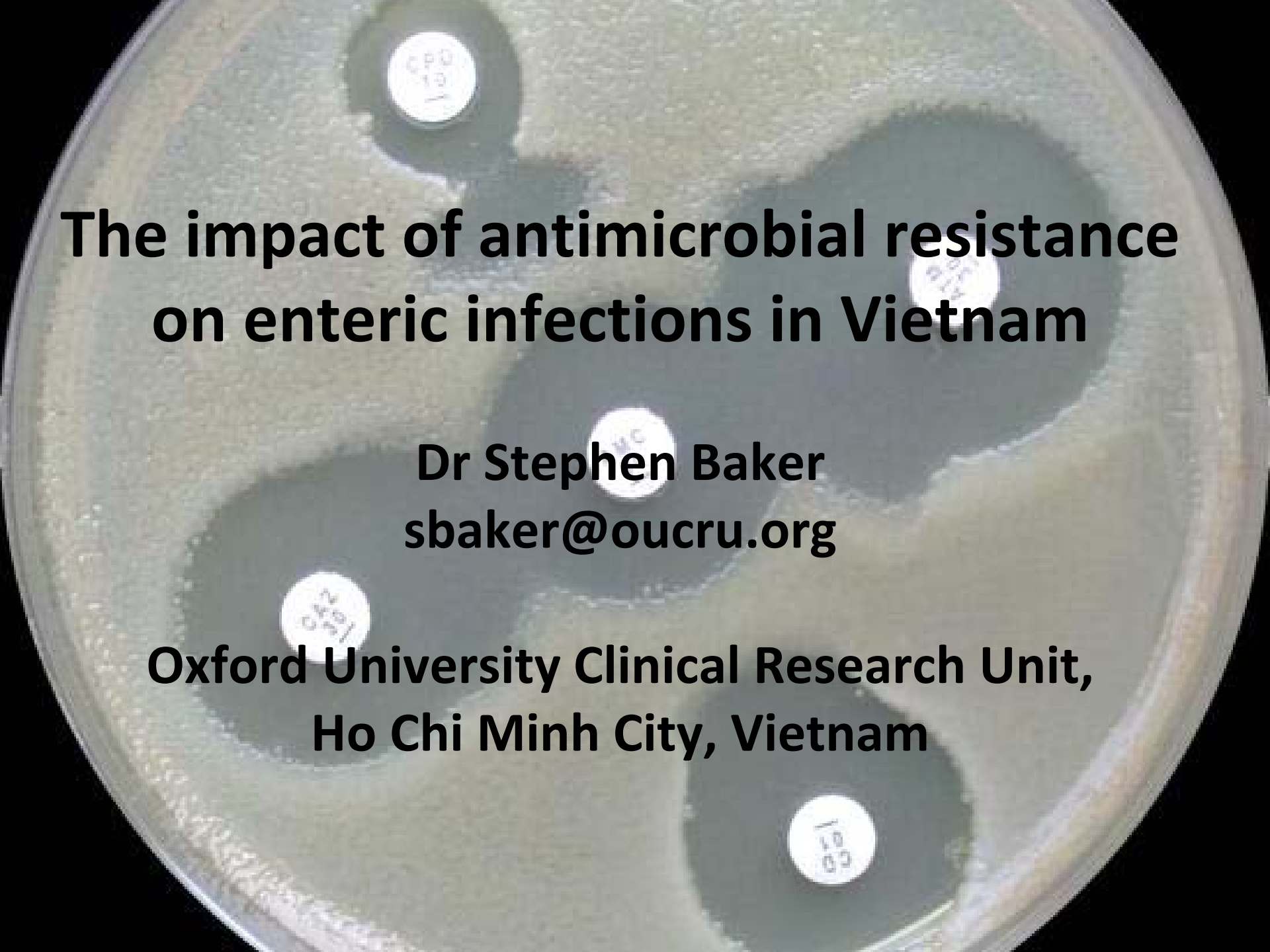


# The impact of antimicrobial resistance on enteric infections in Vietnam

**Dr Stephen Baker**
**sbaker@oucru.org**

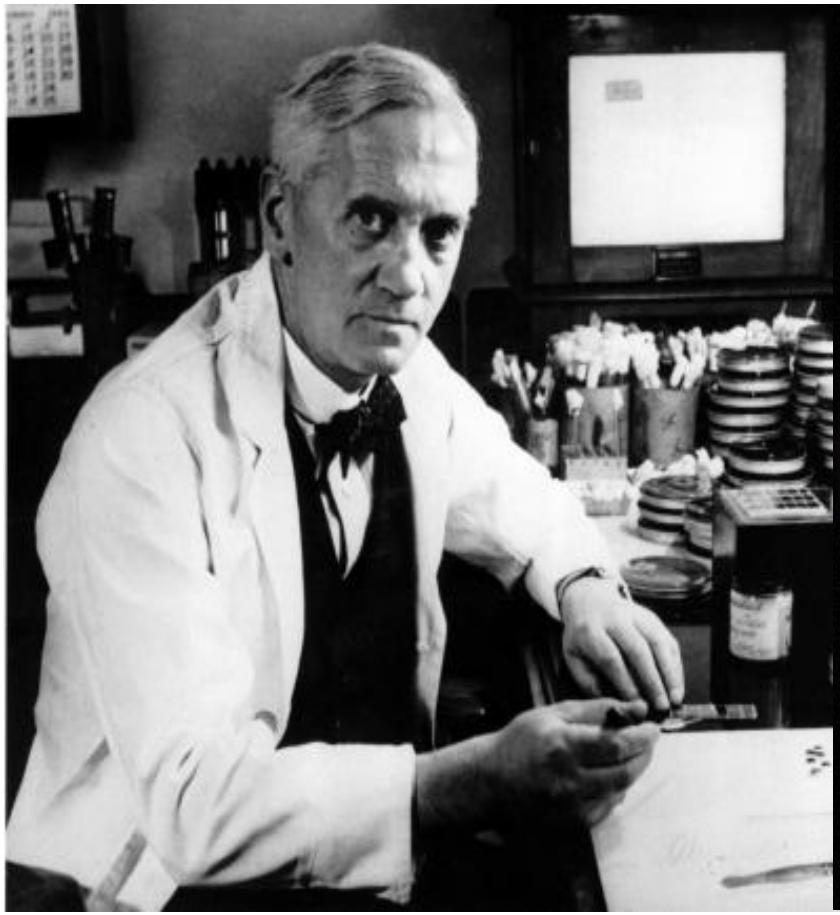
**Oxford University Clinical Research Unit,**
**Ho Chi Minh City, Vietnam**

# Outline

- The impact of antimicrobial resistance
- The drivers of antimicrobial resistance
- (Fluoro)quinolone resistance in typhoid
- ESBL producers
- Resistance in commensal organisms
- Carbepenemases
- Preventative measures

# Microorganisms

# From the miracle…….



**1928……1942……1947**

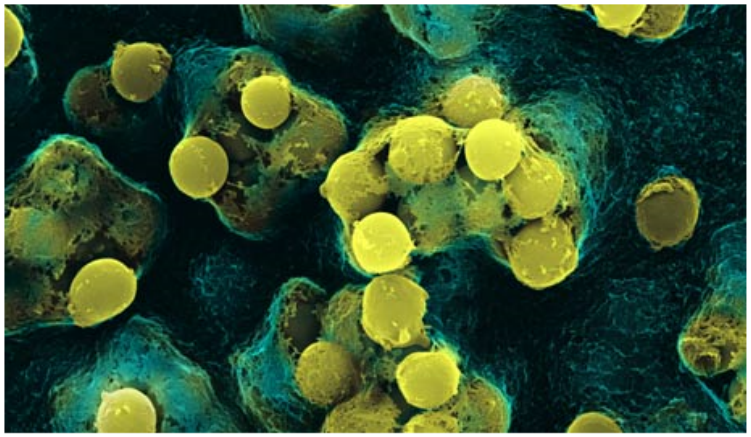
# …….To This

News › Society › Antibiotics

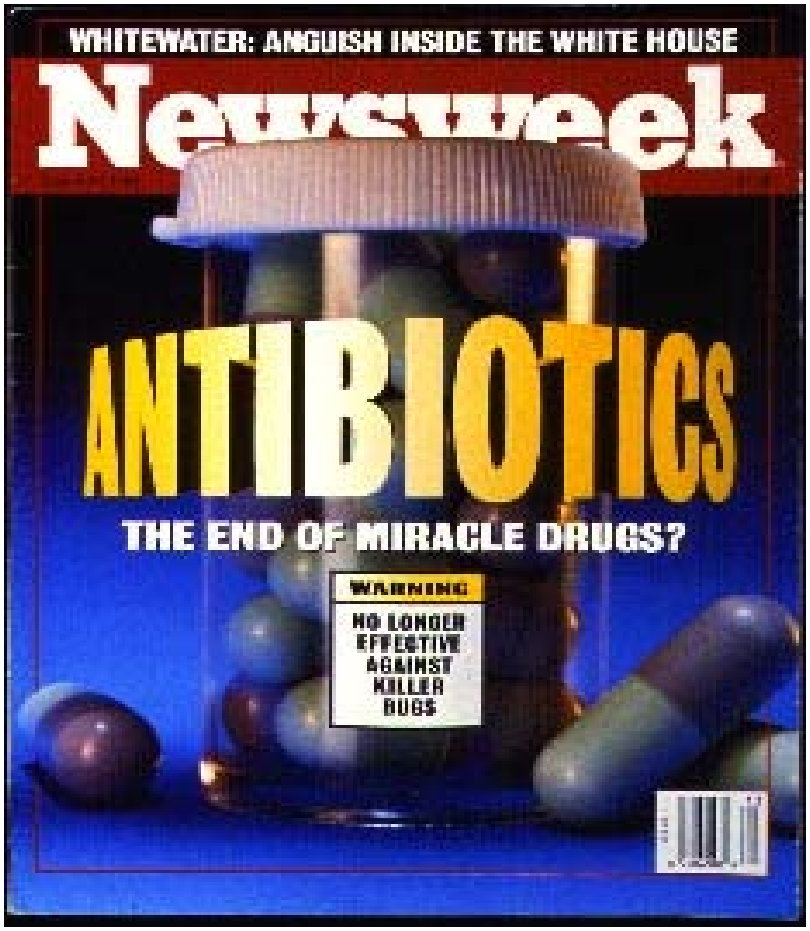
## Are you ready for a world without antibiotics?

Antibiotics are a bedrock of modern medicine. But in the very near future, we're going to have to learn to live without them once again. And it's going to get nasty

Sarah Boseley
The Guardian, Thursday 12 August 2010
Article history



Streptococcus pyrogens bacteria. Photograph: S Lowry/University of Ulster/Getty Images



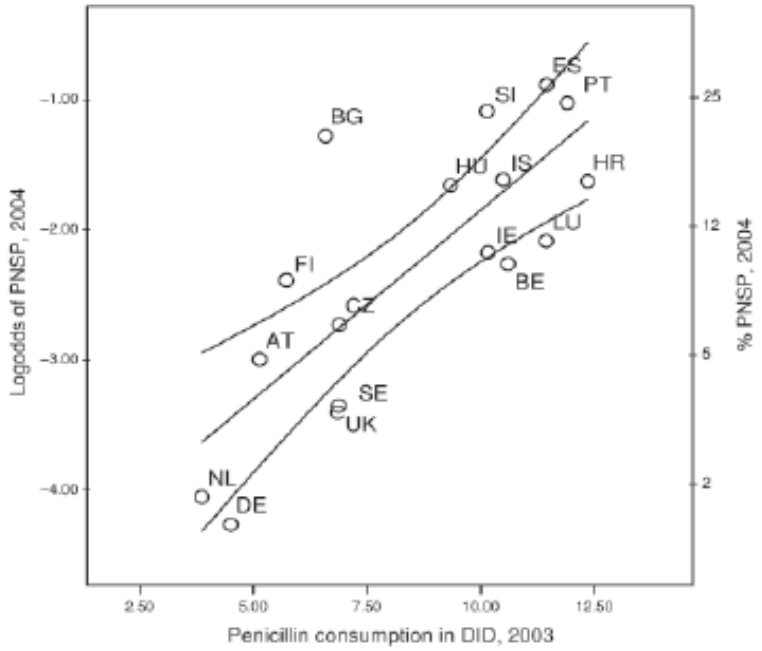

# In less than 50 years

# Impact of resistance

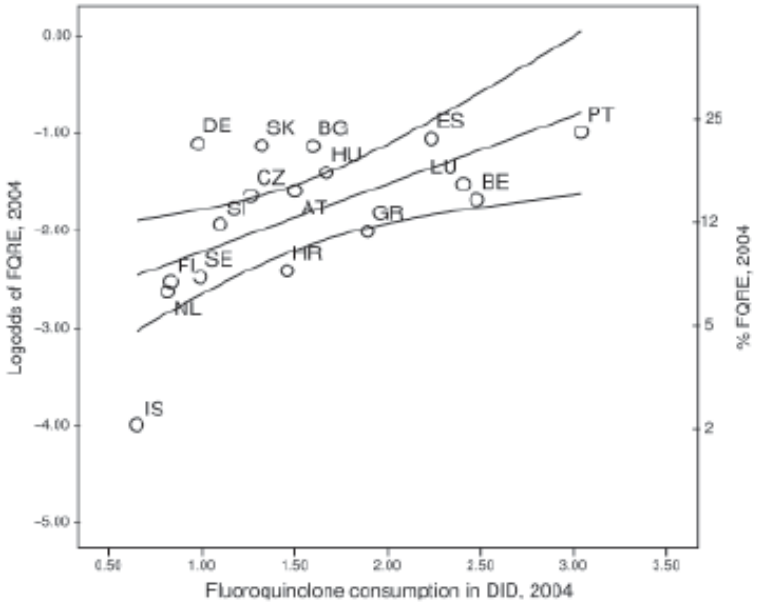
- 70% of neonatal sepsis cannot be treated with antibiotics recommended by WHO due to resistance. *Lancet 2005.*
- 67% died with resistant bacteria as compared to 26% with sensitive. *BMC Pediatrics 2010.*
- Antimicrobial resistance has global health implications
- Pathogenic bacteria that exhibit antimicrobial resistance are a widespread phenomenon and arguably constitute an uncontrollable global epidemic.

