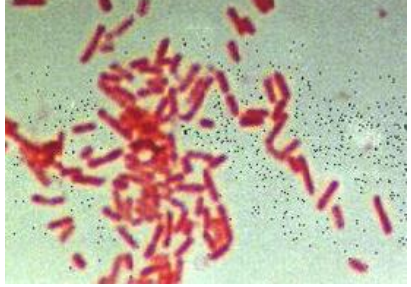

Antibiotic resistance and what can be done

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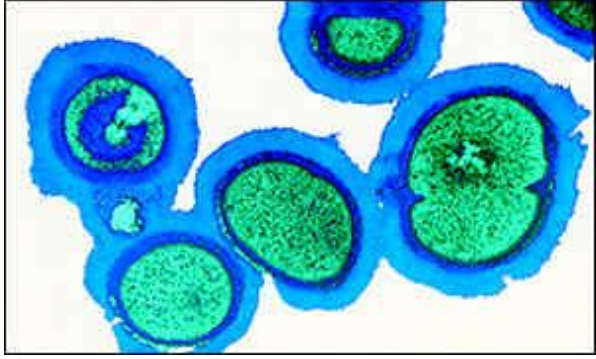
http://idmic.net

Overview

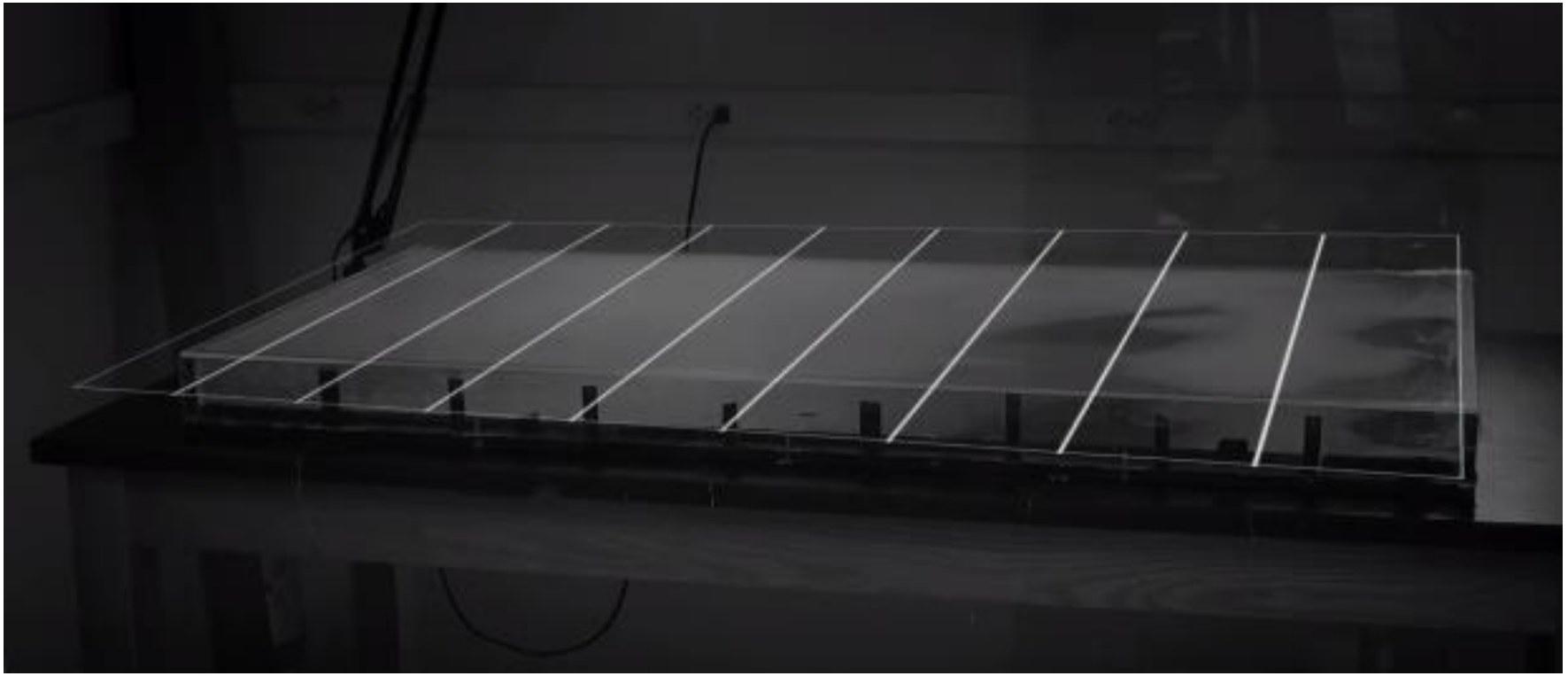
1. Antimicrobial resistance
2. Clinical impact of resistance
3. Laboratory detection of resistance
4. Nepal AMR situation

Bacterial genetic diversity



- 3.5 billion years of evolutionary diversification
- Estimated 10^{21} bacteria; one billion progeny / day ; any given bacterium has 50-50 chance of replicating successfully
- Specific adaptation to innumerable niches
- Sensing of environment; cooperative behaviours; adaptive stress responses
- Humans carry around 2-3 kg of bacteria!

Watching antibiotic resistance evolve…



https://www.youtube.com/watch?v=yybsSqcB7mE

Macroscale influences on bacterial and niche diversity

- Human mobility
- Population, urbanisation
- Food technology, cultural changes
- Land use changes
- Climate change
- Antibiotic use

How does resistance arise?

1. mutational change in bacterial chromosome with clonal expansion of a resistant subpopulation

AND/OR

2. acquisition of new resistance gene(s) from another bacterial species or genus by direct transfer and recombination

   = horizontal transfer

Stepwise mutations in *Klebsiella* species extends the spectrum of β-lactamase enzymes through changes in amino acid sequence at three locations

