# The significance of in vitro antibiotic resistance

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# What is ‘antibiotic resistance’ ?

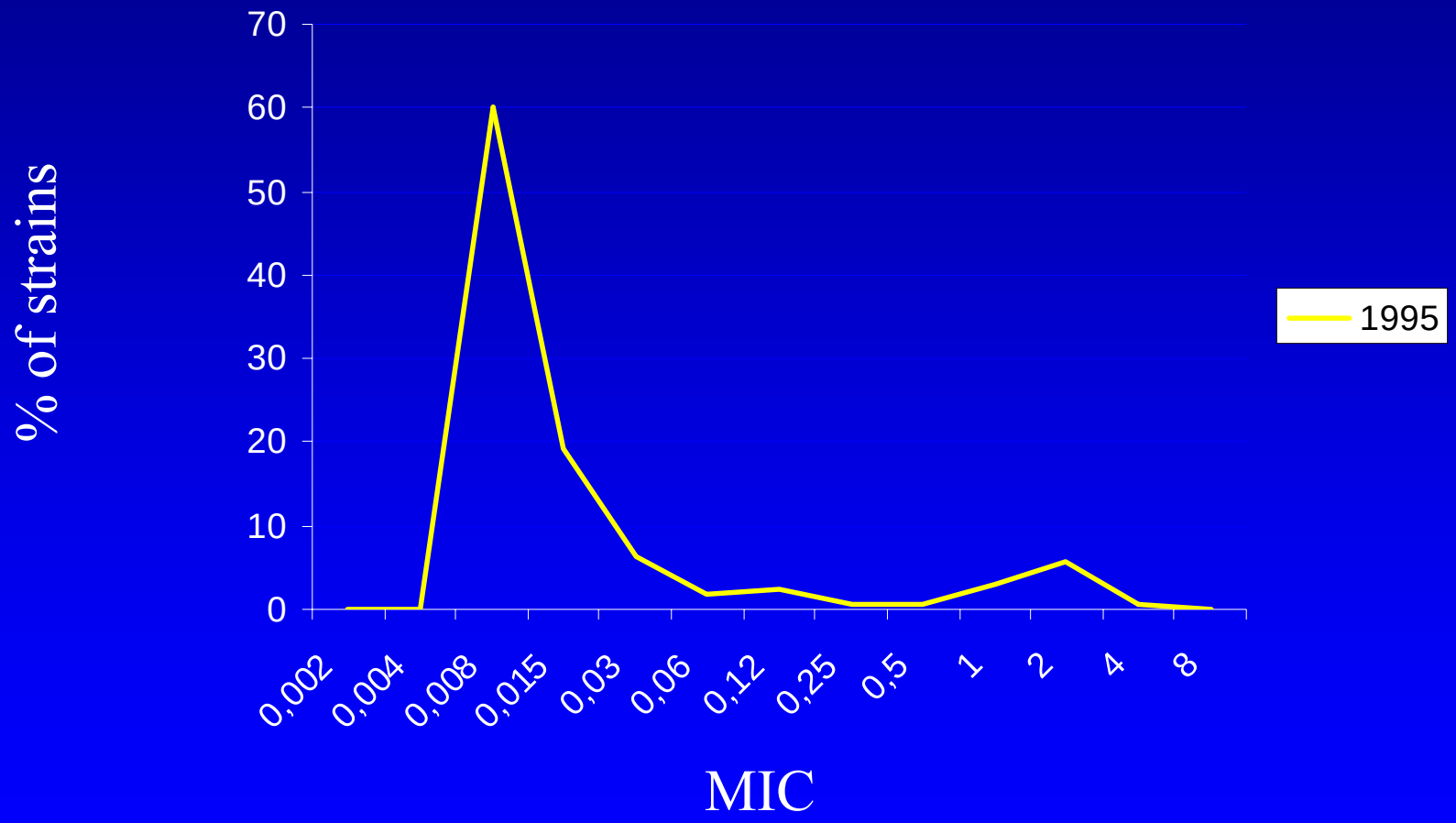
- ‘Antibiotic resistance’ means different things to different people :
  - Epidemiological resistance
  - Clinical microbiology resistance or *in vitro* resistance
  - Clinical resistance or *in vivo* resistance