# Occurrence of antimicrobial resistance is associated with antimicrobial usage

Data for primary blood culture isolates of *S. pneumoniae* and *E. coli*




Occurrence of penicillin-nonsusceptible *Streptococcus pneumoniae* (PNSP) plotted against outpatient use of penicillins in 17 European countries including 95% confidence intervals. DID. defined daily doses per 1,000 inhabitants




Occurrence of fluoroquinolone-resistant *Escherichia coli* (FQRE) plotted against outpatient use of fluoroquinolone antimicrobial agents in 17 European countries including 95% confidence intervals. DID, defined daily doses/1,000 inhabitants.

# MRSA - Methicillin resistant *Staphylococcus aureus*

S. aureus is a common bacterium that can be found on the skin of many healthy people

Typically is causes only minor infections, in

“pimples” but can also cause serious diseases (e.g. pneumonia)

First report of resistance to penicillin in 1947

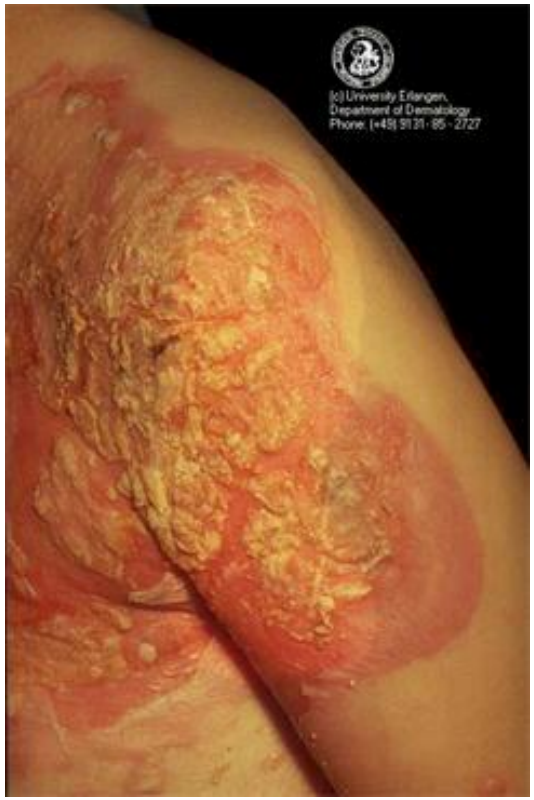
MRSA is also resistant to ampicillin and other

penicillins, erythromycin, tetracycline

can only be treated with Vancomycin

Vancomycin-resistant strains have

already been found and isolated



News Front Page
World
UK
England
Northern Ireland
Scotland
Wales
Business
Politics
Health
Medical notes
Education
Science/Nature
Technology
Entertainment
Have Your Say
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In Pictures
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Last Updated: Thursday, 24 February, 2005, 11:10 GMT

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# NHS superbug death rate doubles

**The number of deaths in which the superbug MRSA has been cited as a cause has doubled in four years, official statistics show.**

Some of the MRSA increase could be down to better reporting

The Office for National Statistics said in 2003 MRSA was mentioned on 955 death certificates - up from 487 in 1999.

But the figures suggested some of the rise may be down to better reporting of the bug.

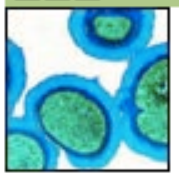
Other statistics revealed the number of HIV diagnoses seems to have levelled off after a decade of increases.

However, it was the MRSA figures which have proved most controversial.

Mortality rates were highest among older people with more men than women dying.

> **"No other country has seen the super bug infection take over its hospitals in the same way as we have in Britain"**

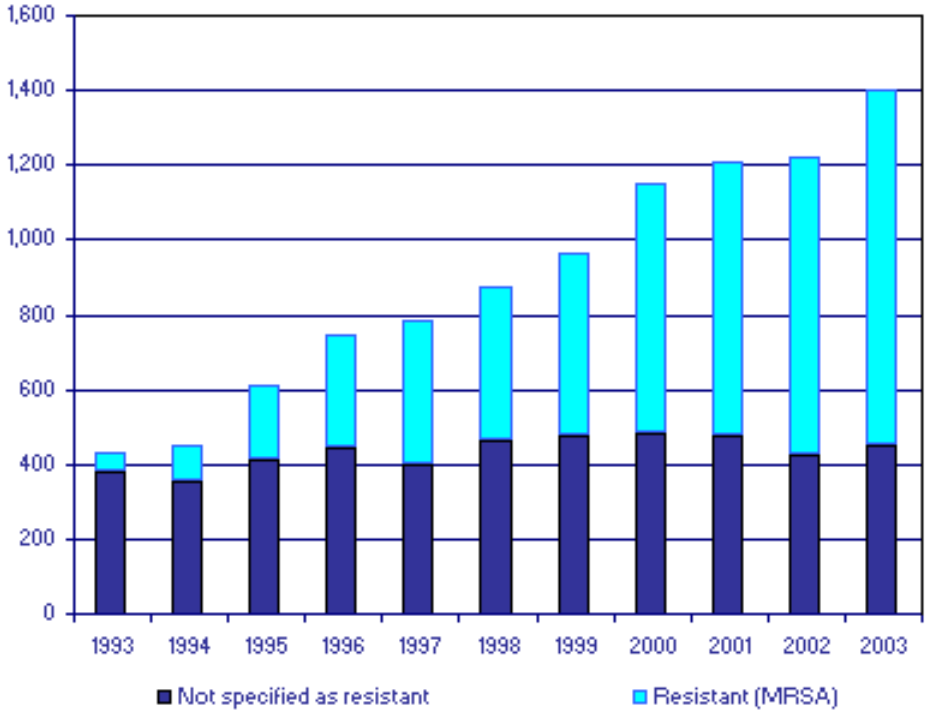
BBC NEWS:VIDEO AND AUDIO
**What hospitals are doing to stop the spread of MRSA**
VIDEO

BBC iCAN

**MRSA infections**
What can you do about the hospital 'superbug'?

SEE ALSO:
- Nasty clones causing MRSA problem
  10 Feb 05 | Health
- 'Clean yourself' advice on MRSA
  26 Jan 05 | Health
- NHS targets 'to blame for MRSA'
  15 Jan 05 | Health
- Genetic blueprint of MRSA cracked
  24 Jun 04 | Health
- Q&A: MRSA 'superbugs'
  13 Dec 02 | Health

# MRSA in the UK

Deaths per year




Source: Health Protection Agency

# Antimicrobial development

## Lack of development of new antimicrobial drugs: a potential serious threat to public health

J Ragnar Norrby, Carl Erik Nord, and Roger Finch, for the European Society of Clinical Microbiology and Infectious Diseases (ESCMID)

Lancet Infect Dis 2005; 5: 115–19

Swedish Institute for Infectious Disease Control, Solna, Sweden; Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden; and Clinical Microbiology and Infectious Diseases, The City Hospital, Nottingham, UK

Correspondence to: Professor J Ragnar Norrby, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden.

**Antimicrobial resistance is threatening the management of infections such as pneumonia, tuberculosis, malaria, and AIDS. In the past, resistance could be handled by development of new drugs active against resistant microbes. However, the pharmaceutical industry has reduced its research efforts in infectious diseases, genomics has not delivered the anticipated novel therapeutics, new regulatory requirements have increased costs, antibiotic use in common infections—eg, bronchitis and sinusitis—is questioned, and, compared with other drugs, return on investments is lower for antimicrobials. To avoid a serious threat to public health, academia, biotechnology and pharmaceutical industry, regulators, and healthcare providers must find solutions to this problem. Academia should concentrate on technologies to unlock new drug targets, and industry on drug candidates. In addition, regulators and pharmaceutical companies should agree on new clinical-trial designs so that information on therapeutic efficacy is generated in fewer patients—eg, by studying pharmacodynamics of antimicrobials in patients with defined infections.**

Since the development of sulphonamides in the 1930s and penicillin in the 1940s, many new classes of antibacterial compounds have been developed. Drugs active against fungi, parasites, and viruses have also been introduced. Within the field of antibiotics, the emergence of resistance was soon realised to represent a considerable clinical problem. However, until the end of the 20th century, the pharmaceutical companies were consistently able to develop new antibiotics with activity against most resistant bacterial strains.

During the past 10–15 years, antibiotic-resistant organisms have steadily increased, and now present a threat to disease management. Examples include meticillin-resistant staphylococci, pneumococci resistant to both penicillin and macrolides, vancomycin-resistant enterococci, multidrug-resistant Gram-negative organisms, and multidrug-resistant strains of *Mycobacterium tuberculosis*. Development of multidrug resistance is now gathering pace and contributing more substantially to the problem. For instance, penicillin-resistant *Streptococcus pneumoniae* was not a major problem until the same organism also became resistant to macrolides. Viruses, particularly HIV, have also developed resistance. Fungi may become resistant but, by contrast with bacteria, resistance cannot be transferred between fungal cells. Resistance has also become a major problem in the treatment of, and prophylaxis against, malaria.

The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has noted with great concern that we now seem to have entered a new era where very few novel antimicrobial drugs are being developed.[1,2] For example, of the new antibiotics introduced during the past decade or that are under development, none has improved activity against multidrug-resistant Gram-negative pathogens, or against mycobacteria (table 1). As a consequence of this lack of new drugs, organisms resistant to most existing antimicrobials may emerge. The result could be that common infections become untreatable, and even lethal. This threat is most obvious in the fields of bacterial infections (including tuberculosis), parasitic infections (especially malaria), and HIV infections.

This article highlights a selection of the issues discussed at an ESCMID symposium on Antimicrobial Treatment in the 21st Century, held in Stockholm in June 2004 (*http://www.escmid.org/sites/index_f.asp?para.3.1.5*). We provide an analysis of the reasons for the current lack of development of new anti-infective drugs, and give some suggestions as to how the problem might be tackled.