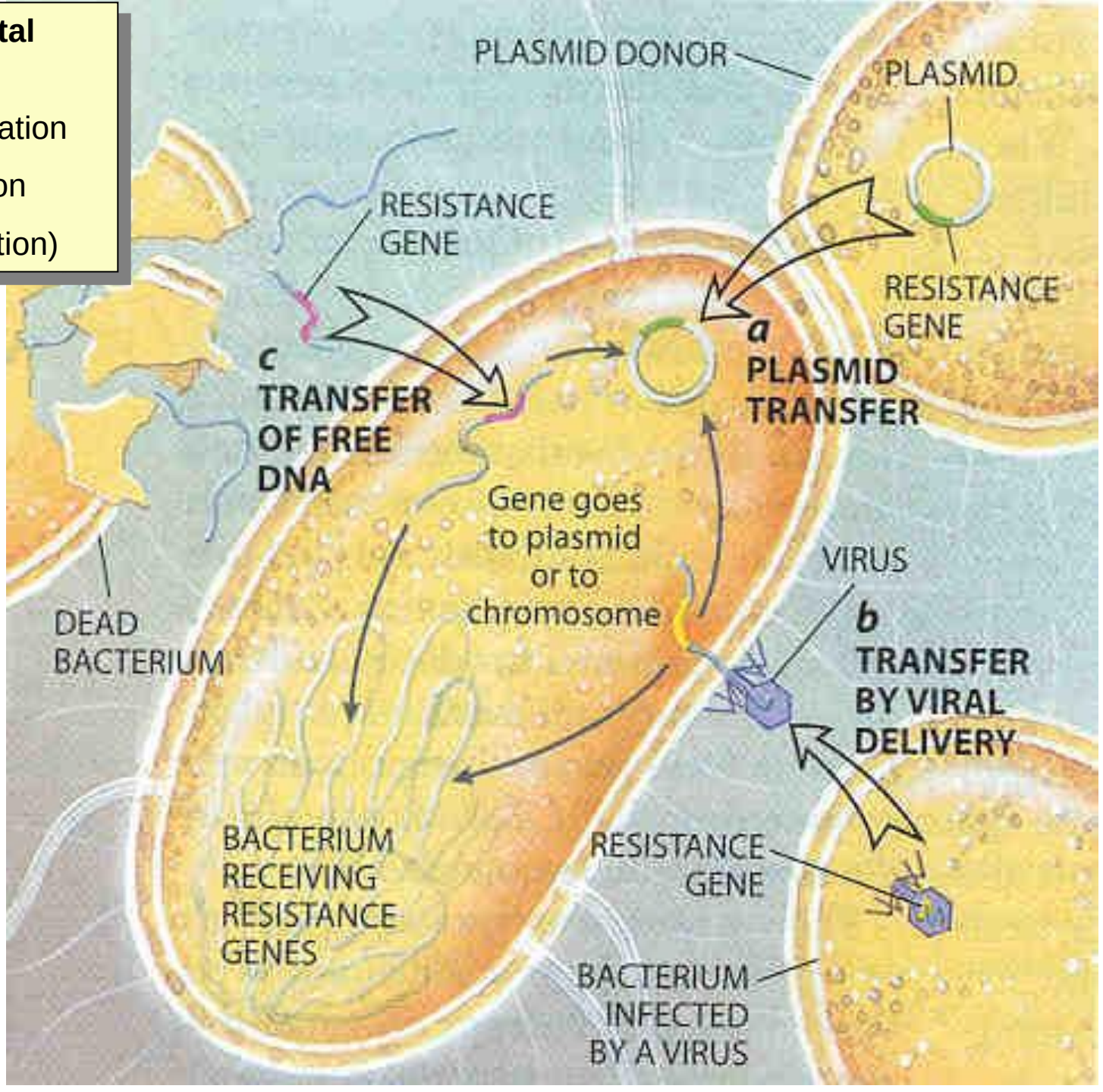
Betalactamase enzyme name	Ceftazadime MIC	102	162	237
TEM-1	<=0.12	GLU	ARG	GLU
TEM-12	4-32	GLU	SER	GLU
TEM-10	64	GLU	SER	LYS
TEM-26	>256	LYS	SER	LYS
TEM-6	256	LYS	HIS	LYS

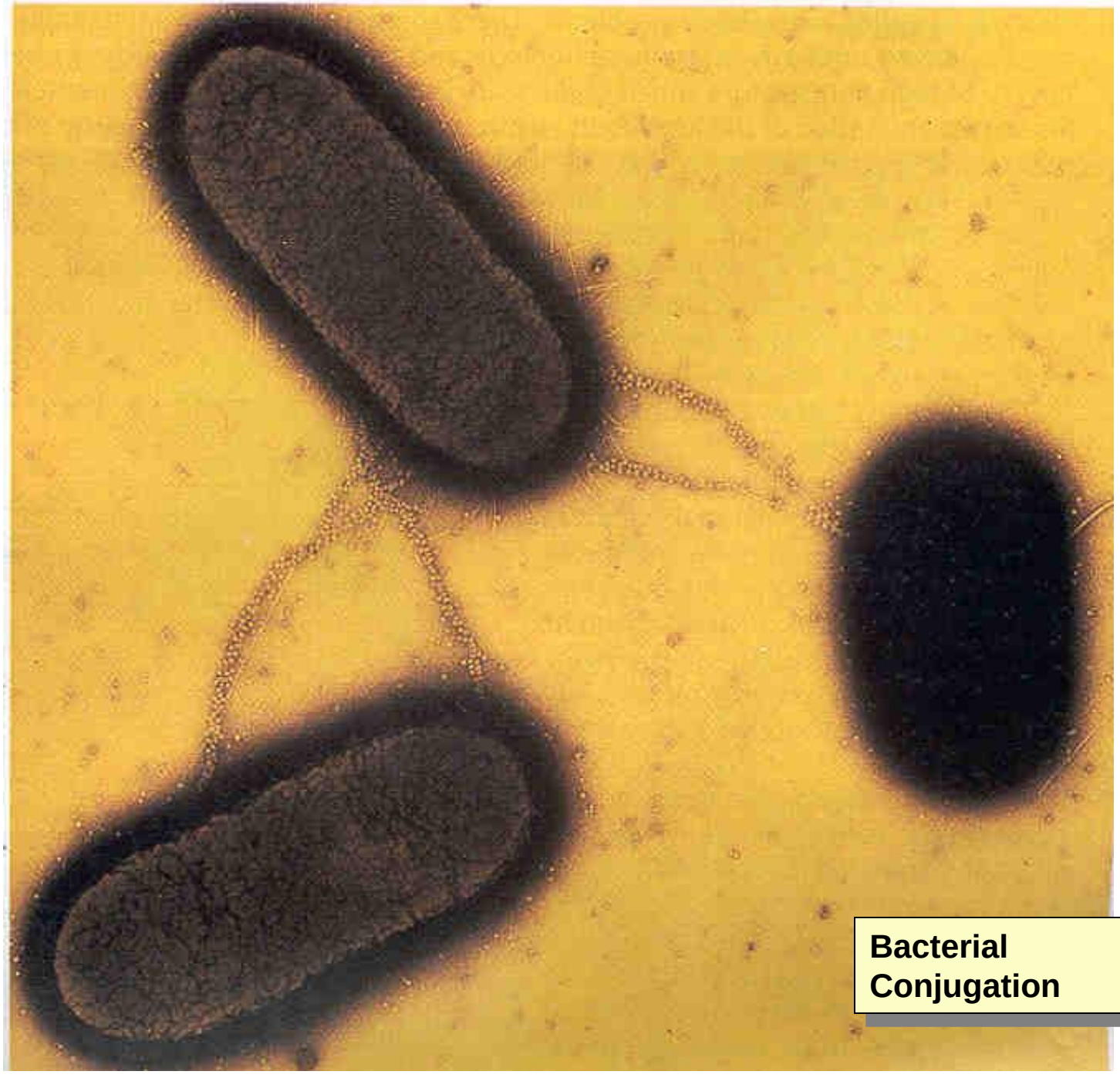
Progressive fluoroquinolone resistance mutations

Mutation	***E. coli***	***Campylobacter***
gyrA at ser83	0.5mg/L	8mg/L
gyrA at asp87	>8mg/L	
parC at 80/84	+	
efflux⇑	++	+
ompF ⇓	+	

2. Horizontal transfer

- transformation
- conjugation
- (transduction)