# What is ‘antibiotic resistance’ ?

- Epidemiological resistance:
  - Reduced susceptibility of bacteria to antibiotics due to the presence of resistance genes / mutations
    - Increase in MIC value / shift in $MIC_{50}$-$MIC_{90}$ values

# What is ‘antibiotic’ resistance ?

## MIC distribution of *S. pneumoniae* in Belgium

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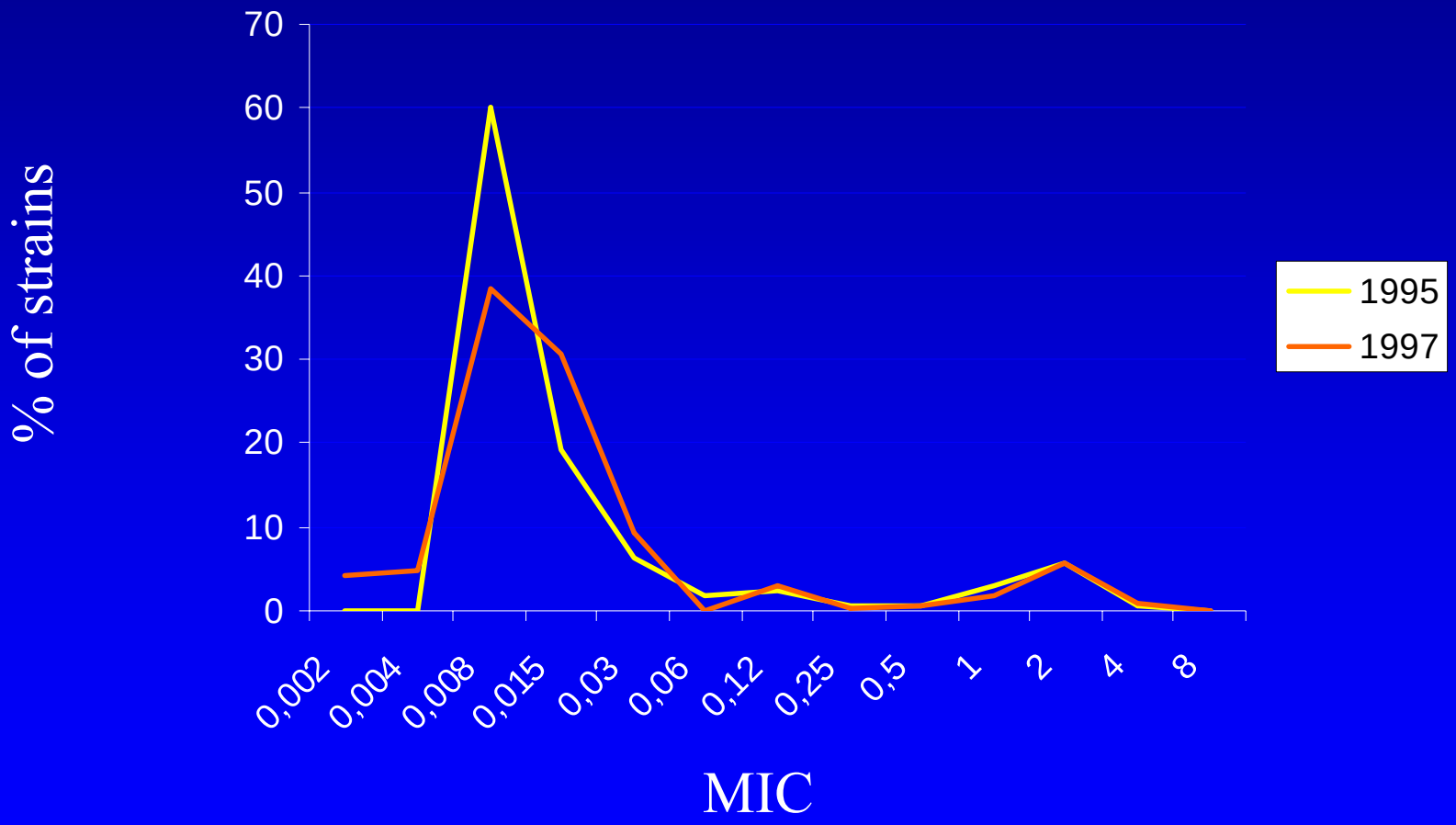