### The roles for academic research, the biotechnology industry, and the pharmaceutical industry

In the past, antibiotics—eg, penicillin, cephalosporin, and streptomycin—were developed by screening natural sources for products. The modern development of a new antimicrobial drug starts by identification of a target that is essential for the microorganism but, preferably, is not present in human beings. This process should, in theory, be facilitated by modern genomic technologies; the complete genomes of more than 100 microorganisms are today mapped. Less than 20% of well-known essential targets have been exploited.[3] However, a genomic-based approach has, so far, failed to deliver novel therapeutics.[3] In fact, in the past three decades,

| Antibiotic | Class | Main Gram-positive/Gram-negative activity |
|---|---|---|
| Quinupristin/dalfopristin | Streptogramin | Gram-positive, including MRSA, VRE |
| Telithromycin | Ketolide | Gram-positive, including MRSA, PRP |
| Linezolid | Oxazolidinone | Gram-positive, including MRSA, PRP, VRE |
| Daptomycin | Lipopeptide | Gram-positive, including MRSA, PRP |
| Moxifloxacin | Fluoroquinolone | Gram-positive, mainly PRP |
| Gatifloxacin | Fluoroquinolone | Gram-positive, mainly PRP |
| Meropenem | Carbapenem | None |

TABLE 1: ANTIBIOTICS ENTERING MARKET 2000

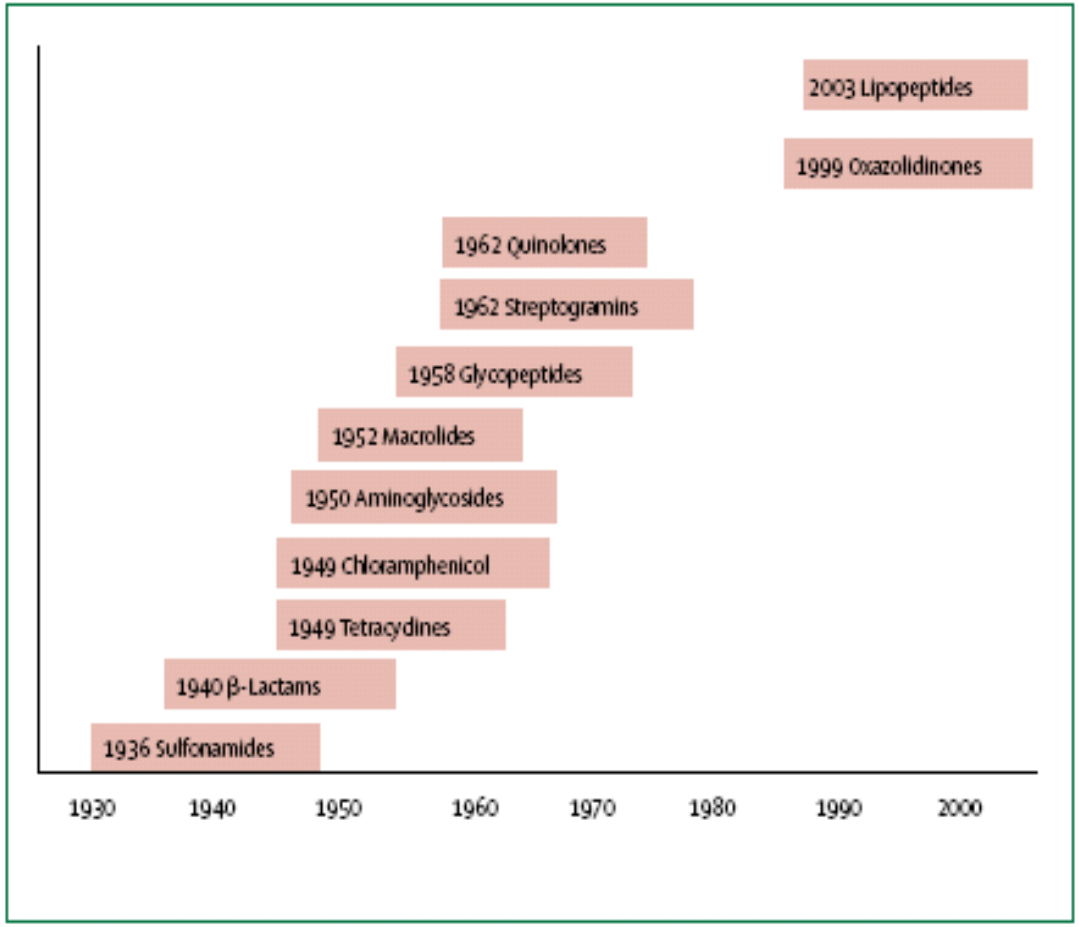


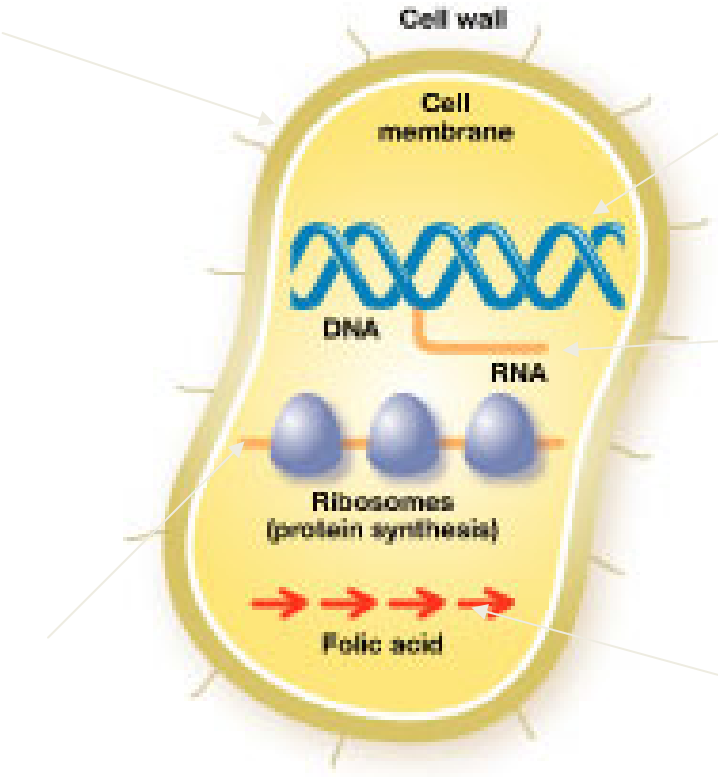

*Figure:* Development of new antibiotics over time



The Lancet Infectious Diseases, February 2005

# Targets of Antimicrobials

**Inhibition of Cell wall synthesis**
Penicillins
Cephalosporins
Carbapenems
Daptomycin
Glycopeptides




**DNA synthesis**
Fluoroquinolones

**RNA synthesis**
Rifampicin

**Protein synthesis**
Macrolides
Chloramphenicol
Tetracycline
Aminoglycosides

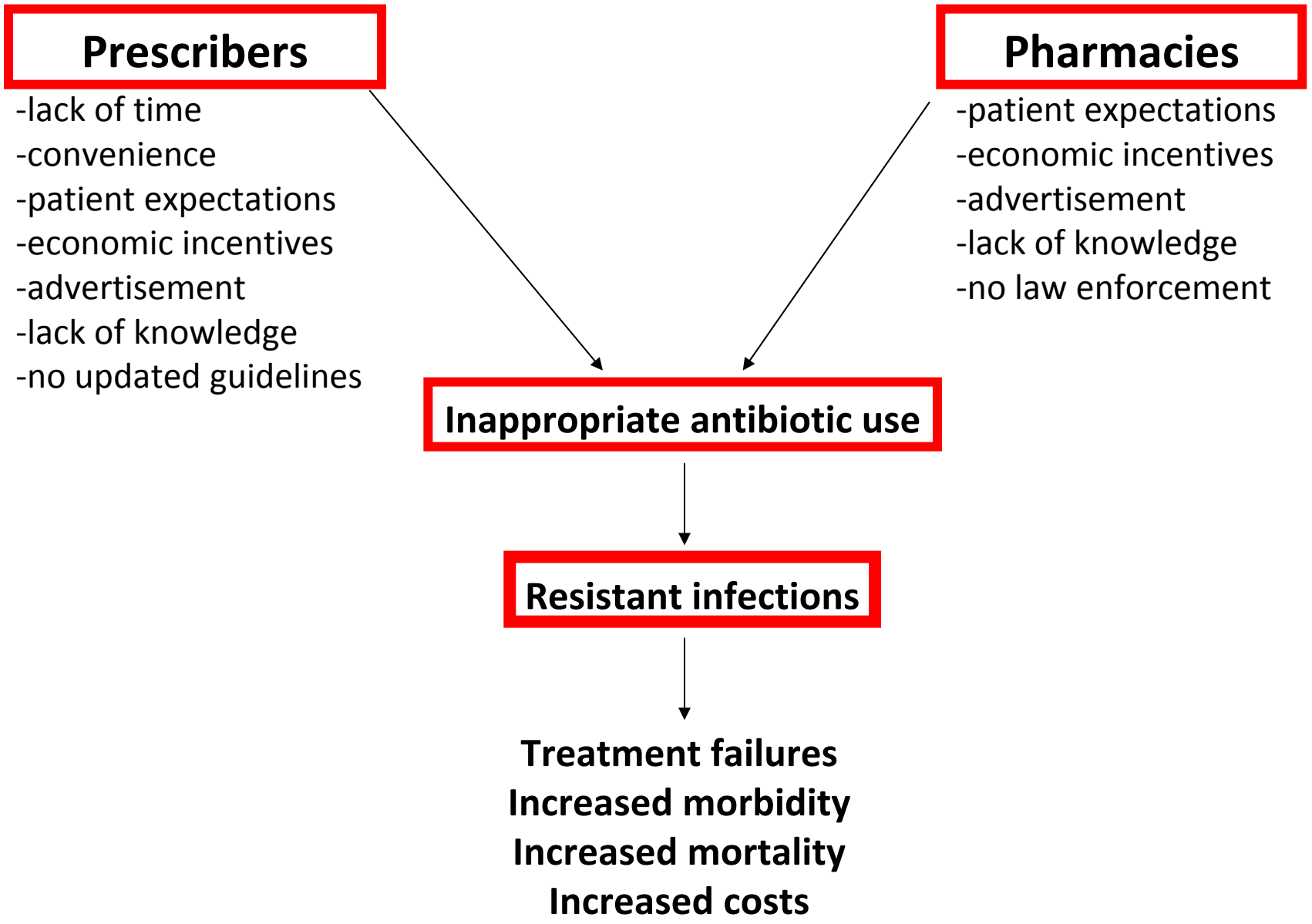
**Folic acid synthesis**
Sulfonamides
Trimethoprim

A lack of novel targets for new antimicrobials?

# Impact of resistance in Vietnam

- Lack of antimicrobial legislation
- Inappropriate community usage
- Inadequate therapy or therapy not required
- Forcing a selective pressure on bacterial populations
- Failing of hospital therapy for severe infections
- Prospect of antimicrobials becoming useless….

# Emergence of Antimicrobial Resistance



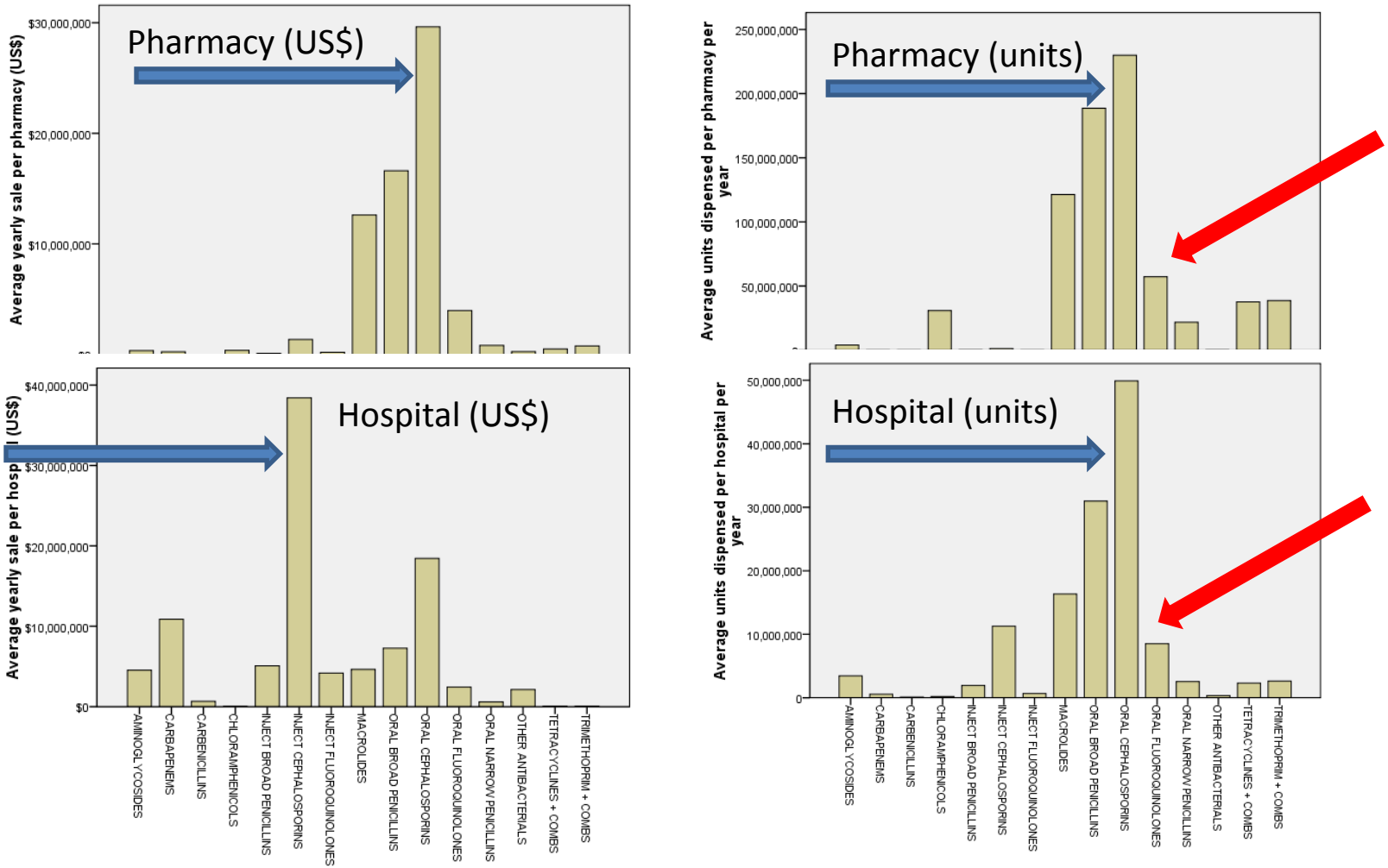
# Important drivers of AB consumption

- High out-of-pocket health expenditure
- Mostly self-medication as is cheaper and quicker
- Despite regulation, AB dispensed without prescription
- No law enforcement