Bacterial Conjugation

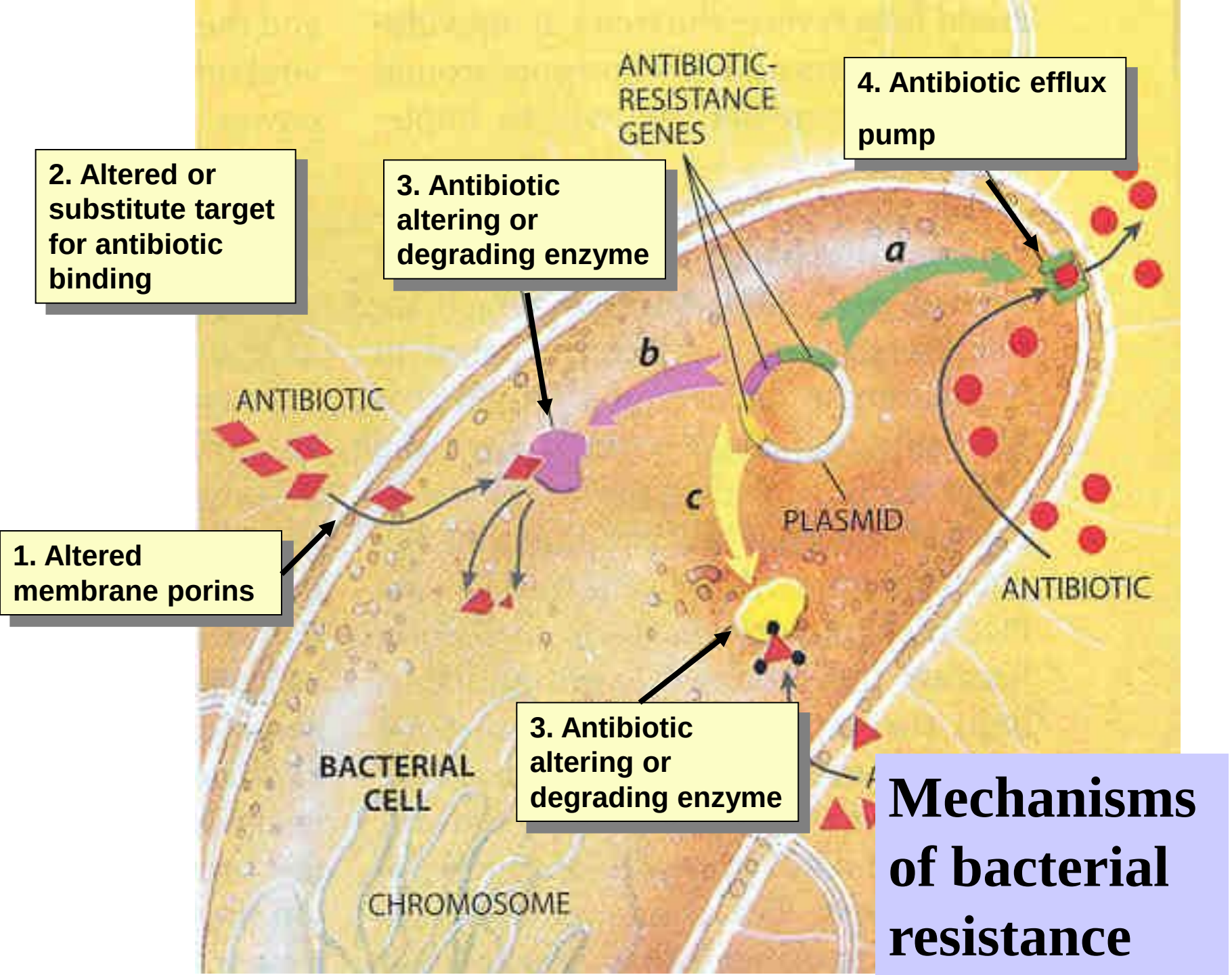
vanB vancomycin resistance in enterococci (VRE)

Transposon Tn*1546* gene cluster (Courvalin 1993)

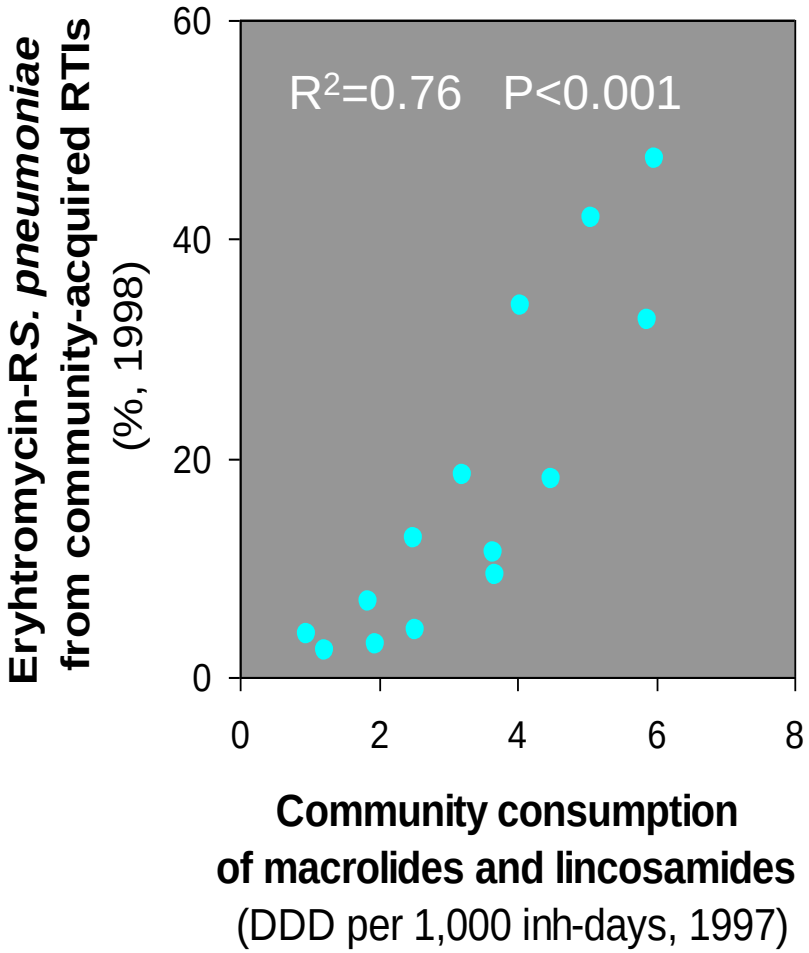
<-ORF1--ORF2-->vanR->vanS->vanH->vanA->vanX-->--vanY-->vanZ--->
transposition regulation glycopeptide resistance accessory

- 9 genes , 10kbase, plasmid or chromosomal location
- May be located within a conjugative plasmid

vanA VRE: similar plasmid borne operon


ANTIBIOTIC-
RESISTANCE
GENES
4. Antibiotic efflux pump
2. Altered or substitute target for antibiotic binding
3. Antibiotic altering or degrading enzyme
a
b
ANTIBIOTIC
c
PLASMID
ANTIBIOTIC
1. Altered membrane porins
3. Antibiotic altering or degrading enzyme
BACTERIAL CELL
CHROMOSOME
Mechanisms of bacterial resistance

Correlation of resistance with Antimicrobial Use in Community-Acquired Infections in Europe, 1997-2000



