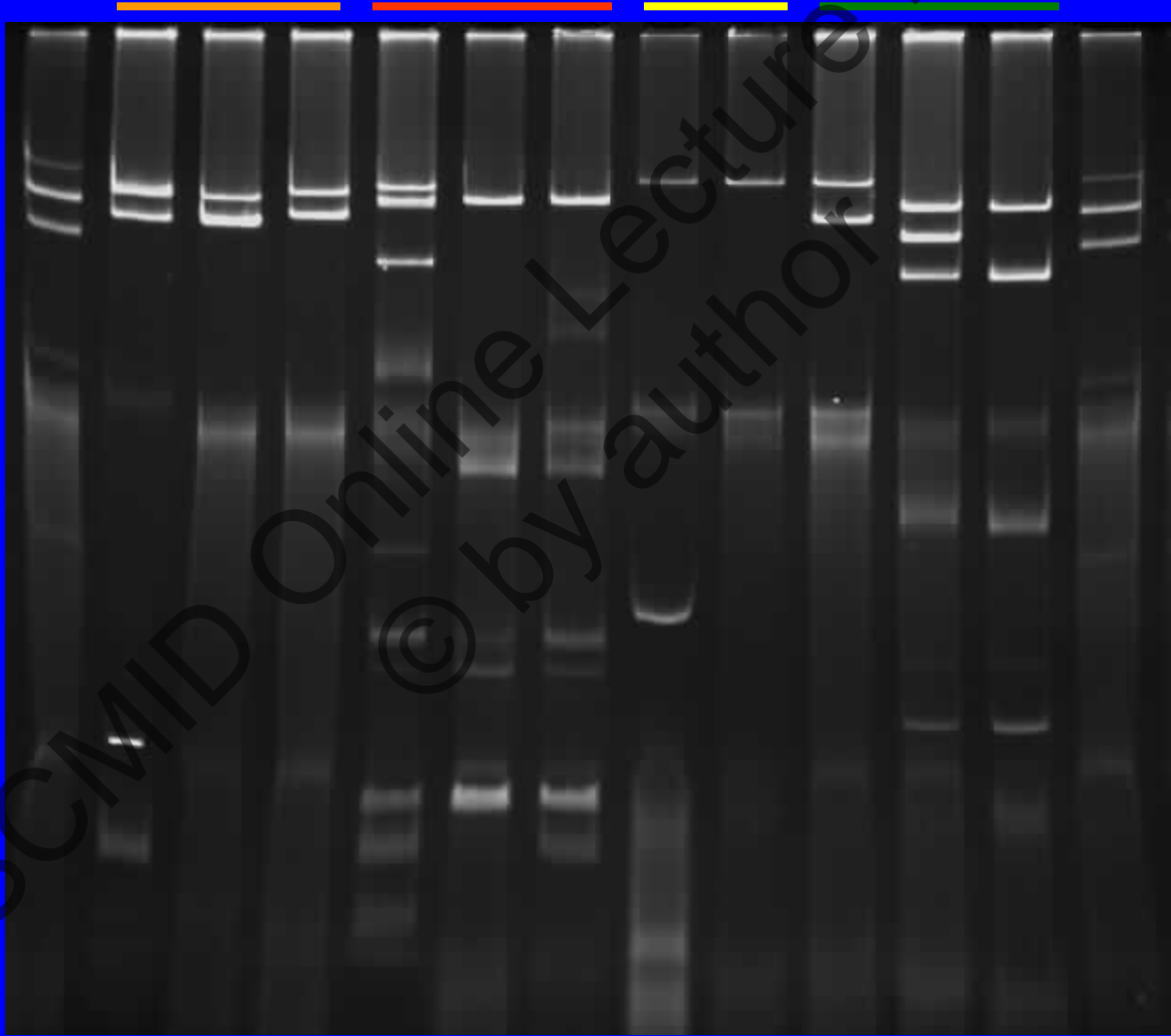


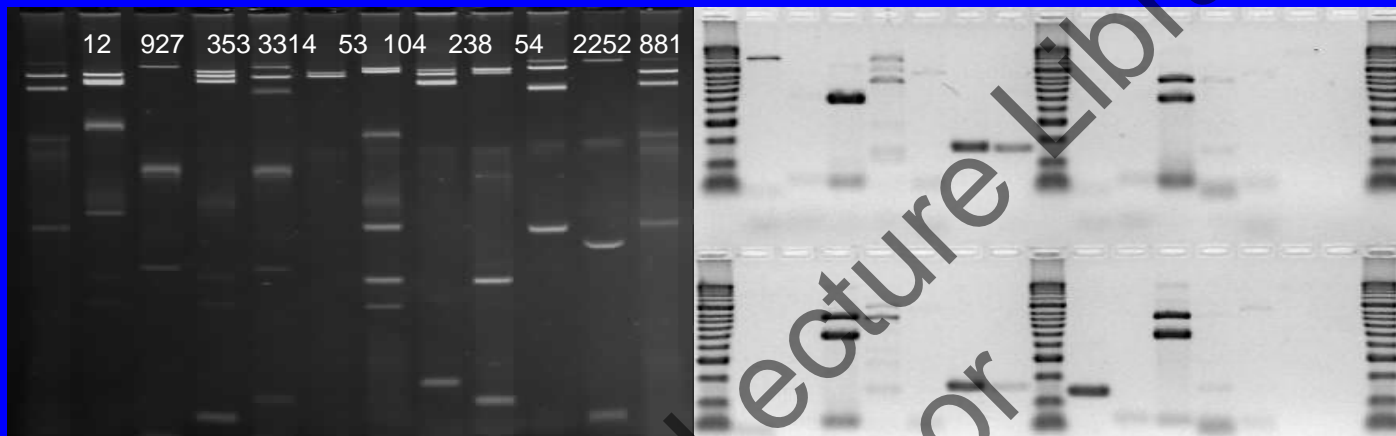
Integrations



Detection of Mobile elements: Plasmids

[*E. coli* CTX-M (+) and derived transconjugants]




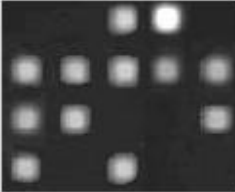


| <i>E.coli</i> BLEE+ | Inc Groups | <i>E.coli</i> MR | Grupos Inc |
|------------------------|------------------|------------------|-----------------|