# Important drivers of AB consumption

- Lack of knowledge: patient, pharmacist, doctor
- Financial incentives: patient, pharmacist, doctor
- Financial incentives health facilities
- Lack of time doctor
- Lack of good diagnostics
- Frequently used for mild ARI
- Advertising

# Antibiotic use pattern is some pharmacies and hospitals in Vietnam




Source: GARP report 2010

# Quinolones

- Family of broad-spectrum antibiotics.
- The majority of quinolones in clinical use belong to the subset of Fluoroquinolones (FQ).
- Inhibit topoisomerases/DNA synthesis
  - DNA gyrase/topo II *(gyrA* and *gyrB)*
  - Primary target in Gram-negatives

  - Topoisomerase IV (*parC and parE*)
  - Primary target in Gram-positives

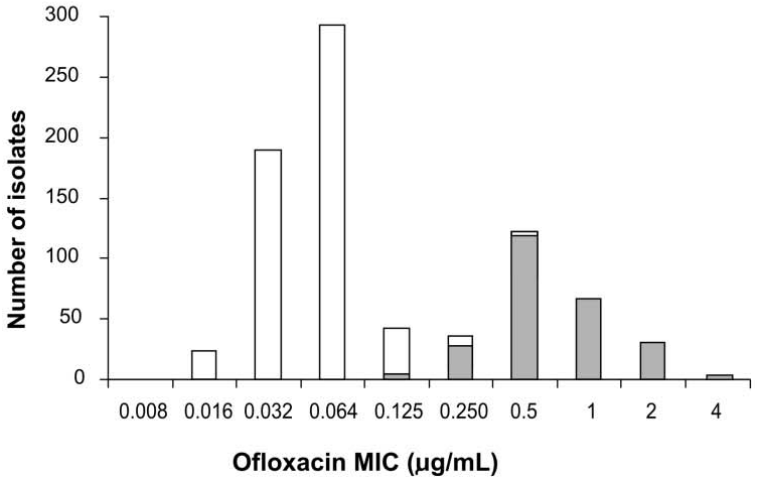
# Resistance to Quinolones

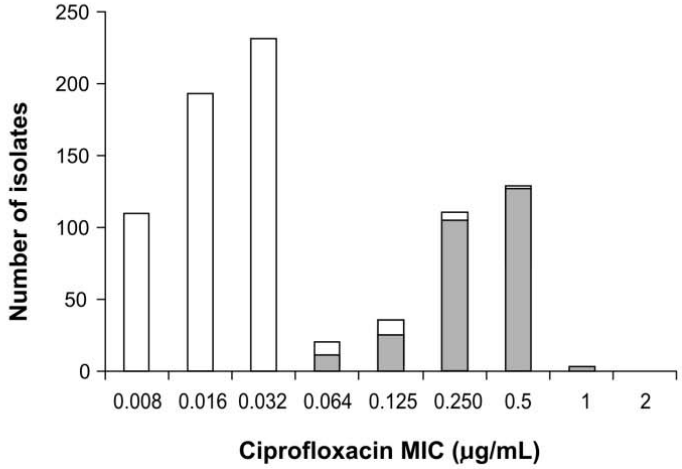
- Mutations in DNA gyrase and topo IV subunits
- Stepwise increase in resistance results from sequential mutations
    - Quinolone resistance determining region(QRDR) is a hotspot for mutation
    - *gyrA* codons 83 and 87 in *Salmonella*
    - Amino acid substitutions within QRDR
    - Hydroxyl group → hydrophobic group
    - Changes in binding site conformation and/or charge

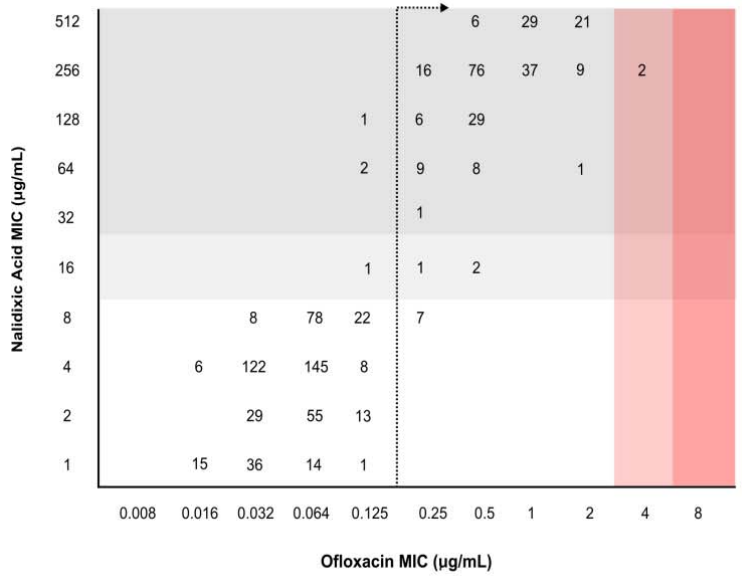
# Plasmid-mediated FQ resistance

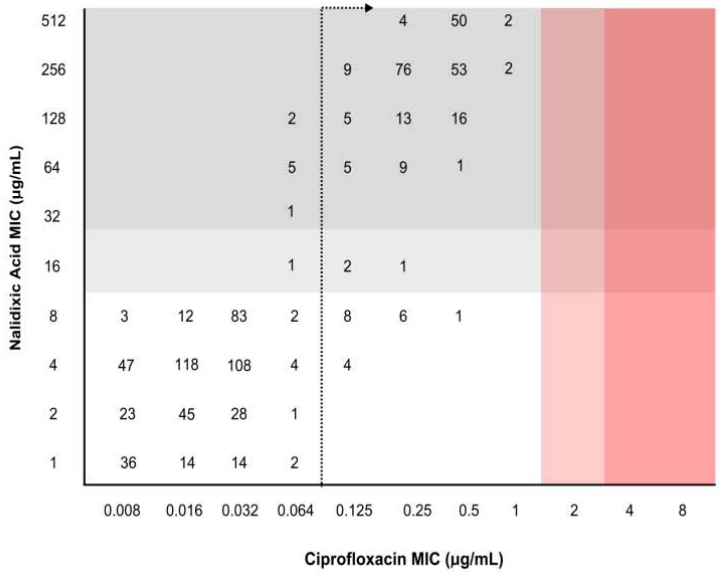
- Plasmid-mediated FQ resistance (*qnrA, B, S*)
- Significant as previously FQ resistance only spread vertically!
- Protects DNA gyrase from ciprofloxacin (CIP) inhibition.
- Nalidixic acid resistance, reduced susceptibility to FQ's
- Association with ESBL producers *(qnrA, B).*