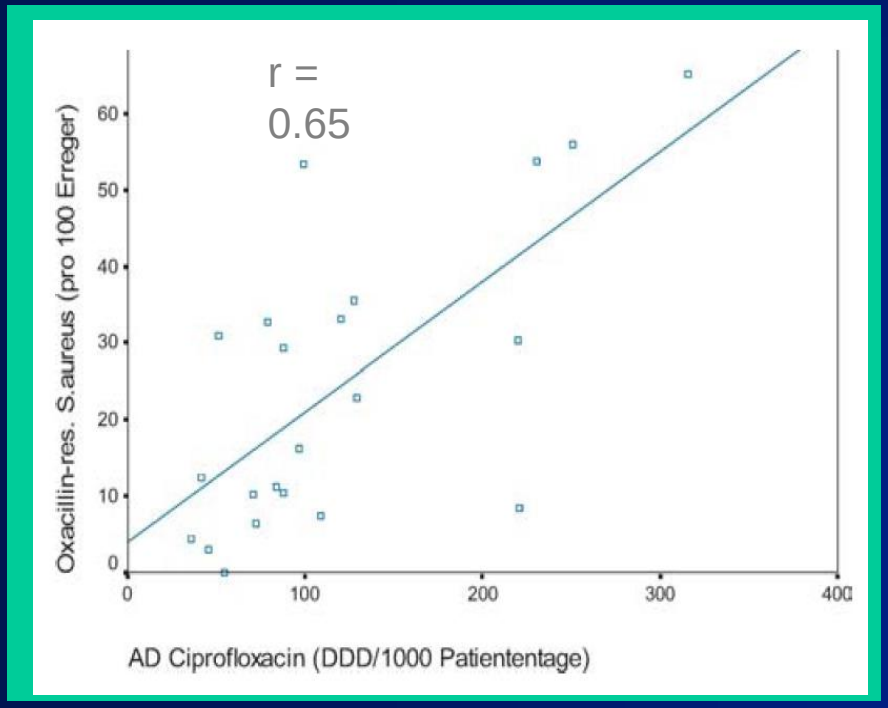
Each dot represents a different European nation

A very tight relationship between overall community consumption and resistance (erythromycin is a macrolide)

Source: Alexander Proj., FINRES, STRAMA, DANMAP and Cars O, et al. Lancet 2001.

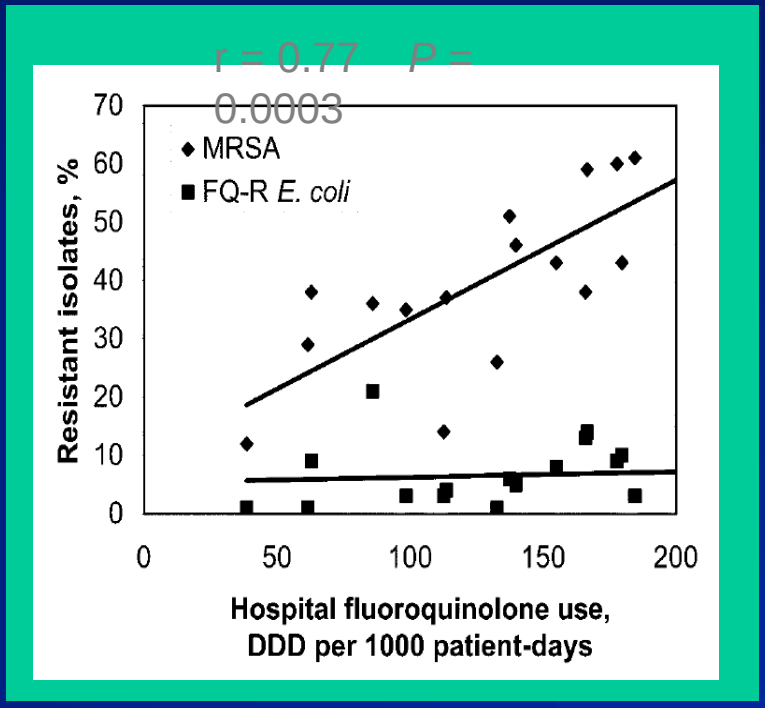
Correlations Between Fluoroquinolone Consumption and %MRSA

22 German ICUs, SARI, 2000-2003



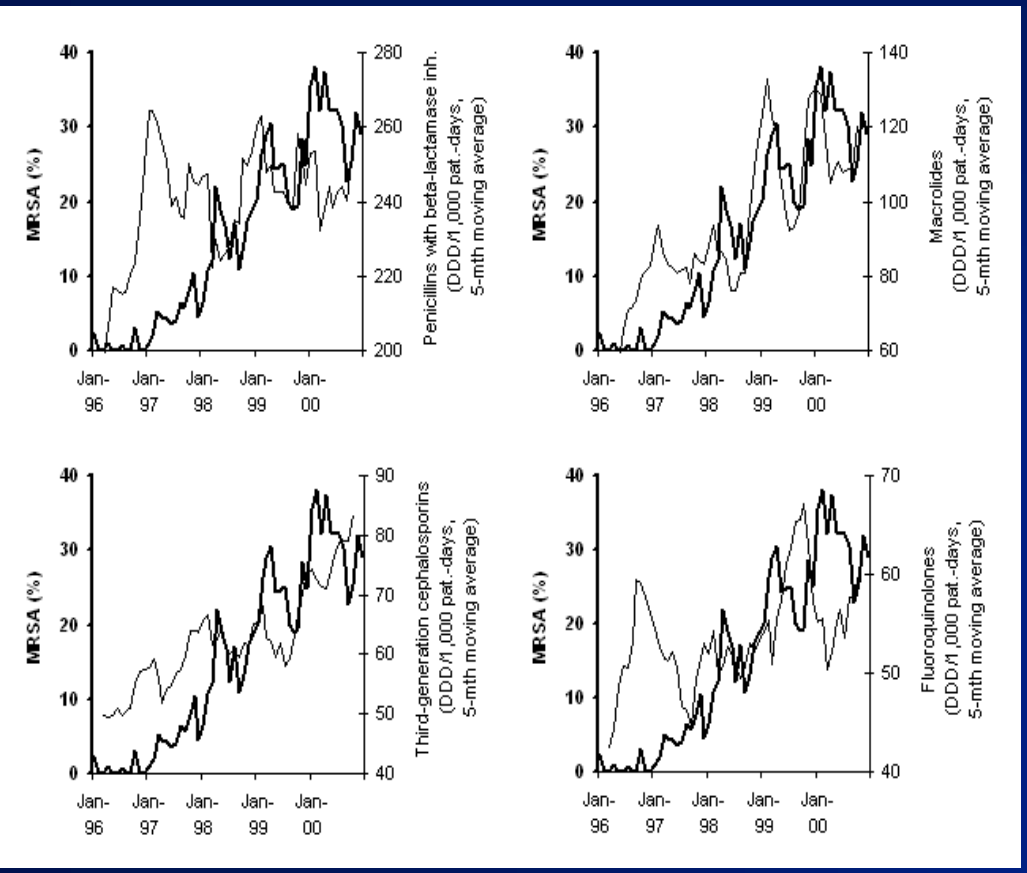
Meyer E, et al.
Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 2004;47:345-351

17 U.S. hospitals, SCOPE-MediMedia , 200



MacDougall C, et al.
Clin Infect Dis 2005;41:435-440.

%MRSA and Monthly Use of Macrolides, Third-Generation Cephalosporins and Fluoroquinolones, Aberdeen Royal Infirmary, 01/1996-12/2001



Explaining variable for monthly %MRSA	Lag (months)	Estimated coefficient
%MRSA	1	0.420
Macrolide use	1,2,3	0.165
Third-generation cephalosporin use	4,5,6,7	0.290
Fluoroquinolone use	4,5	0.255
Constant	-	- 36.7