| 12 | FIA, K, B/O | 927 | FIA, FIB |
| 353 | FIA, FIB, K, B/O | 3314 | I1-Iγ, FIA, FIB |
| 53 | FIB, Y, K | 104 | FIB, Y, B/O |
| 238 | FIB, FIA, K | 54 | FIB, FIA |
| 2252 | I1-Ig, FIB, P | 881 | FIB, FIA |

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Table 1

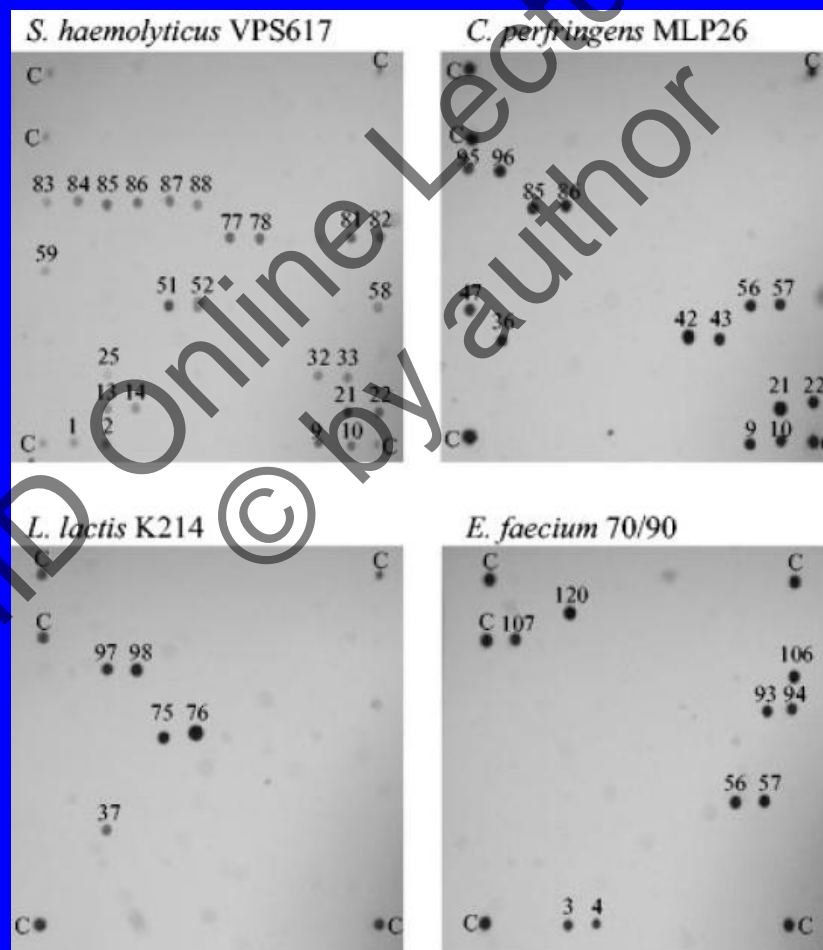
Platforms in use for microbial diagnostic microarrays.