# Fluoroquinolones in Typhoid in Vietnam

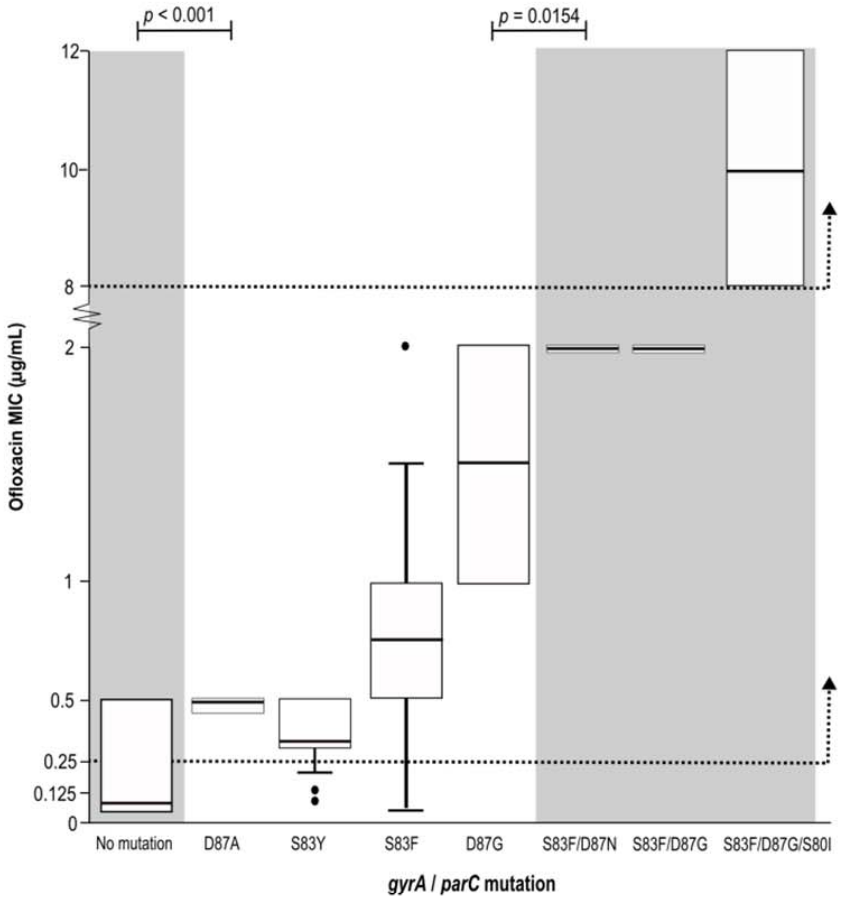


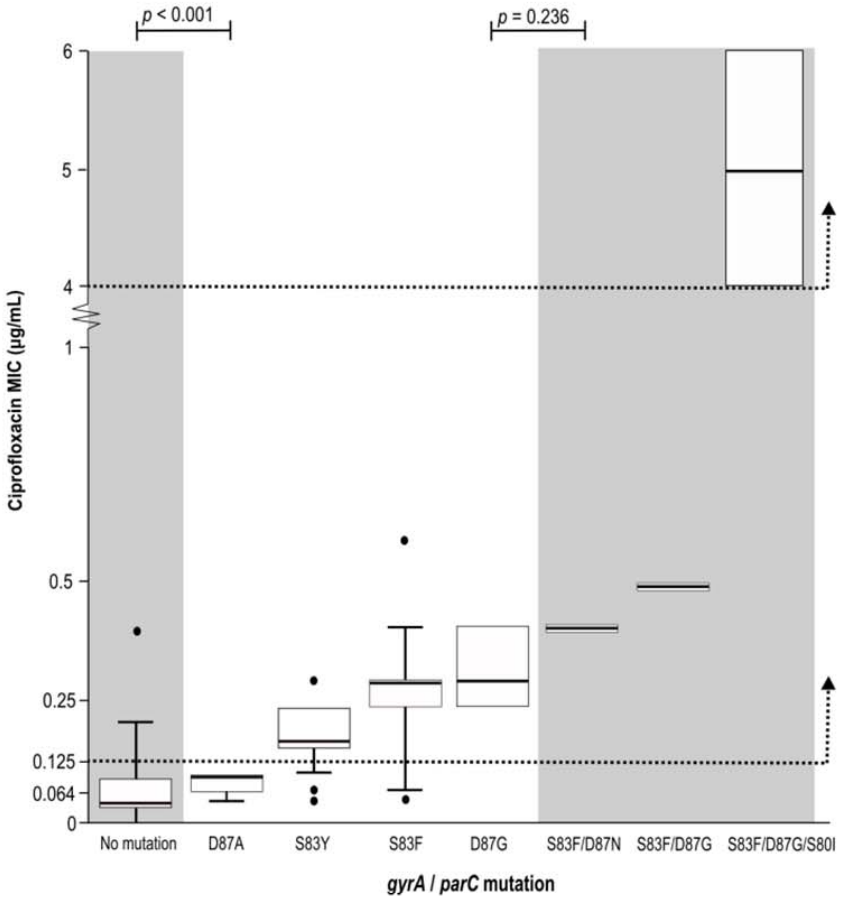



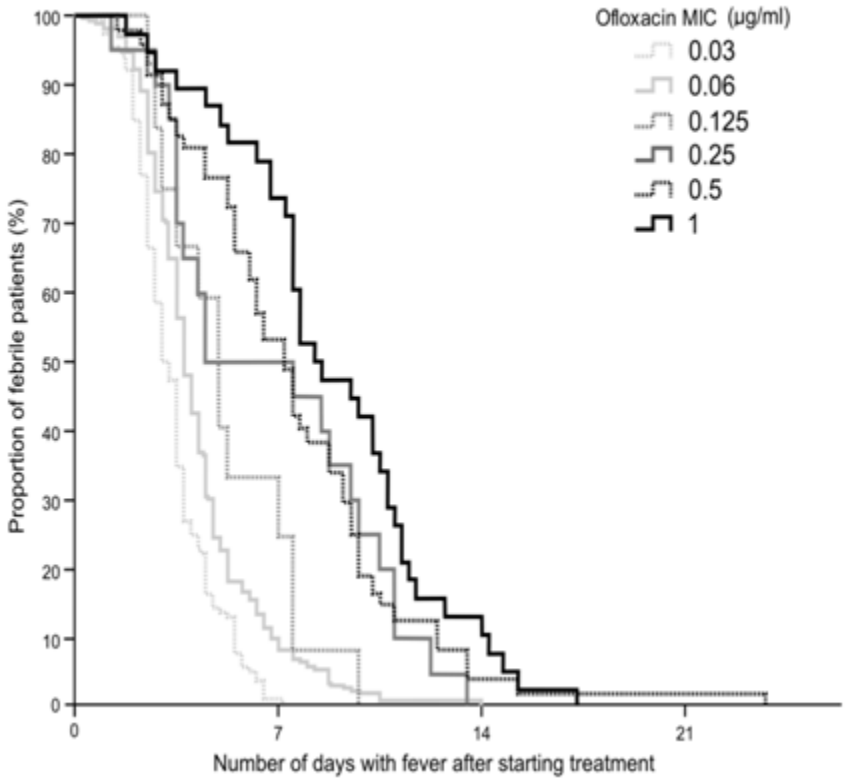



# Fluoroquinolones in Typhoid



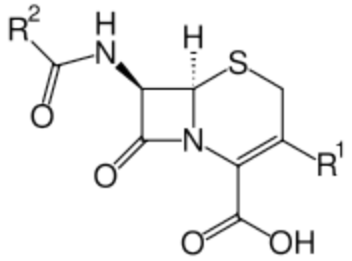



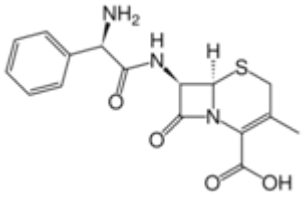
# Clinical typhoid response

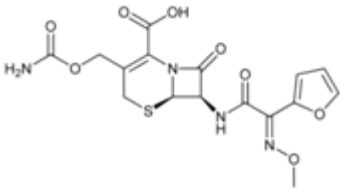


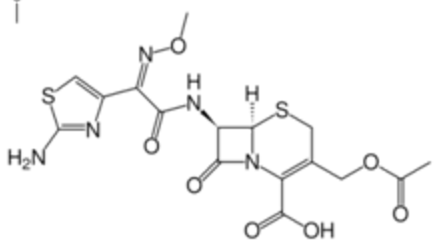

| Breakpoint value | Number successfully treated/ total number treated (%) | Odds ratio (95% CI) |
|---|---|---|
| Ofloxacin MIC < 0.06 µg/mL<br>Ofloxacin MIC ≥ 0.06 µg/mL | 148/152 (97.4 %) 144<br>338/388 (87.1 %) 314 | 5.47 (1.95-21.20) |
| Ofloxacin MIC < 0.12 µg/mL<br>Ofloxacin MIC ≥ 0.12 µg/mL | 406/423 (96.0 %)<br>80/117 (68.4 %) | 11.05 (5.71-21.88) |
| Ofloxacin MIC < 0.25 µg/mL<br>Ofloxacin MIC ≥ 0.25 µg/mL | 417/435 (95.9 %)<br>69/105 (65.7 %) | 12.09 (6.24-23.81) |
| Ofloxacin MIC < 0.50 µg/mL<br>Ofloxacin MIC ≥ 0.50 µg/mL | 432/455 (94.9 %)<br>54/85 (63.5 %) | 10.78 (5.6-20.77) |
| Ofloxacin MIC < 1.00 µg/mL<br>Ofloxacin MIC ≥ 1.00 µg/mL | 466/502 (92.8 %)<br>20/38 (52.6 %) | 11.65 (5.26-25.36) |
| Nalidixic acid susceptible<br>Nalidixic acid resistant | 417/434 (96.1 %)<br>69/106 (65.1 %) | 13.15 (6.74-26.20) |

# Cephalosporins









1st Generation

e.g. Cephalexin

2nd Generation

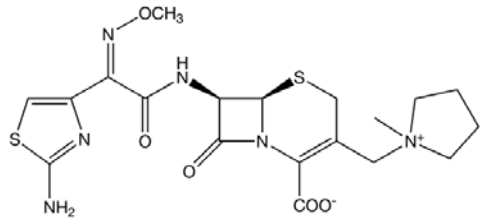
e.g. Cefuroxime, cefoxitin

3rd Generation

e.g. Cefotaxime, cefpodoxime, ceftriaxone, cefoperazone, ceftazidime

4th Generation

Cefopime, Cefquinome



# What Are ESBLs?

- Molecular class A or D b-lactamases
- Hydrolyse oxyiminio cephalosporins
- Have an active site serine
- Generally inhibited by b-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam)
- Most often associated with *E.coli* and *Klebsiella pneumoniae* but can be produced by other enteric bacilli
- >170 types

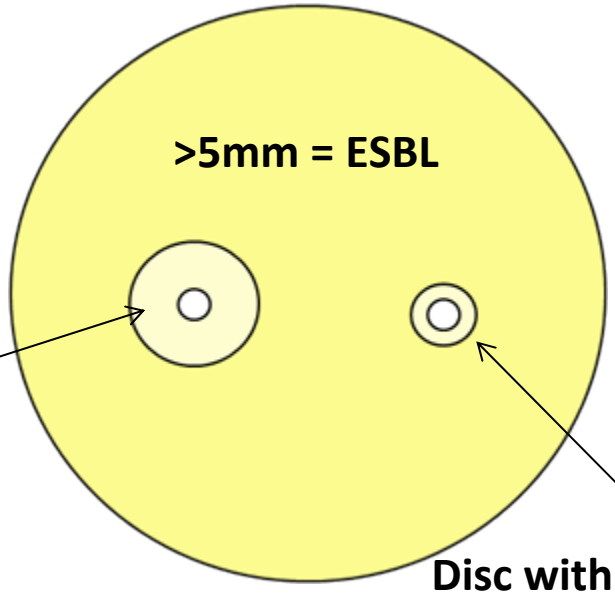
# Extended-spectrum β-lactamases

- Plasmid-mediated enzymes found in *Enterobacteriaceae*
- Hydrolyze 3rd generation cephalosporins
  - But not carbapenems or cephamycins (cefoxitin)
- Encoded on large plasmids (>100Kb)
  - Multi-drug resistance
- Mostly Ambler class A
  - TEM, SHV, CTX-M
- More rarely
  - Ambler Class D
  - OXA (can also be resistant to cefipime)
  - VEB, PER (resistant to b-lactamase inhibitors)

# Laboratory Detection

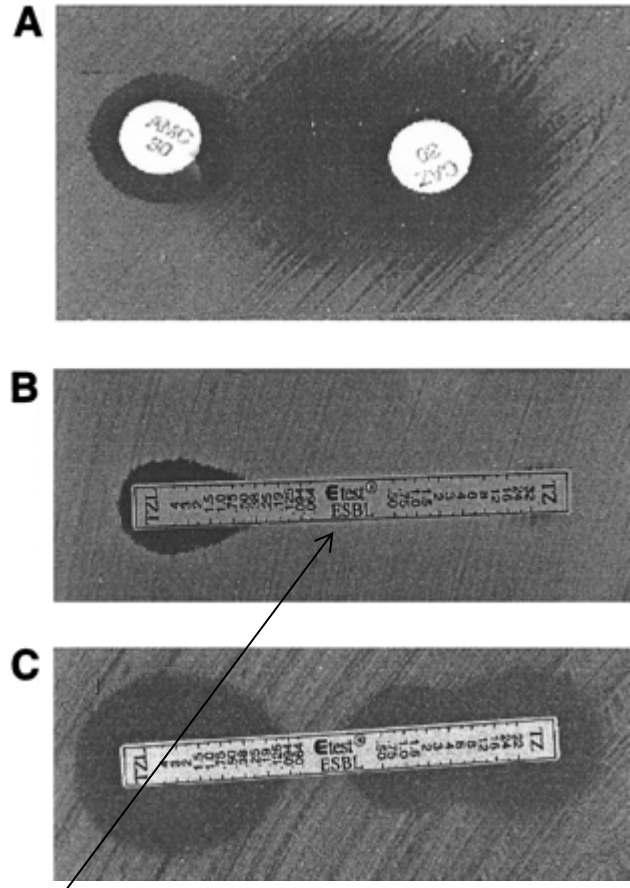
- Increased global reporting of resistant Enterobacteriacae
  - AmpC
  - ESBL
- Many labs still fail to routinely test for ESBLs
- No methodology without its problems