R^2=0.902

ViResiST

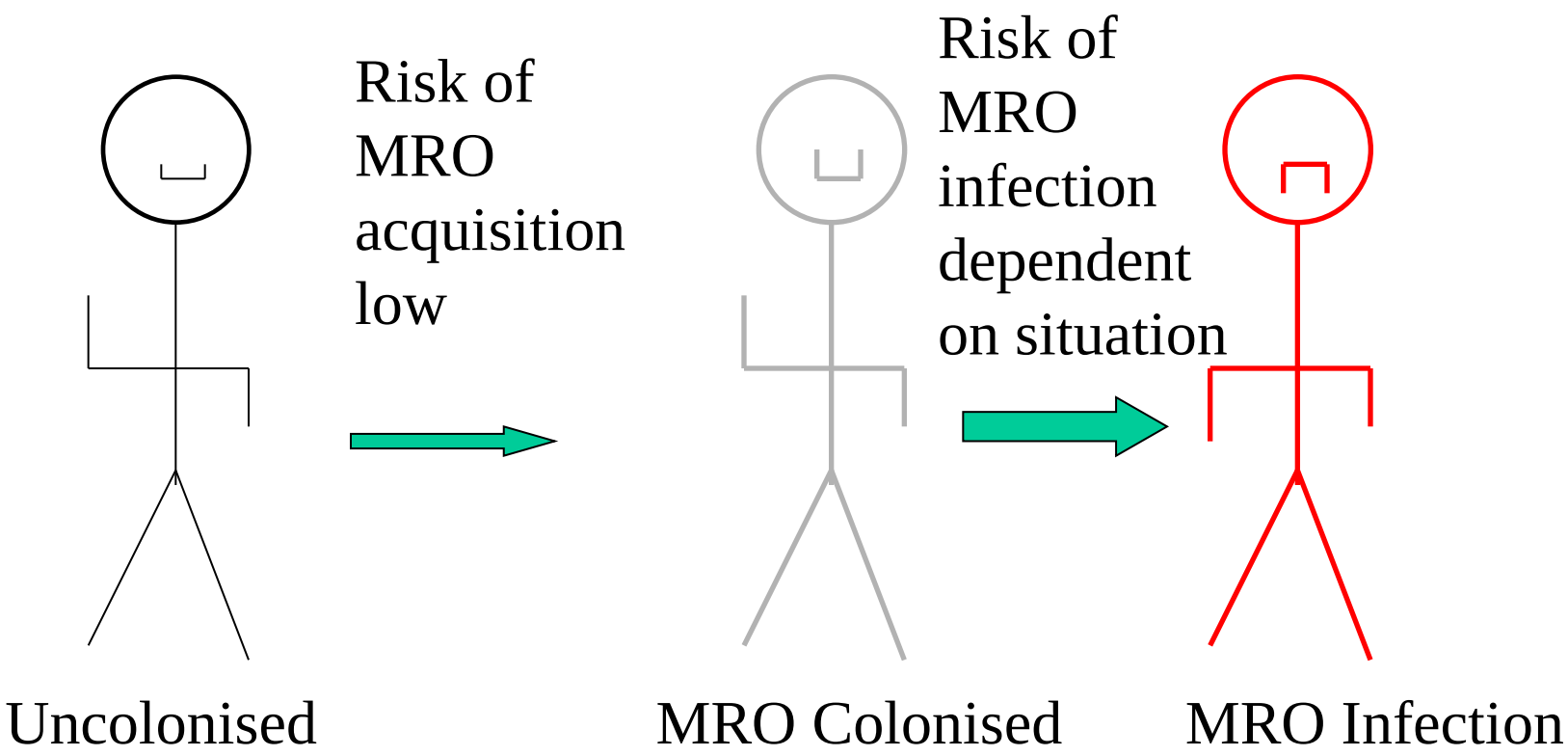
Source: Monnet DL, et al. Emerg Infect Dis 2004;10:1432-1441.

Antibiotic usage drives resistance

- Agriculture, food production, animals- zoonotic pathogens – *Campylobacter*, *Salmonella*, *Enterococcus*, *E. coli*. Resistance genes transfer from animal bowel flora to human strains within human gut.
- Humans- outpatients – over the counter availability, mostly unnecessary, frequent low dosing, poor compliance
- Humans – hospitals – 50%+ unnecessary or too prolonged. Frequent inappropriate agent selection, dosage, route or duration

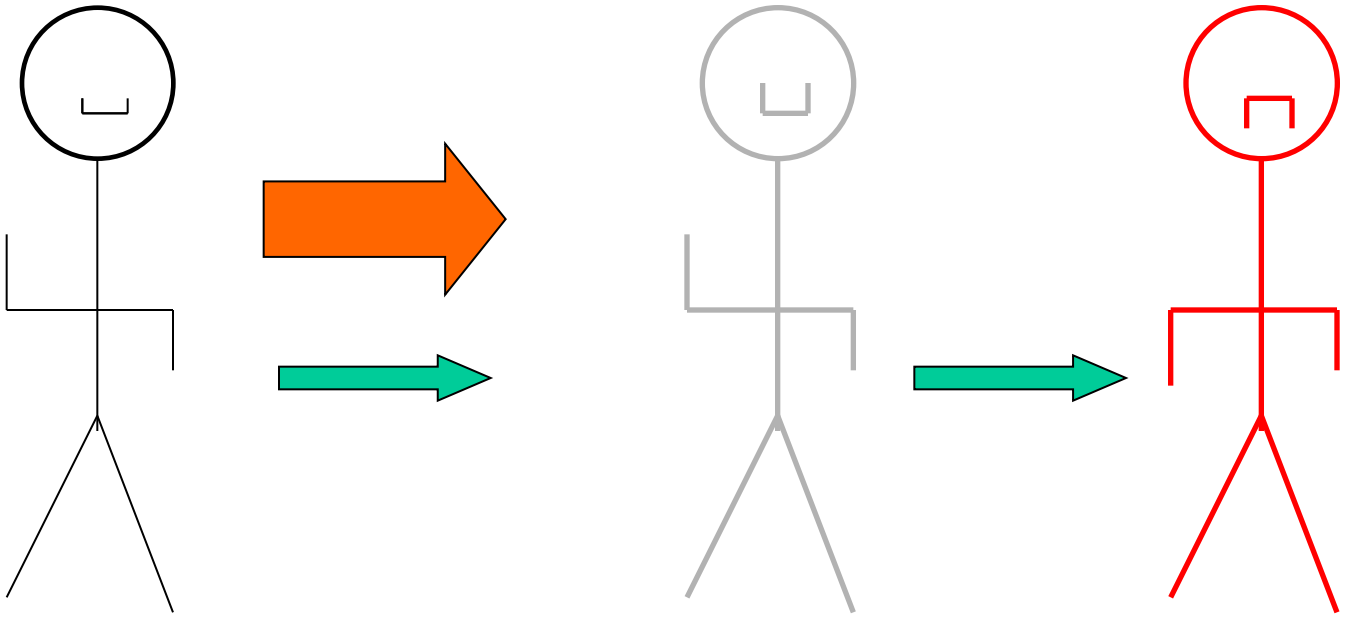
Multi-resistant organism (MRO) colonisation/infection sequence