| | Planar glass microarray | Macroarray | Affymetrix gene-chip | Three-dimensional platforms ^a |
|---|--|---|--|--|
| Density | Low/Medium | Low/Medium | High | Low/Medium |
| Open access | + | + | - | - |
| Flexibility | + | + | - | +/- |
| Price | Moderate | Moderate | High | ? |
| Throughput | High | Moderate | High | High |
| Real-time detection (on-chip melting curve analysis) | - | - | - | + |
| |  |  |  |  |

^aGel-pad microarrays (Magi-Chip[®] or IMAGE Chip); Pamgene[®]

Microarray-Based Detection of 90 Antibiotic Resistance Genes of Gram-Positive Bacteria

Vincent Perreten,^{1*} Lorianne Vorlet-Fawer,¹ Peter Sliemers,² Ralf Ehrlich,²
Peter Kuhnert,¹ and Joachim Frey¹



Molecular Methods: Advantages

Major impact in both empirical and directed treatment:

[Usually] faster than phenotypic methods. Particularly useful for noncultivable agents, or for slow growing organisms: *M. tuberculosis*,...)

Increased sensitivity

Definition of actual underlying mechanisms

Multifactorial phenotypes

Similar phenotypes determined by different causes

Molecular Methods: Limitations

Risk of false resistance:

Unexpressed gene

Incomplete/partial gene

Limited ability to detect resistance genes:

Only those the actually considered

New/divergent alleles may not be detected

Simultaneous detection of multiple alleles

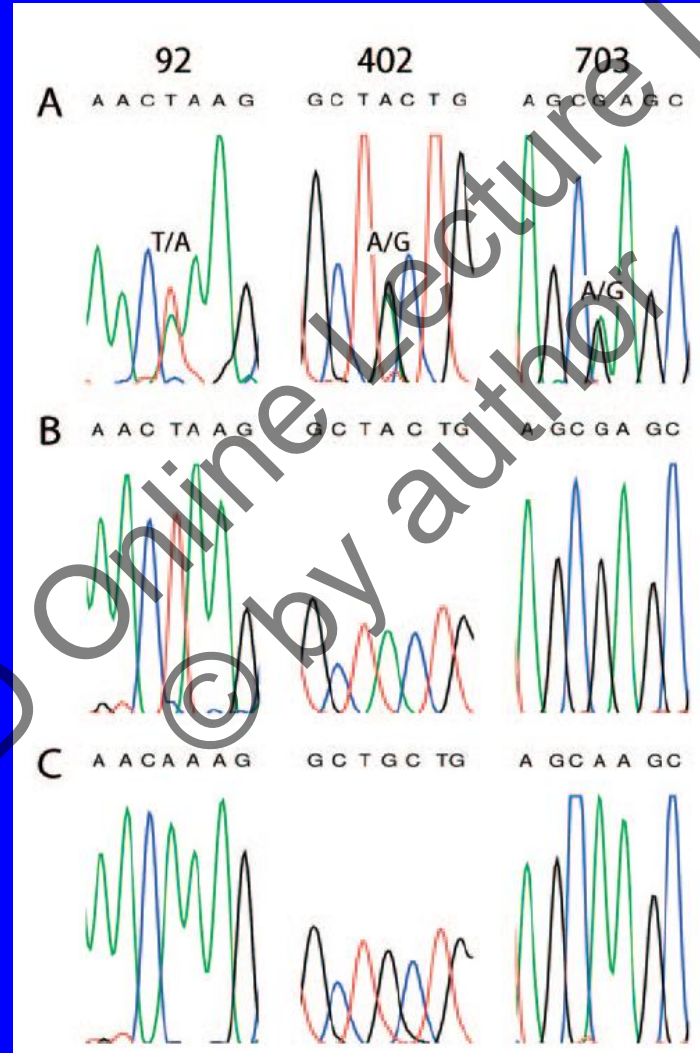
Impossibility of detect not described genes