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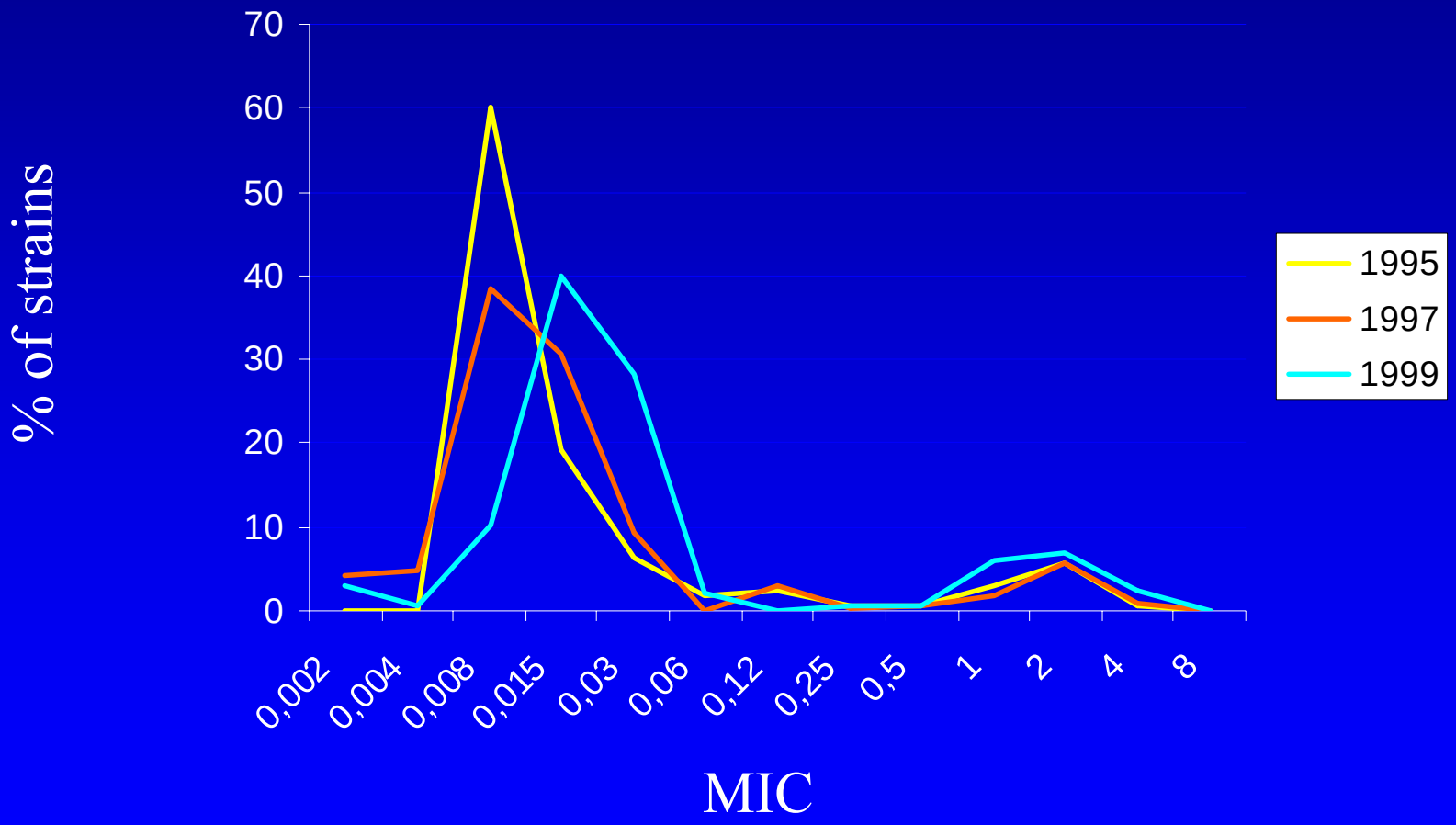