A. Situation without antibiotic exposure



Multi-resistant organism (MRO) colonisation/infection sequence

Antibiotic exposure increases risk of acquiring MRO



Uncolonised

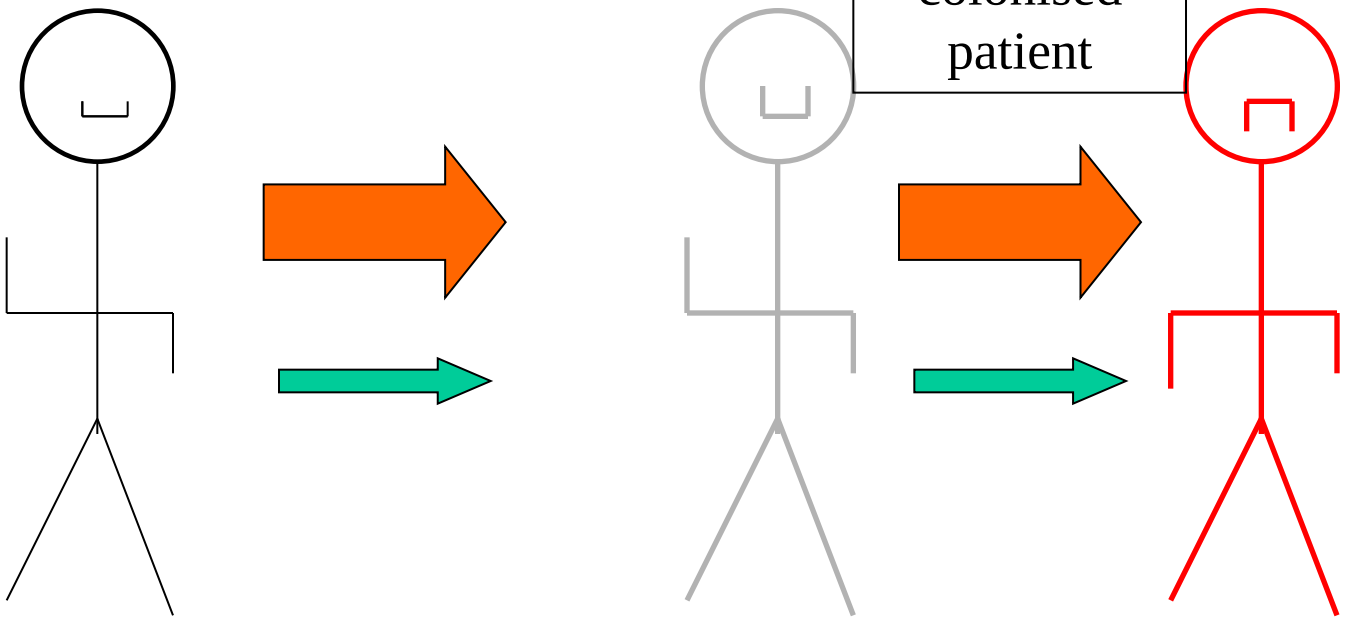
MRO Colonised

MRO Infection

Multi-resistant organism (MRO) colonisation/infection sequence

Antibiotic exposure increases risk of acquiring MRO

Antibiotic exposure increases risk that infection will occur in MRO-colonised patient



Uncolonised

MRO Colonised

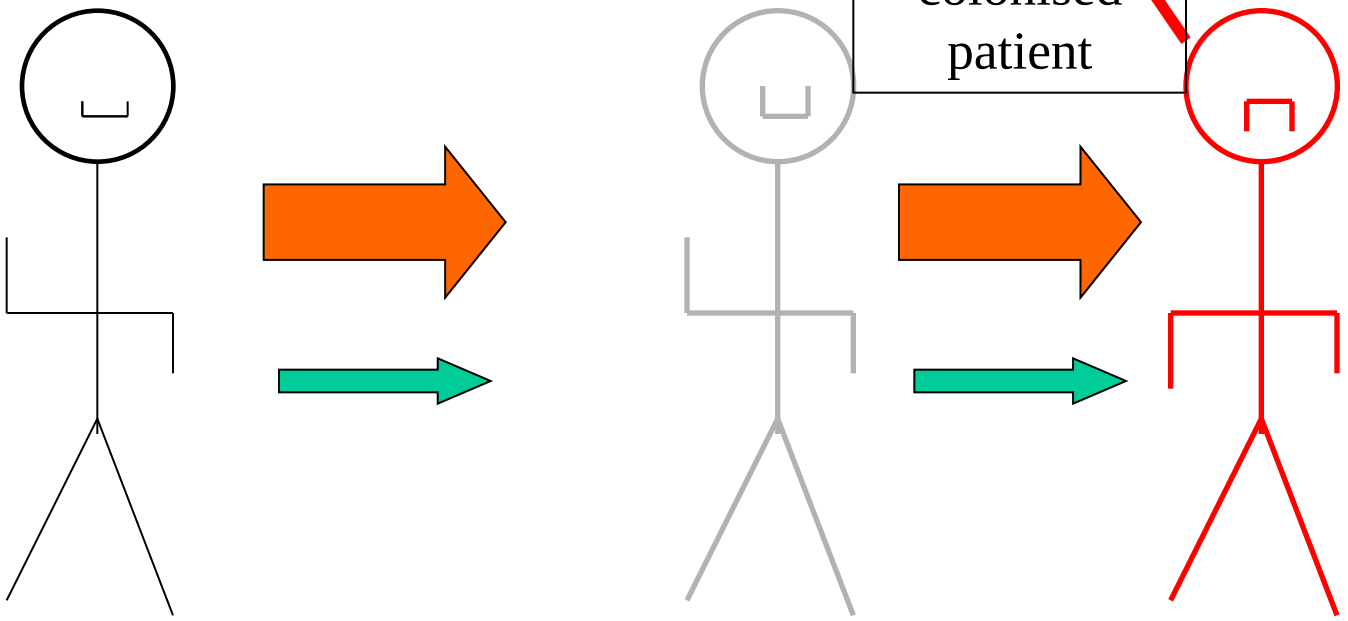
MRO Infection

Multi-resistant organism (MRO) colonisation/infection sequence

Antibiotic exposure increases risk of acquiring MRO

Antibiotic exposure increases risk that infection will occur in MRO colonised patient

AVOID using antibiotics in patients without infection!



Uncolonised

MRO Colonised

MRO Infection

Why is antimicrobial resistance important?

1. **Antimicrobial resistance kills-** mortality higher for resistant pathogens
2. **AMR hampers the control of infectious diseases** – prolonged infectivity – eg. resistant-TB cases
3. **AMR increases the costs of health care –** MDR-TB cost of treatment 200 times greater
4. **Achievements of modern medicine are put at risk by AMR-** eg. Oncological treatment, organ transplantation, prosthesis insertion
5. **AMR threatens health security, damages trade and economies**

Important pathogens ….

Gram negatives

(multi-resistant isolates with one or more of plasmid ampC enzymes, ESBL, carbapenem, quinolone, aminoglycoside resistance)

- *Shigella*, *Salmonella* species
- *E.coli*, *Klebsiella*, *Enterobacter* species
- *Pseudomonas aeruginosa*, *Acinetobacter baumannii*

Gram positives

- *Mycobacterium tuberculosis*- mdr and xdr
- *Staphylococcus aureus* – MRSA, (VRSA)
- *Enterococcus* species – vancomycin resistance - VRE
- *Streptococcus pneumoniae- penicillin +/- ceftriaxone resistance*

Carbapenem resistance

TABLE 1. Species distribution of clinically relevant acquired carbapenemases

Organism	MBLs (class B)	Class A KPC (GES)	OXA (class D)
Pseudomonads			
Pseudomonas aeruginosa	**++**	+	+
Pseudomonas putida	+	+	
Acinetobacter baumannii	+[a]		**++**
Acinetobacter spp.	+		+
Enterobacteria			
Klebsiella pneumoniae	+[a]	**++**	+
Escherichia coli	+	+	+
Proteus mirabilis	+		+
Providencia spp.	+		
Klebsiella oxytoca	+	+	
Serratia marcescens	+[a]	+	
Enterobacter spp.	+[a]	+	
Citrobacter freundii	+	+	
Morganella morganii	+		
Salmonella enterica		+	
Raoultella spp.		+	

MBL, metallo-β-lactamase.
++, prevalent species–enzyme type combinations; +, occasionally reported species–enzyme type combinations.
[a]Endemic in certain regions.
Crosses in bold denote higher prevalence in the respective species.