Usually more expensive than conventional methods:

Equipment, reagents...

Technical skills for staff and technicians

Sequencing of *bla*_{SHV} genes



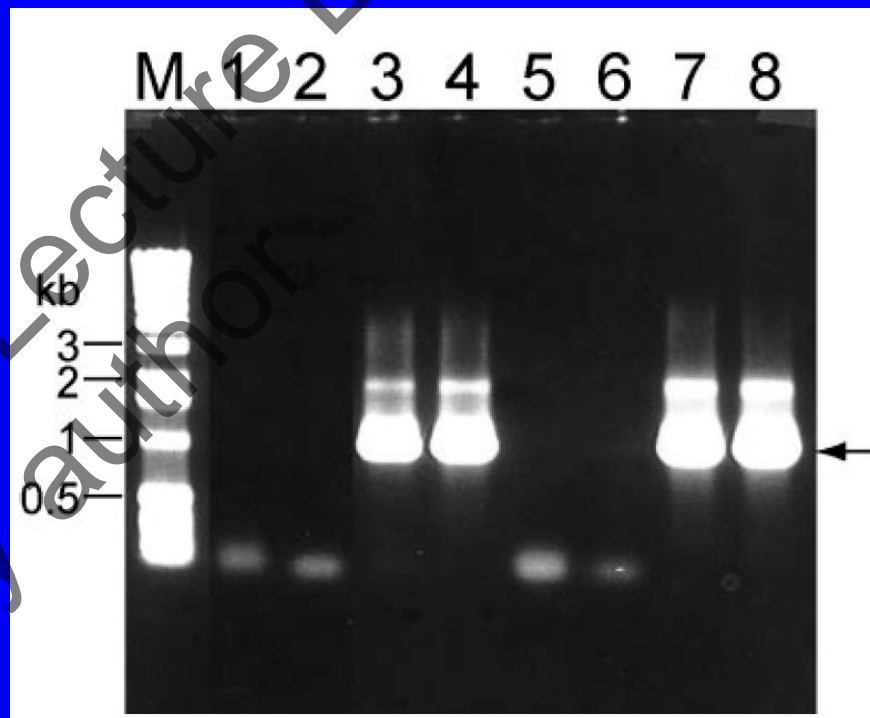
PCR Amplicon

SHV-12

SHV-2

Presence of *bla*_{TEM-1} DNA in commercial *Taq* polymerase

| LANE | PCR Buffer | <i>Taq</i> |
|---------|------------|------------|
| 1 and 2 | B | B |
| 3 and 4 | B | A |
| 5 and 6 | A | B |
| 7 and 8 | A | A |



PCR products amplified from water (DNA-free) and primers for *bla*_{TEM}

Molecular Methods: Local or Regional/National Lab?

Factors to be considered:

Local prevalence of resistance mechanisms

Immediate benefits for patients

Needs for resistance surveillance

Investigation of new resistance mechanisms