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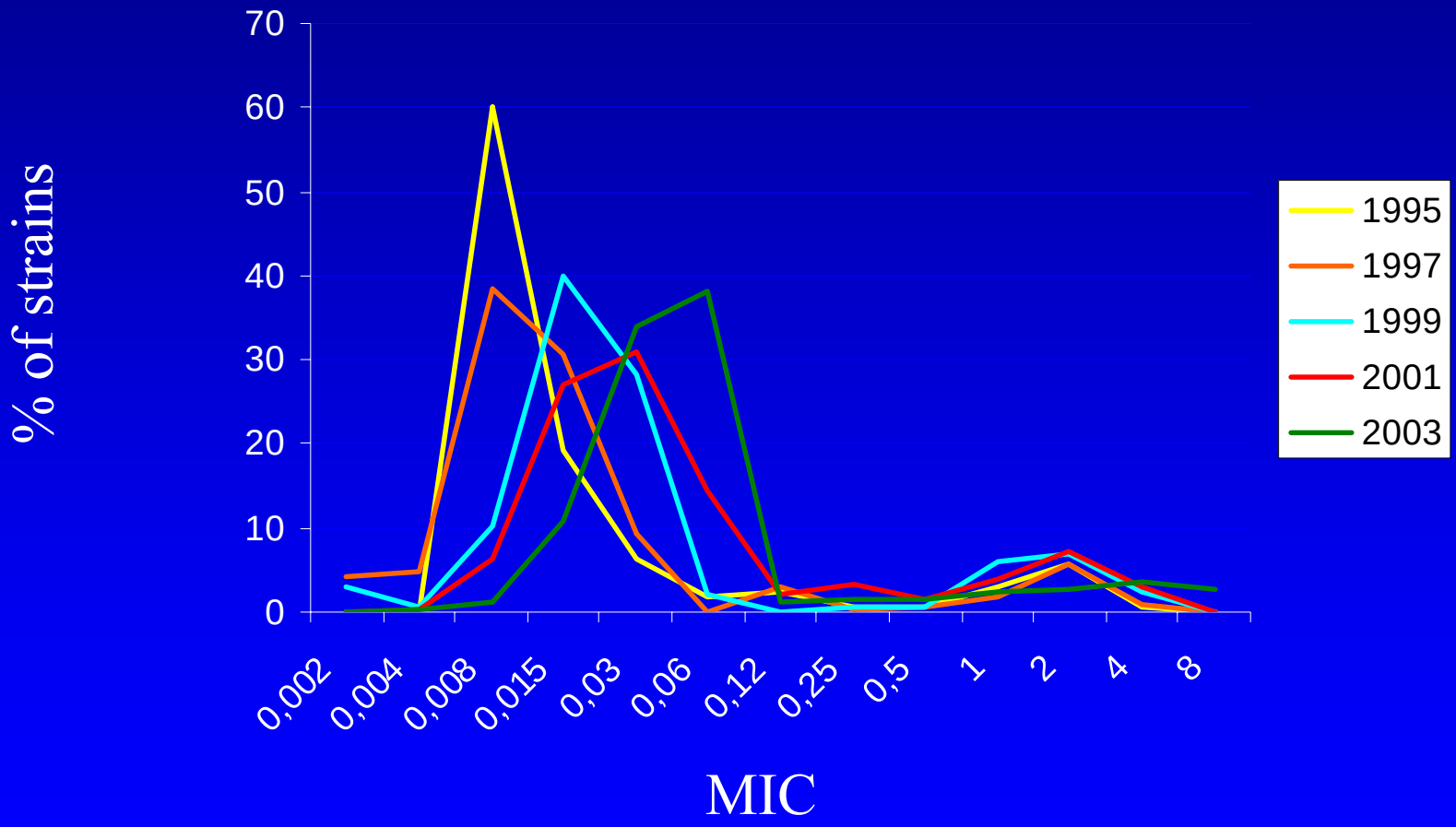


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# What is antibiotic resistance

- 'antibiotic resistance' means different things to different people:
  - Clinical microbiology resistance or '*in vitro*' resistance
    - Breakpoint resistance
      - Reduced susceptibility of bacteria to antibiotics above predefined nationally / internationally accepted limits
  - Epidemiological resistance:
  - Clinical resistance or 'in vivo' resistance