# ESBL detection




Disc with cephalosporin and clavulanic acid

Disc with cephalosporin alone



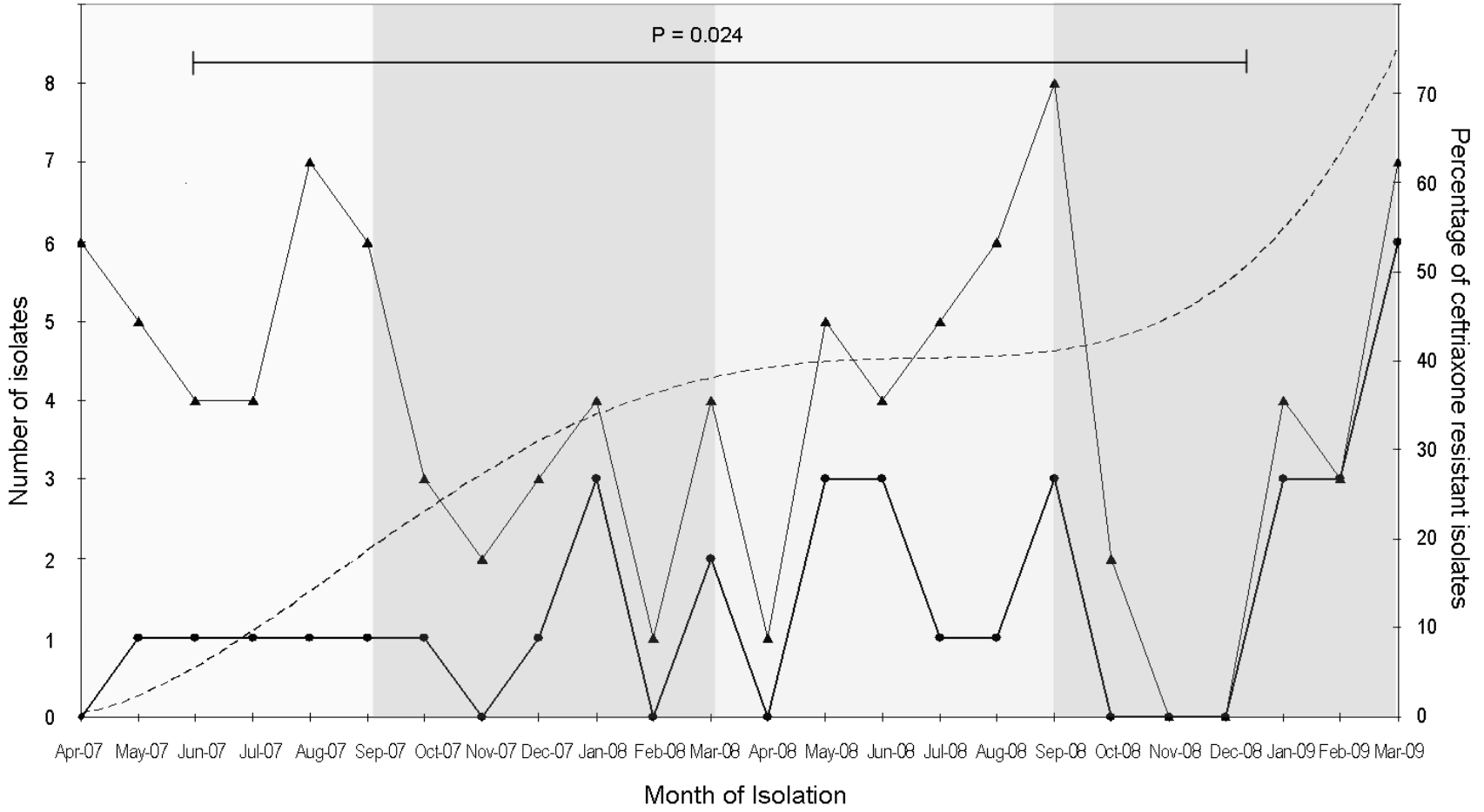

- Double disc synergy
- Combination disc test
- E-test

3-fold reduction of MIC=ESBL

# Laboratory detection

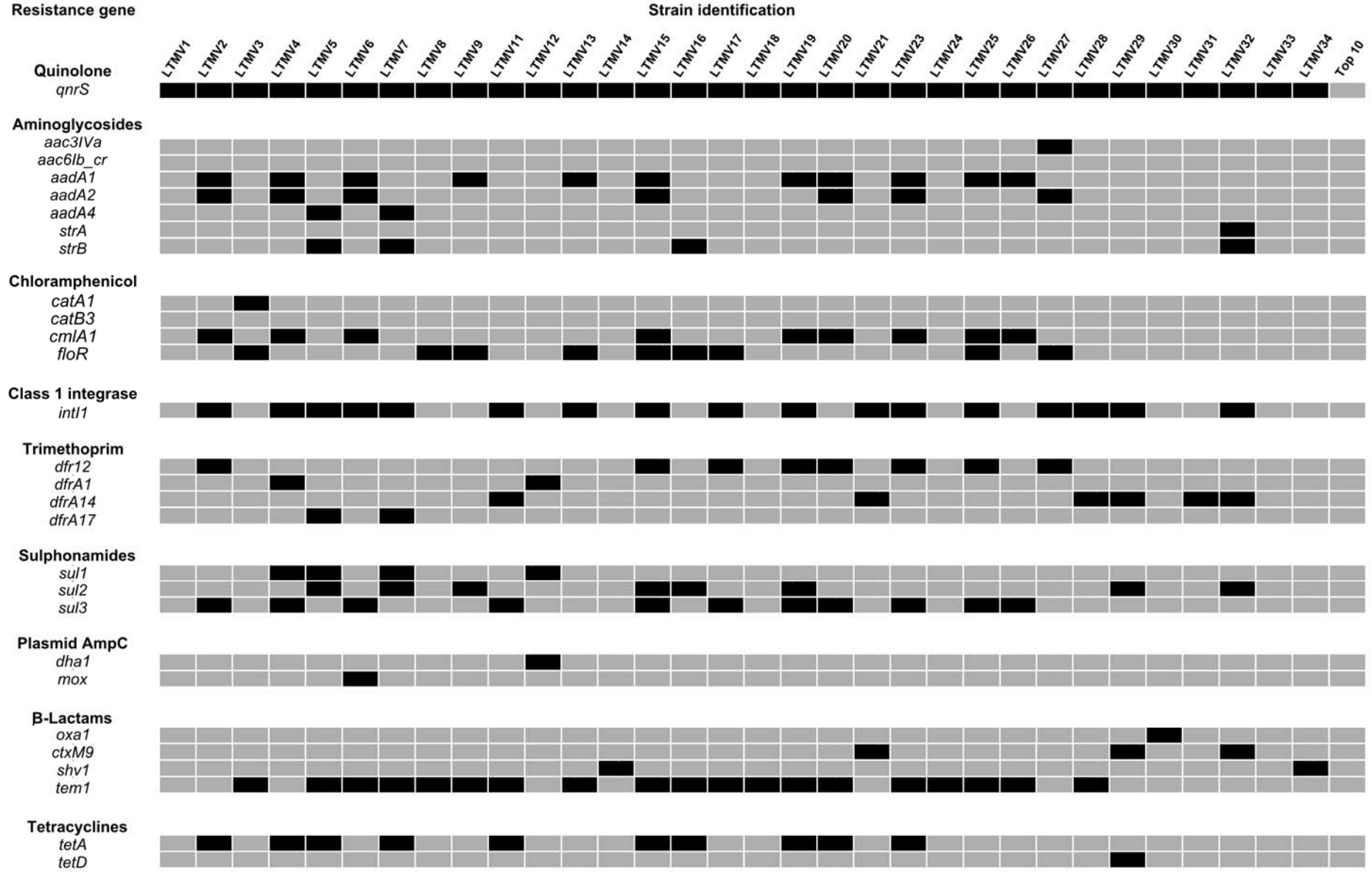
- Initial decreased susceptibility to oxyamino cephalosporins
    - Will not detect all ESBLs (TEM7,TEM12,SHV2)
- Further phenotypic (and genotypic) testing of isolates for ESBL production
- Testing with ceftazidime alone may miss CTX-M isolates
- Cefoxitin susceptibility will exclude the presence of AmpC-type beta-lactamase

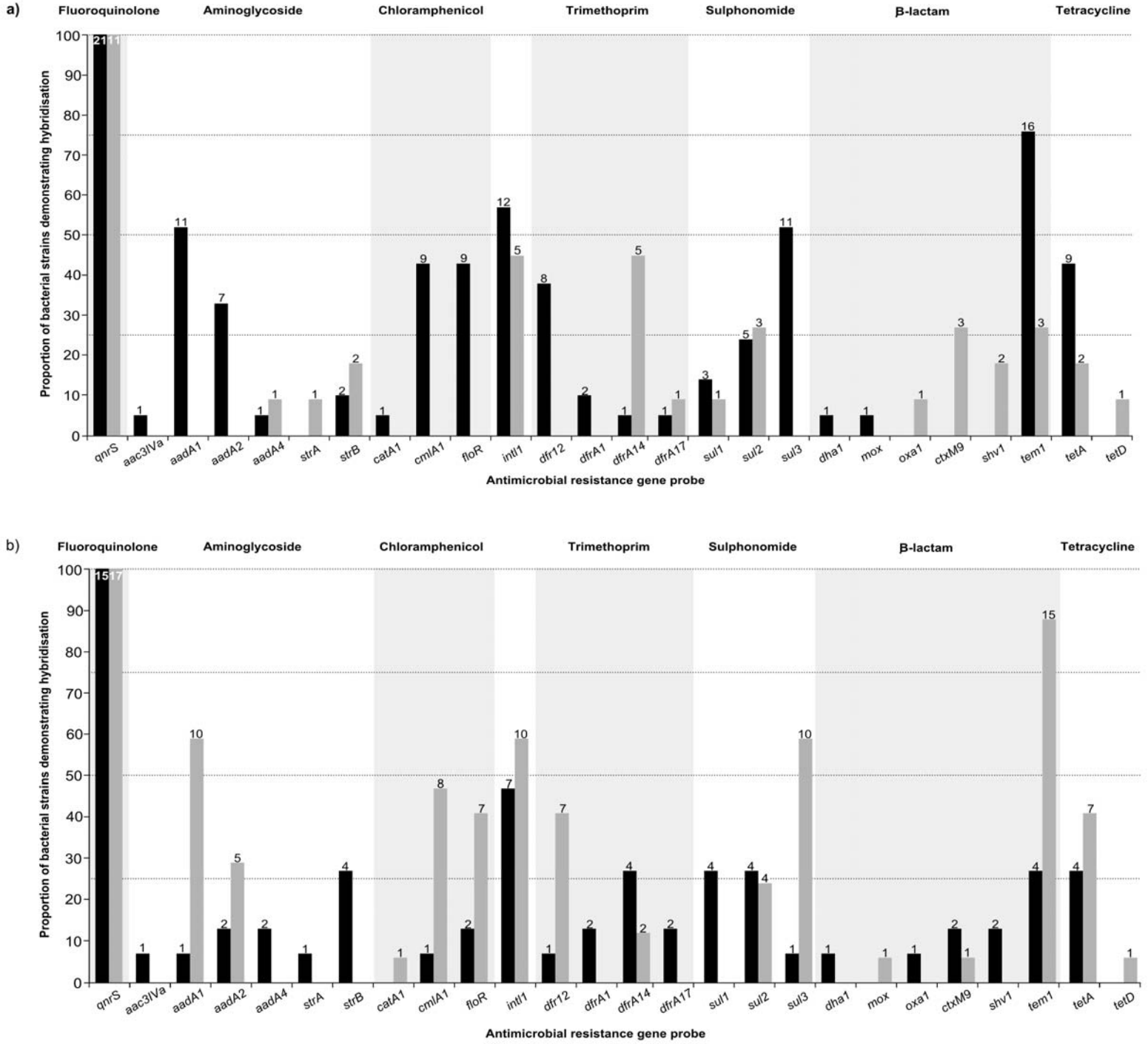
# Extended-spectrum β-lactamases in *Shigella* in Ho Chi Minh City



# Resistance in commensal enteric bacteria

| | Antimicrobial tested | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **AMP** | **GEN** | **CHL** | **TET** | **SXT** | **CRO [c]** | **FEP [c]** | **KAN** | **TIC** |
| **Number of resistant organisms (from 32) [n (%)]** | 23 (71.9) | 21 (65.6) | 18 (56.3) | 15 (46.9) | 15 (46.9) | 6 (18.8) | 5 (15.6) | 2 (6.3) | 2 (6.3) |
| **Resistant *E. coli* (from 17) [n(%)]** | 13 (76.5) | 9 (52.9) | 13 (76.5) | 8 (47.1) | 8 (47.1) | 1 (5.9) | 1 (5.9) | 2 (11.8) | 1 (5.9) |
| **Resistant *K. pneumoniae* (from 15) [n(%)]** | 10 (66.7) | 13 (86.7) | 4 (26.7) | 7 (46.7) | 6 (40.0) | 5 (33.3) | 4 (26.7) | 1 (6.7) | 1 (6.7) |
| ***p* value [a]** | 0.6989 | 0.0605 | 0.0118* | 1 | 0.7345 | 0.0755 | 0.1609 | 1 | 1 |
| **Resistant organisms community (from 21) [n(%)]** | 15 (68.2) | 11 (50.0) | 17 (77.3) | 12 (54.5) | 11 (50.0) | 0 (0.0) | 0 (0.0) | 2 (9.1) | 1 (4.5) |
| **Resistant organisms hospital (from 11) [n(%)]** | 8 (72.7) | 10 (90.9) | 1 (9.1) | 3 (27.3) | 4 (36.4) | 6 (54.5) | 5 (45.5) | 0 (0.0) | 1 (9.1) |
| ***p* value [b]** | 1 | 0.0273* | 0.0005* | 0.2659 | 0.712 | 0.0004* | 0.0019* | 0.5417 | 1 |