Carbapenemase genes may be from three of the four molecular classes of betalactamases

NDM- class B metallobetalactamase

Carbapenem resistance

TABLE I. Species distribution of clinically relevant acquired carbapenemases

Organism	MBLs (class B)
Pseudomonads	
Pseudomonas aeruginosa	**++**
Pseudomonas putida	+
Acinetobacter baumannii	+[a]
Acinetobacter spp.	+
Enterobacteria	
Klebsiella pneumoniae	+[a]
Escherichia coli	+
Proteus mirabilis	+
Providencia spp.	+
Klebsiella oxytoca	+
Serratia marcescens	+[a]
Enterobacter spp.	+[a]
Citrobacter freundii	+
Morganella morganii	+
Salmonella enterica	
Raoultella spp.	

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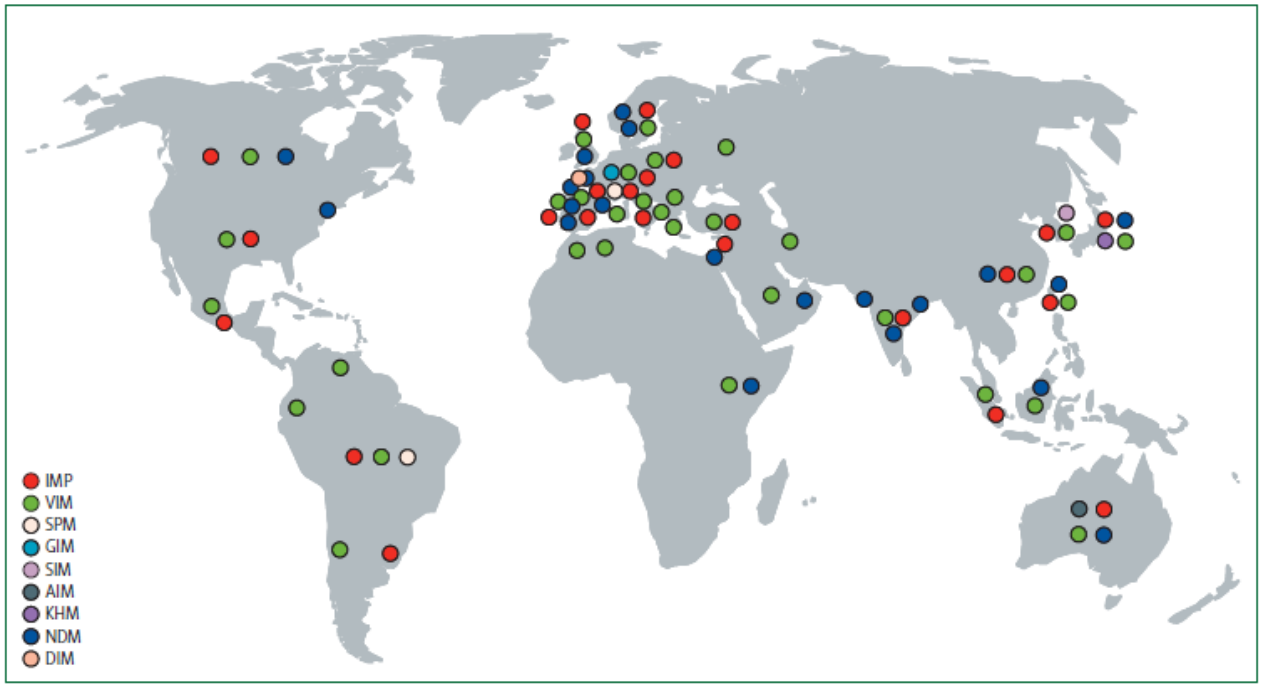
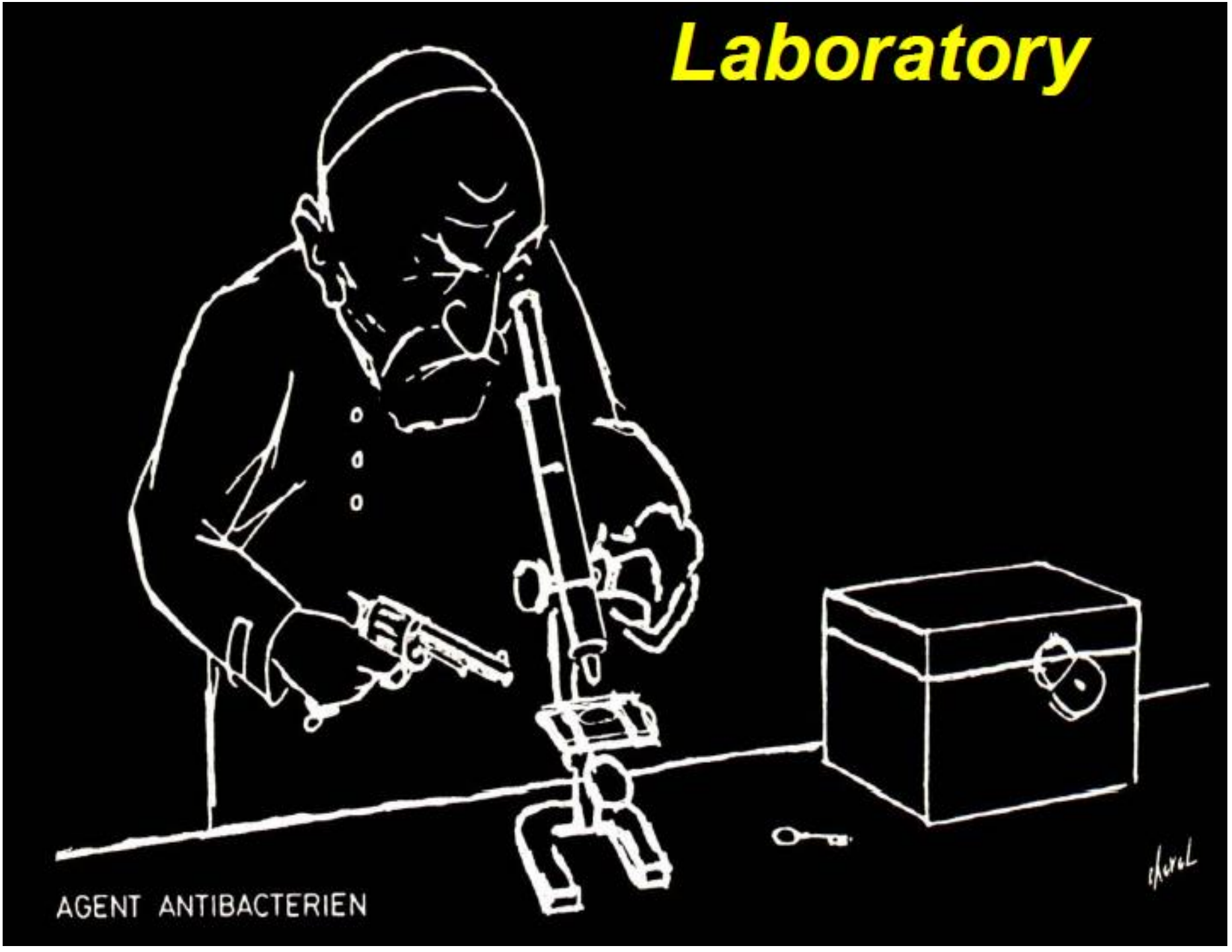


Figure 2: Worldwide dissemination of different types of metallo-β-lactamases

These isolates usually are pan-resistant; high mortality.