# What is ‘antibiotic’ resistance ?

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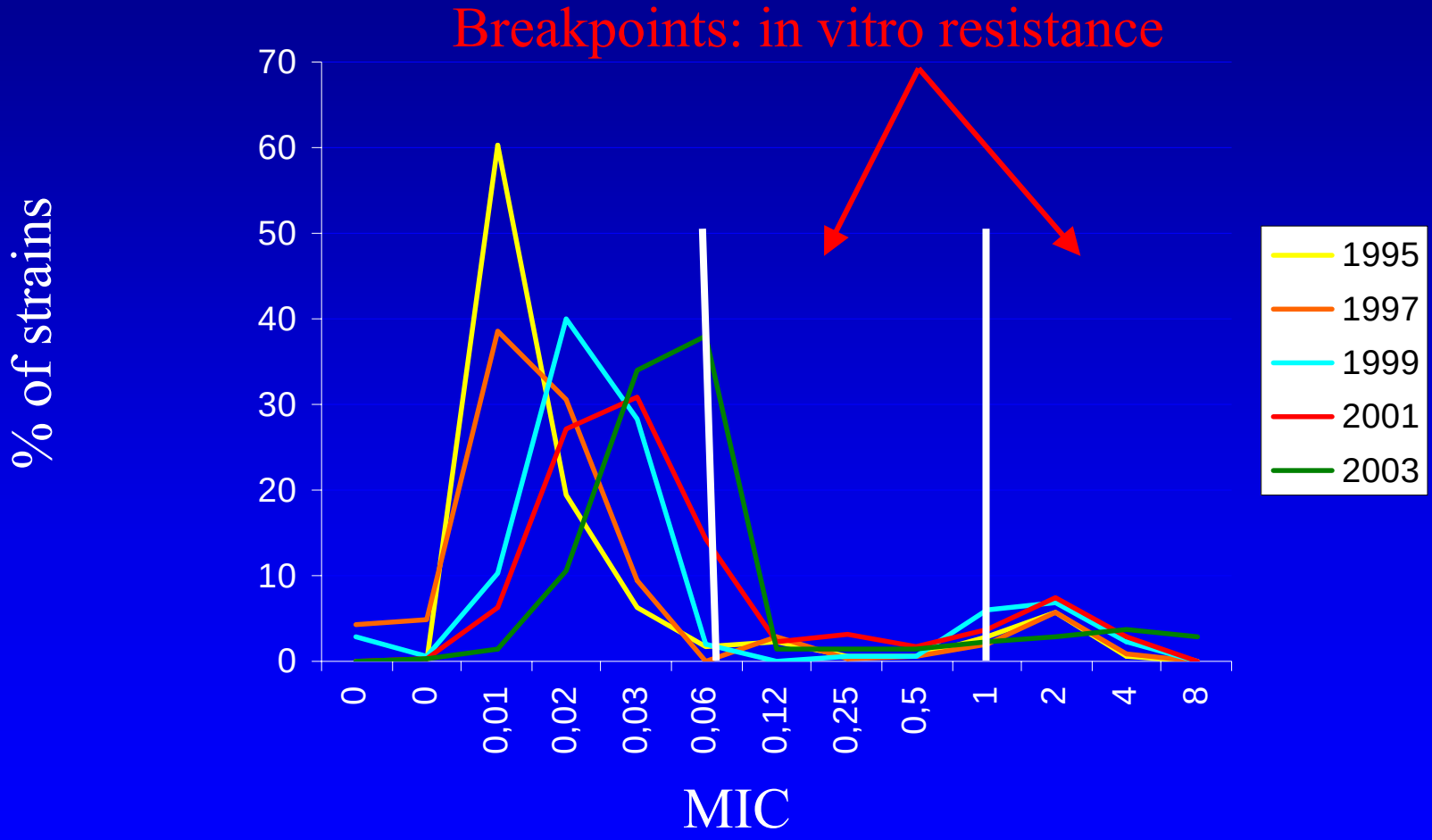