Resistance gene
Strain identification
LTMV1
LTMV2
LTMV3
LTMV4
LTMV5
LTMV6
LTMV7
LTMV8
LTMV9
LTMV11
LTMV12
LTMV13
LTMV14
LTMV15
LTMV16
LTMV17
LTMV18
LTMV19
LTMV20
LTMV21
LTMV23
LTMV24
LTMV25
LTMV26
LTMV27
LTMV28
LTMV29
LTMV30
LTMV31
LTMV32
LTMV33
LTMV34
Top 10
Quinolone
qnrS
Aminoglycosides
aac3IVa
aac6Ib_cr
aadA1
aadA2
aadA4
strA
strB
Chloramphenicol
catA1
catB3
cmlA1
floR
Class 1 integrase
intI1
Trimethoprim
dfr12
dfrA1
dfrA14
dfrA17
Sulphonamides
sul1
sul2
sul3
Plasmid AmpC
dha1
mox
β-Lactams
oxa1
ctxM9
shv1
tem1
Tetracyclines
tetA
tetD


a)
Fluoroquinolone
Aminoglycoside
Chloramphenicol
Trimethoprim
Sulphonomide
β-lactam
Tetracycline
Proportion of bacterial strains demonstrating hybridisation
Antimicrobial resistance gene probe
qnrS
aac3IVa
aadA1
aadA2
aadA4
strA
strB
catA1
cmlA1
floR
intI1
dfr12
dfrA1
dfrA14
dfrA17
sul1
sul2
sul3
dha1
mox
oxa1
ctxM9
shv1
tem1
tetA
tetD
b)
Fluoroquinolone
Aminoglycoside
Chloramphenicol
Trimethoprim
Sulphonomide
β-lactam
Tetracycline
Proportion of bacterial strains demonstrating hybridisation
Antimicrobial resistance gene probe
qnrS
aac3IVa
aadA1
aadA2
aadA4
strA
strB
catA1
cmlA1
floR
intI1
dfr12
dfrA1
dfrA14
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mox
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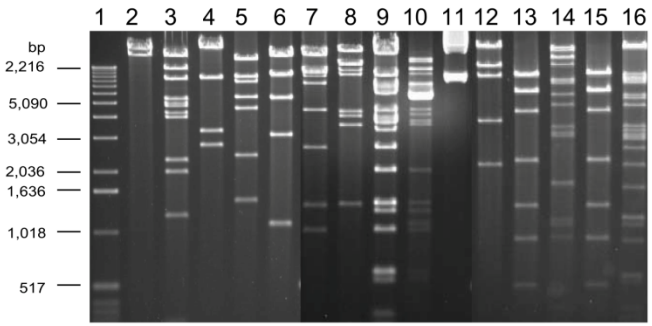
shv1
tem1
tetA
tetD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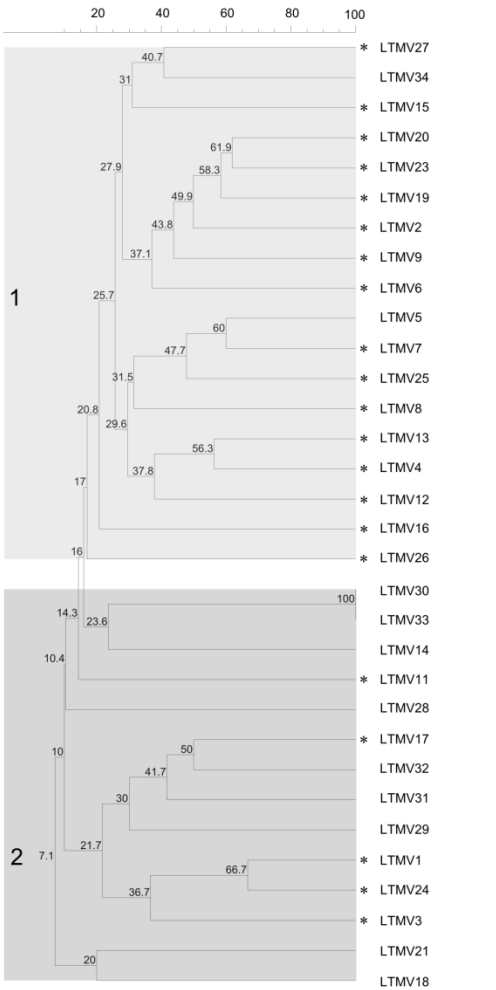
bp
2,216
5,090
3,054
2,036
1,636
1,018
517


20 40 60 80 100
1
2
LTMV27
LTMV34
LTMV15
LTMV20
LTMV23
LTMV19
LTMV2
LTMV9
LTMV6
LTMV5
LTMV7
LTMV25
LTMV8
LTMV13
LTMV4
LTMV12
LTMV16
LTMV26
LTMV30
LTMV33
LTMV14
LTMV11
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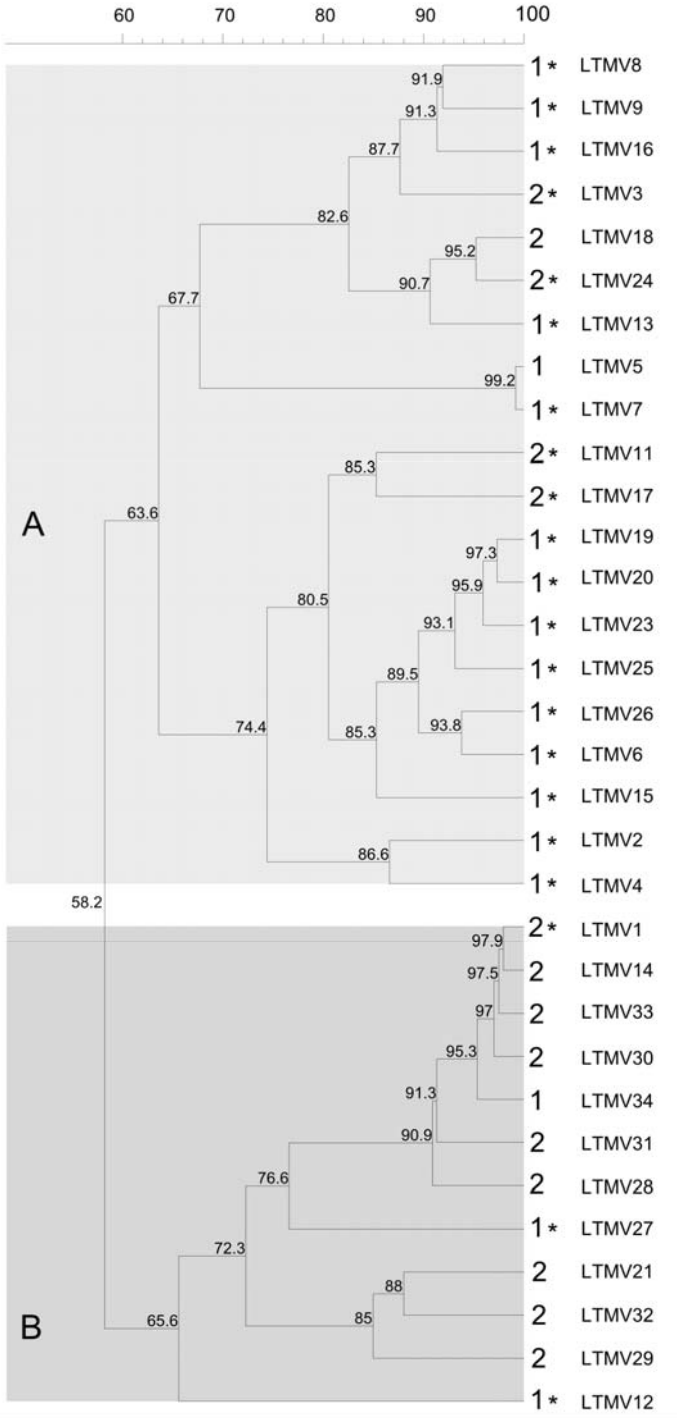
LTMV1
LTMV24
LTMV3
LTMV21
LTMV18


60 70 80 90 100
A
B
LTMV8
LTMV9
LTMV16
LTMV3
LTMV18
LTMV24
LTMV13
LTMV5
LTMV7
LTMV11
LTMV17
LTMV19
LTMV20
LTMV23
LTMV25
LTMV26
LTMV6
LTMV15
LTMV2
LTMV4
LTMV1
LTMV14
LTMV33
LTMV30
LTMV34
LTMV31
LTMV28
LTMV27
LTMV21
LTMV32
LTMV29
LTMV12

# Carbapenemases

- Class A
  - Chromosomally-encoded IMI, NMC-A and SME
  - Plasmid-encoded KPC and GES
  - Clavulanic acid-inhibited
- Class B
  - Metallo-β-lactamases (inhibited by EDTA)
  - IMP, VIM, NDM-1
  - Integron encoded (transposon and plasmid)
- Class D
  - OXA types (mostly *Acinetobacter baumannii)*
  - Sensitive to b-lactamase inhibitors

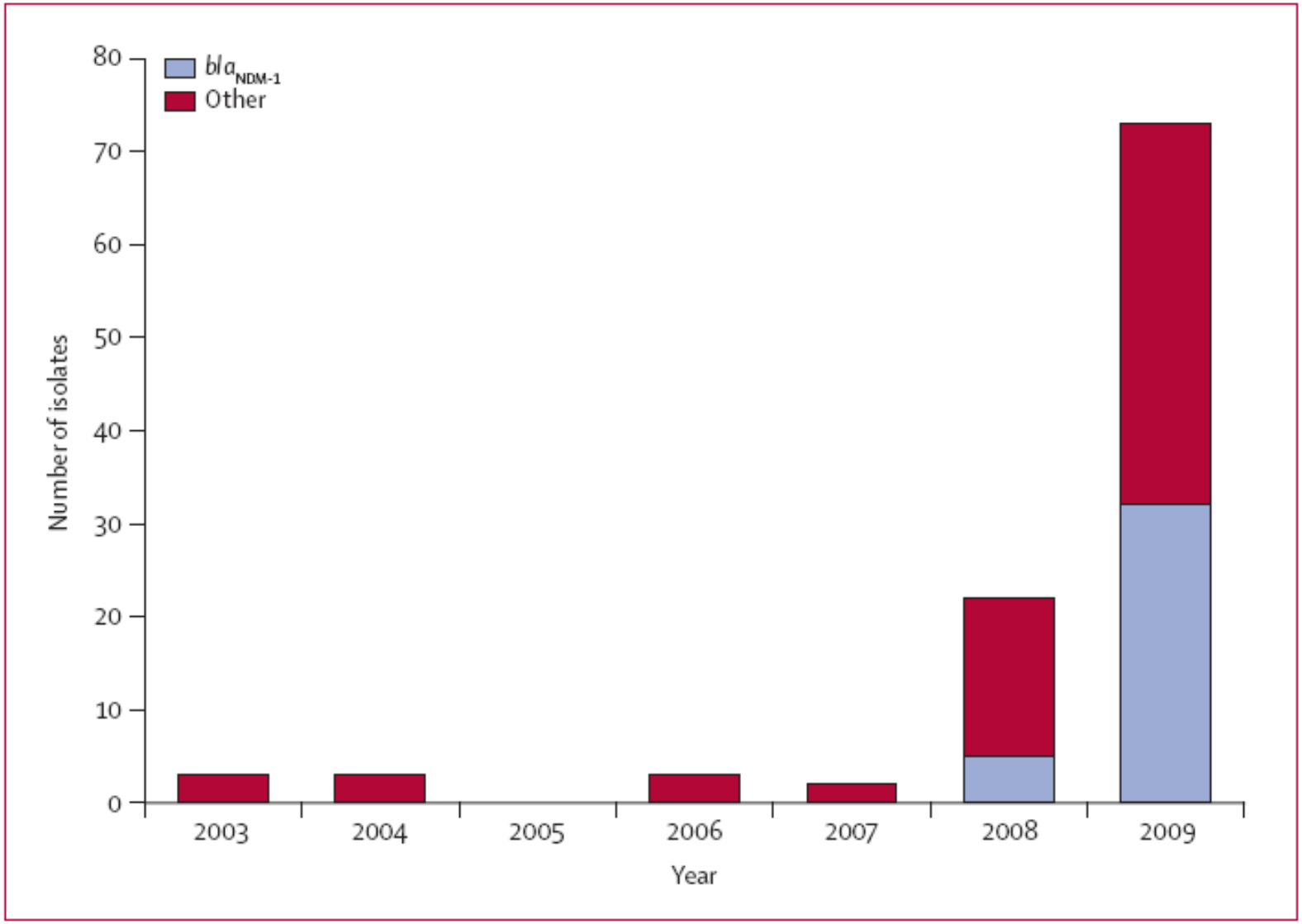



***Figure 1*: Numbers of carbapenemase-producing Enterobacteriaceae referred from UK laboratories to the UK Health Protection Agency's national reference laboratory from 2003 to 2009**

The predominant gene is $bla_{NDM-1}$, which was first identified in 2008. The other group includes diverse producers of KPC, OXA-48, IMP, and VIM enzymes.