Laboratory



Detection of antibiotic resistance

- *In vivo* :
  - Clinical response to therapy
  - Eradication of the bacterium from the site of infection e.g.. Re-culture blood or CSF after therapy.
- Phenotypic
  - disc diffusion – S or R breakpoint zone size
  - agar diffusion MIC – E-test (right)
  - broth microdilution MIC – the reference
- Genotypic
  - PCR e.g.. *mecA* gene presence (MRSA)
  - Gene or whole genome sequencing

The *in vivo* susceptibility test

- Scenario 1: patient treated with antibiotic appears to be improving – what are the possible explanations?
  - The antibiotic did it ! Take credit…
  - The patient had a self-limited illness and improve despite the treatment that you prescribed

The *in vivo* susceptibility test

- Scenario 1: patient treated with antibiotic appears to be improving – what are the possible explanations?
- Scenario 2: patient is failing to respond to antibiotic treatment.
  - They have a non-bacterial infection
  - They don't have an infection!
  - The antibiotic is inactive or the wrong one
  - The infection is due to a multi-resistant organism that is not susceptible to the treatment
  - The illness does not get better that quickly! E.g. typhoid takes a median of 4 days of treatment before temp falls
  - Non compliant patient

Gram negative laboratory testing

- *AMPc* cephalosporinase producers (chromosomal- ESCPPM group- *Enterobacter*, *Serratia*, *Citrobacter* etc), also plasmid-borne- wider range including *E. coli*
  - third generation cephalosporins **may test initially as S but then resistance may emerge during treatment – don't report result**
  - Can report piperacillin+tazocin or ticarcillin+clavulanate
  - Meropenem resistance possible if outer membrane porin changes also present
- ESBL producers = resistance to third generation cephalosporins
  - piperacillin+tazocin or ticarcillin+clavulanate may test as S **but probably inferior for treatment – don't report results**
- Carbapenem resistance
  - **Reliable detection is problematic- meropenem disc test is recommended as a screen**

Salmonella Typhi and *S. paratyphi*

- **Perfloxacin is used to screen for quinolone resistance .** This predicts treatment failure with ciprofloxacin. Naladixic acid can also be used but inferior.
- At low levels of resistance, testing of ciprofloxacin = ‘susceptible’ – a false result
- Gatifloxacin, moxifloxacin retain activity for perfloxacin resistant isolates
- Ceftriaxone testing important

Staphylococcus aureus : MRSA detection

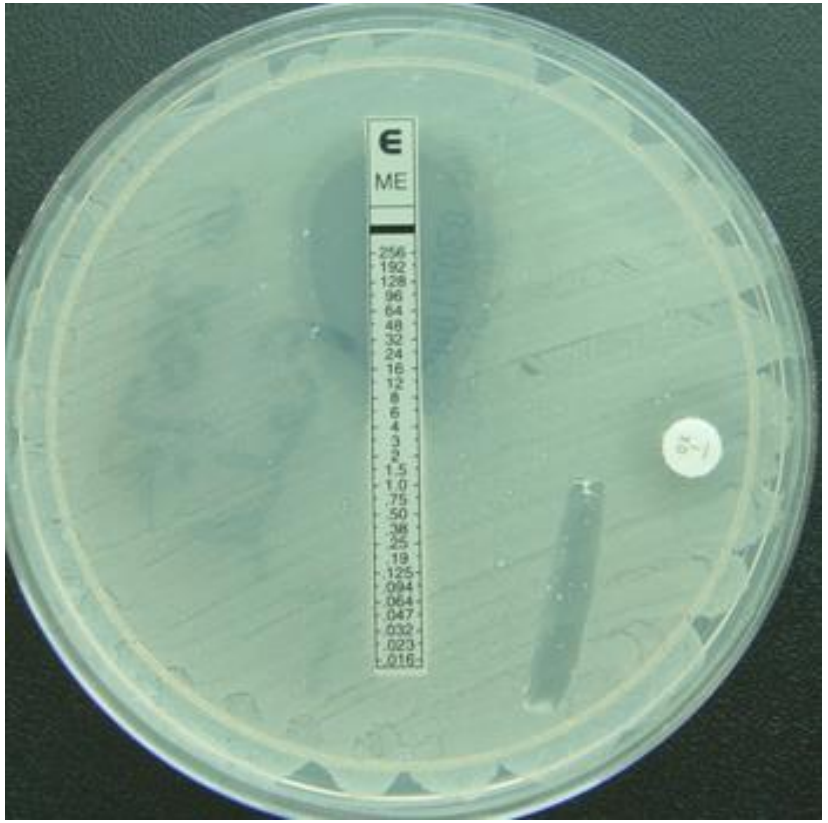
Cefoxitin 30 microgram disc screen is required for adequate sensitivity of detection of MRSA

- Other betalactams should not be tested– may get falsely susceptible result!
- Cefoxitin susceptible isolates are susceptible to dicloxacillin, cloxacillin, cephalosporins, imipenem etc.
- MRSA should be considered resistant to all currently available betalactams (ceftaroline is the exception)
- Laboratory should also effectively screen for high level vancomycin-resistant *Staphylococcus aureus* (VRSA)- **30 microgram vancomycin disc screen is effective**

Streptococcus pneumoniae **betalactam resistance detection**

Oxacillin 1µg disc test reliably detects isolates with raised penicillin MIC (> 0.12mg/L).

- Oxacillin resistance predicts treatment failure with penicillin in meningitis
- Penicillin and ceftriaxone minimal inhibitory concentration (MIC) may be done with an ‘E-test strip’.



Laboratory testing: ensuring a reliable result

- Before testing :
  - Clinician formulates a differential diagnosis
  - Selection of correct tests PRIOR to treatment
  - Correct specimen collection and transport to the laboratory
- In the laboratory
  - Trained staff following standard procedures
  - Quality controlled media for culture
  - Correct AST methods used
  - Internal quality control – special control bacterial strains tested regularly against the media & antibiotic discs in use
  - External quality assurance- unknown samples sent to the laboratory for identification and AST

Laboratory testing: ensuring a reliable result

- After testing- reporting and liaison
  - Timely report provided and communicated to the clinician
  - Explanatory comments provided – distinguish colonisation from infection, explain extrapolation of AST results, provide treatment guidance or direct clinician to specific guidelines
  - Direct liaison with clinician occurs for critical results – positive blood cultures, highly resistant isolates

Gram negative resistance: survey of Nepal literature (2011)

- No referenced quality control systems for testing
- Variable resistance rates seen
- High quinolone resistance in East Nepal studies
- MDR *Salmonella enterica* detected

Gram positive resistance: published work from Nepal

- *Staphylococcus aureus* (MRSA): high nasal carriage rates in children; prevalent in hospitalised patients (no paediatric studies located)
- *Streptococcus pneumoniae*: relatively low rates of penicillin resistance in several studies (testing not correct though). Rates of ceftriaxone resistance not documented.
- Laboratory methodology issues are important and may lead to underestimation of resistance

Search your literature

- Searching Pubmed is easiest; also contact the Bir Hospital laboratory for local data
- Evaluate the microbiological quality of the study:
  - Are the isolates studied from an appropriate range of clinical specimens?
  - Have likely contaminants or colonising bacterial been eliminated from analysis
  - Under methods, what quality control is documented?
  - For AST, do they reference CLSI or EUCAST standards for testing and have the correct antibiotics been tested?
  - Does the laboratory concerned participate in the EQA program from national public health laboratory?

Further presentations and resources

- Basnyat B et al Nepal Global Antibiotic Resistance Partnership, 2015 AMR situation analysis. J Nepal Health Res Counc 2015 May - Aug;13(30): 102-11

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http://idmic.net

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