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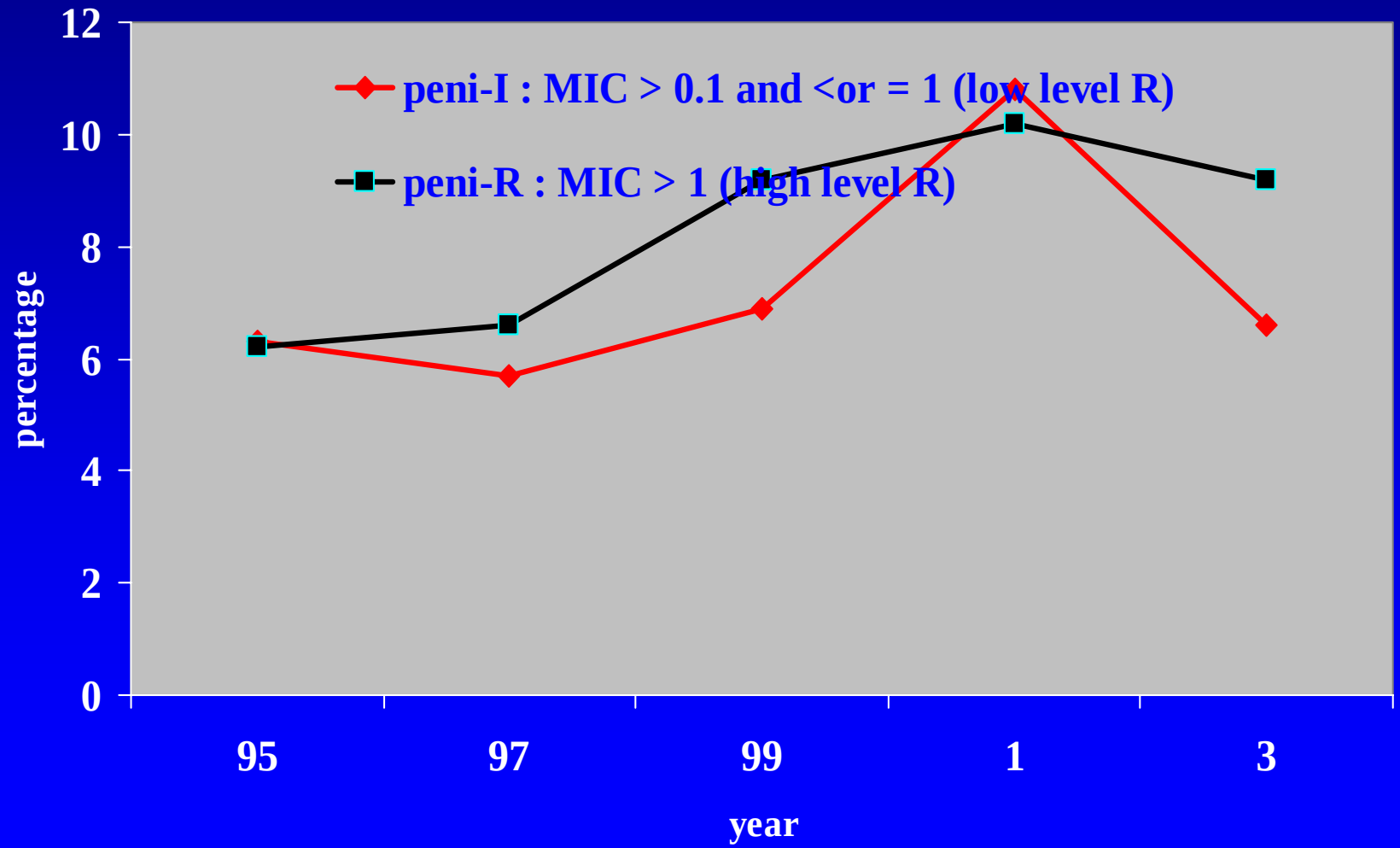
# Evolution of *S. pneumoniae* 'in vitro' resistance rates to penicillin (Belgium)



# Determination of breakpoints

- Based on ill-defined mix of microbiological, pharmaco-kinetic, pharmaco-dynamic, clinical data
  - Periodically re-evaluated ?
- Different organisations establish (different) breakpoints
  - USA: FDA, NCCLS
  - UK: BSAC; France: SFM, Germany: DIN, Spain, Sweden, The Netherlands
  - EUCAST
- Impact on treatment guidelines