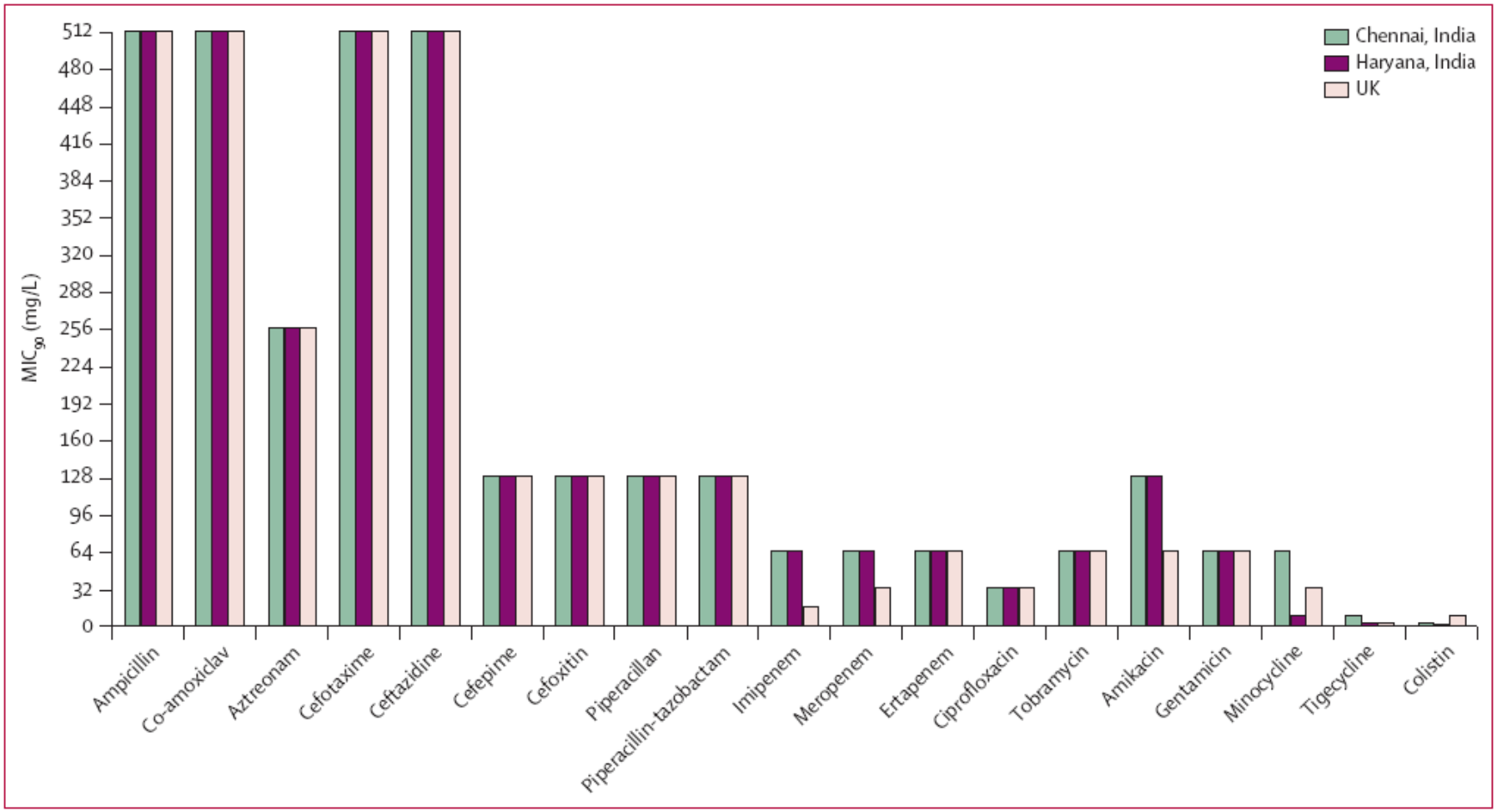
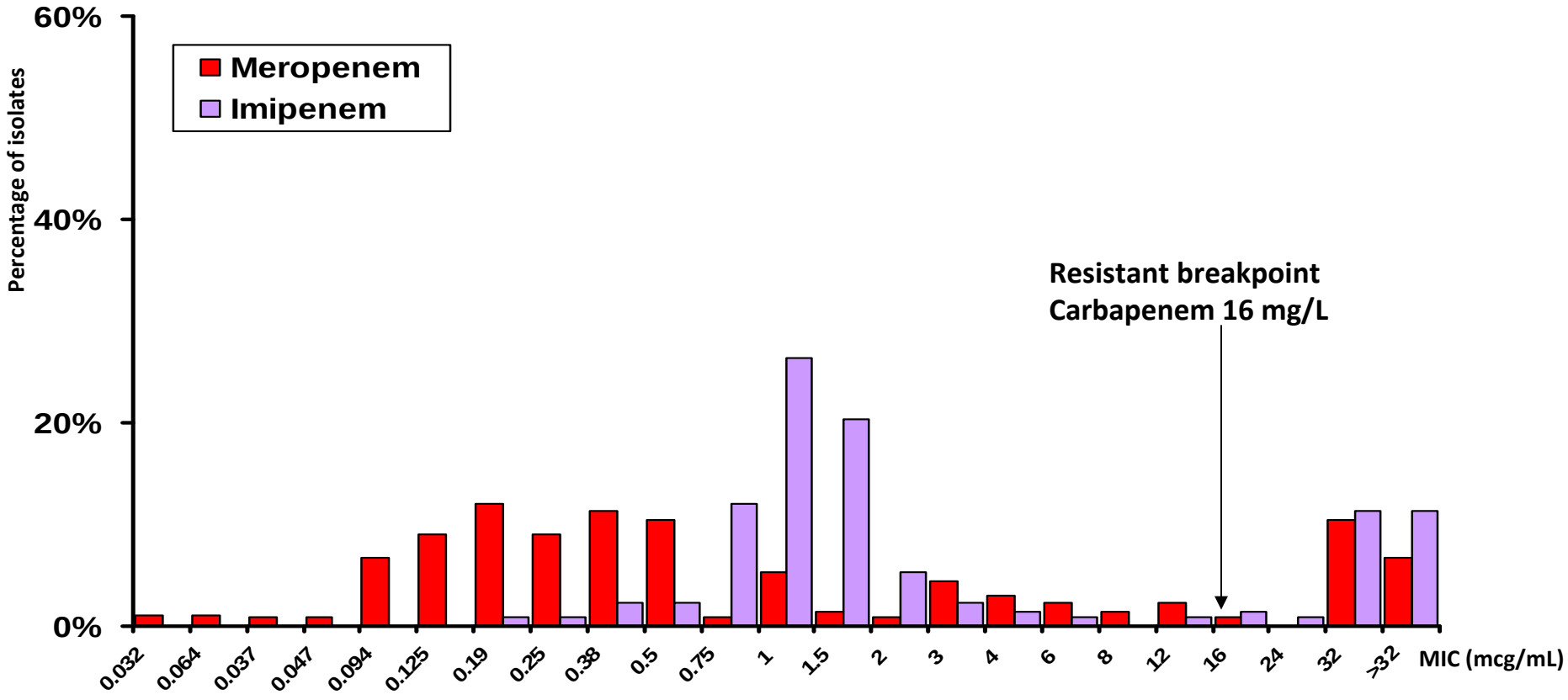



*Figure 2*: 90% minimum inhibitory concentration ($MIC_{90}$) for Enterobacteriaceae from Chennai and Haryana, India, and the UK

# Carbapenem resistance *P. Aeruginosa* Cause of hospital pneumonia

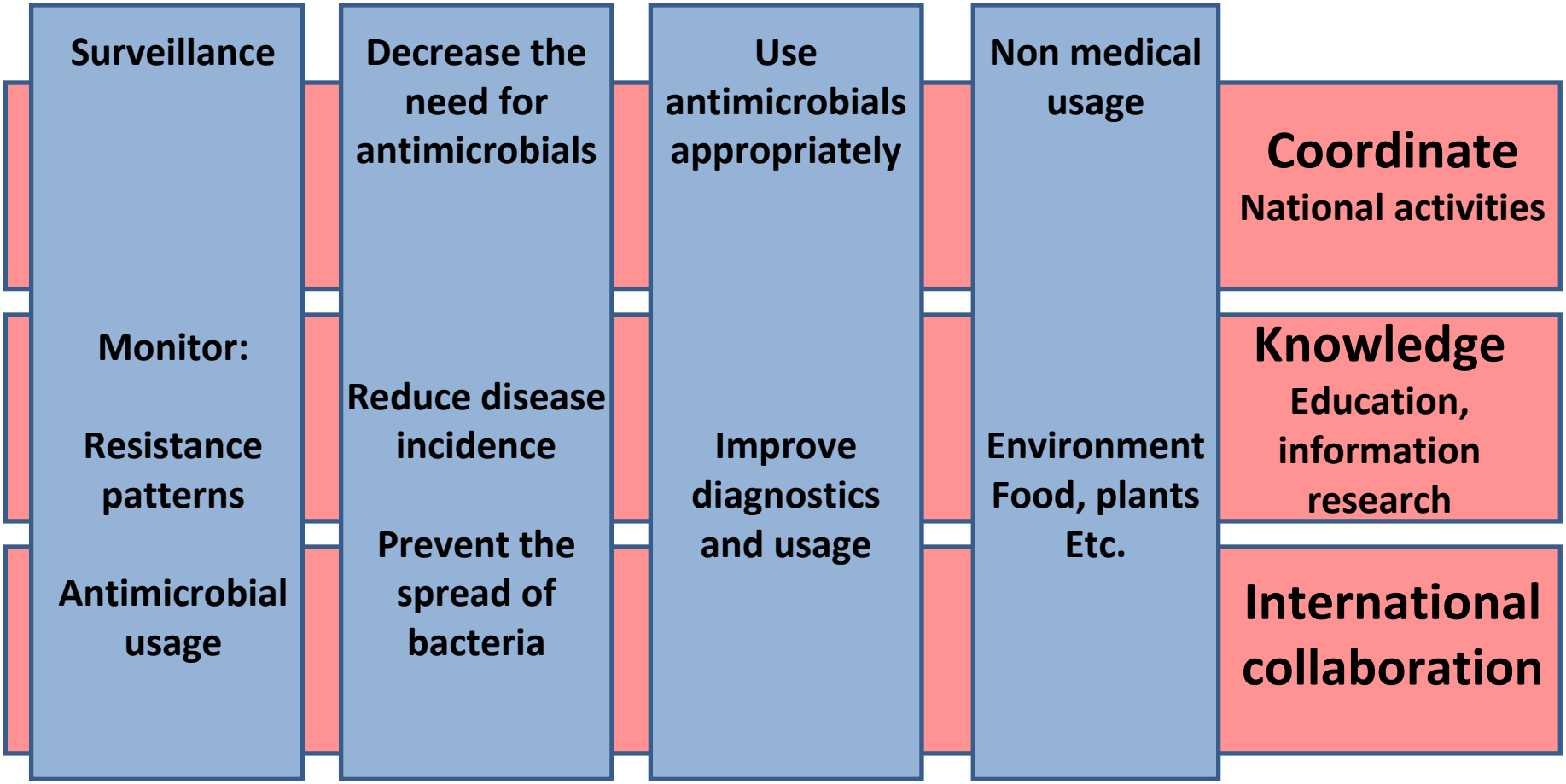
**(133 strains isolated in 6 hospitals - 2008)**

- **Emerging carbapenem resistance**




Source: Dr Doan Mai Phuong, Bach Mai Hospital

# Tools to control use and resistance



# What surveillance can be done now?

- **Antibiotic consumption surveillance**
  - main driving force for development of resistance
  - Surveillance provides data to implement interventions
  - Hospital, community, agriculture

- **Antibiotic resistance surveillance**
  - Monitor of prescribing practices and interventions
  - Early warning of important resistance trends
  - Helps prescribers to give the right antibiotic
  - Hospital, community, agriculture

# Proposals to Combat Antimicrobial Resistance

- Speed development of new antibiotics
- Develop alternatives
- Track resistance data nationwide
- Restrict antimicrobial use
- Directly observe dosing (TB)
- Use more narrow spectrum antibiotics
- Use antimicrobial cocktails

# In addition

- Education
- Guidelines and Pathways
- Antimicrobial cycling
- Antimicrobial order forms
- Combination therapy
- De-escalation of therapy
- Dose Optimisation
- IV/Oral
- Controlling community usage.....

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- Ms. Vien Le Thi Minh
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