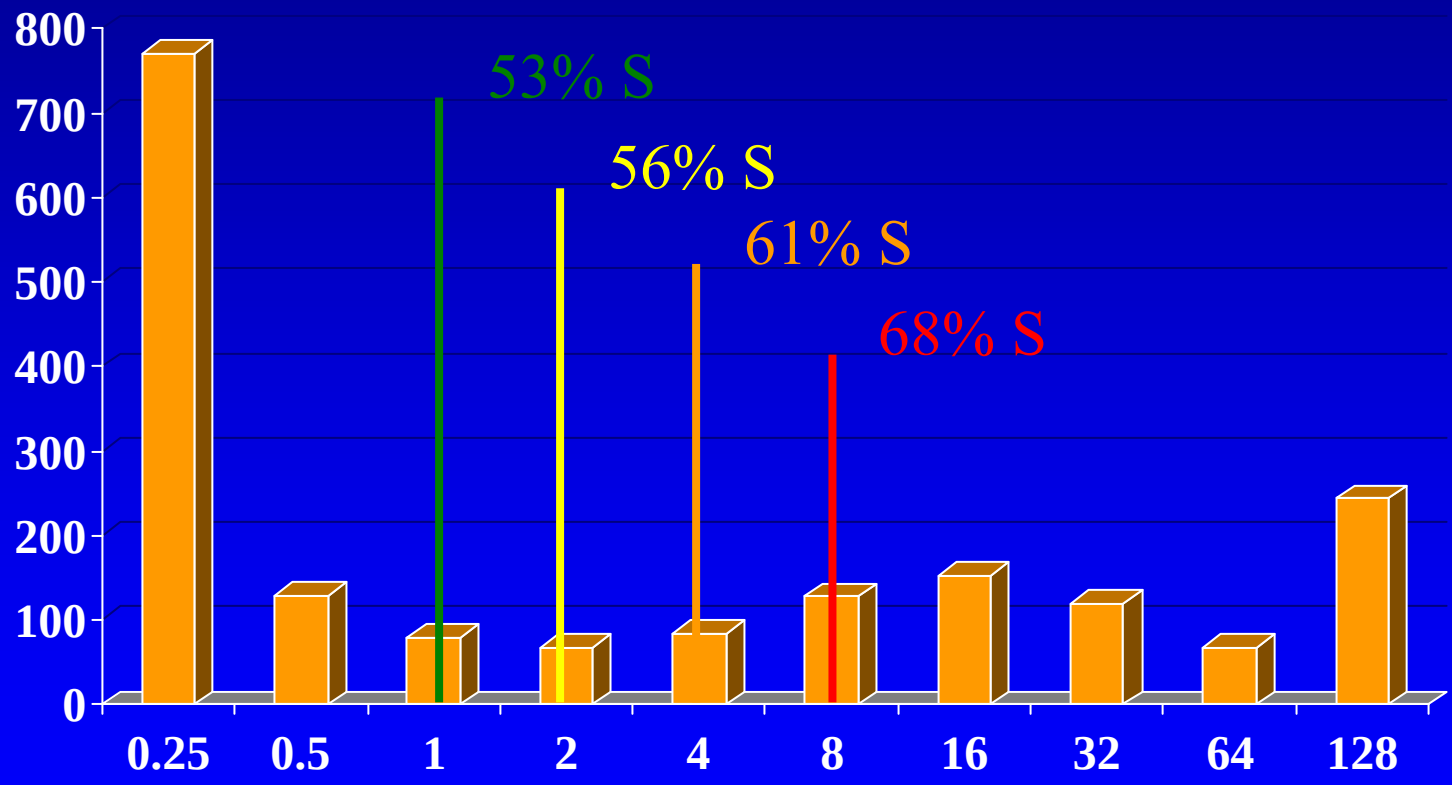
# Breakpoints for enterobacteriaceae

| Country | MIC breakpoint, ug/mL | |
|---|---|---|
| | cefotaxime | ceftazidime |
| United States | ≤ 8 | ≤ 8 |
| France | ≤ 4 | ≤ 4 |
| Norway | ≤ 2 | ≤ 2 |
| England | ≤ 1 | ≤ 1 |
| Sweden | ≤ 0.5 | ≤ 2 |
| Spain | ≤ 1 | ≤ 1 |

# Breakpoints for enterobacteriaceae:

ceftria MIC frequency distribution

inducible enterobacteriaceae NPRS 3,4,5,6



# Breakpoint resistance and epidemiology of resistance

- ‘In vitro’ resistance or breakpoint resistance is often a crude and rather insensitive measure of reduced susceptibility of bacteria to antibiotics
- Different breakpoints hinder comparison between national resistance rates

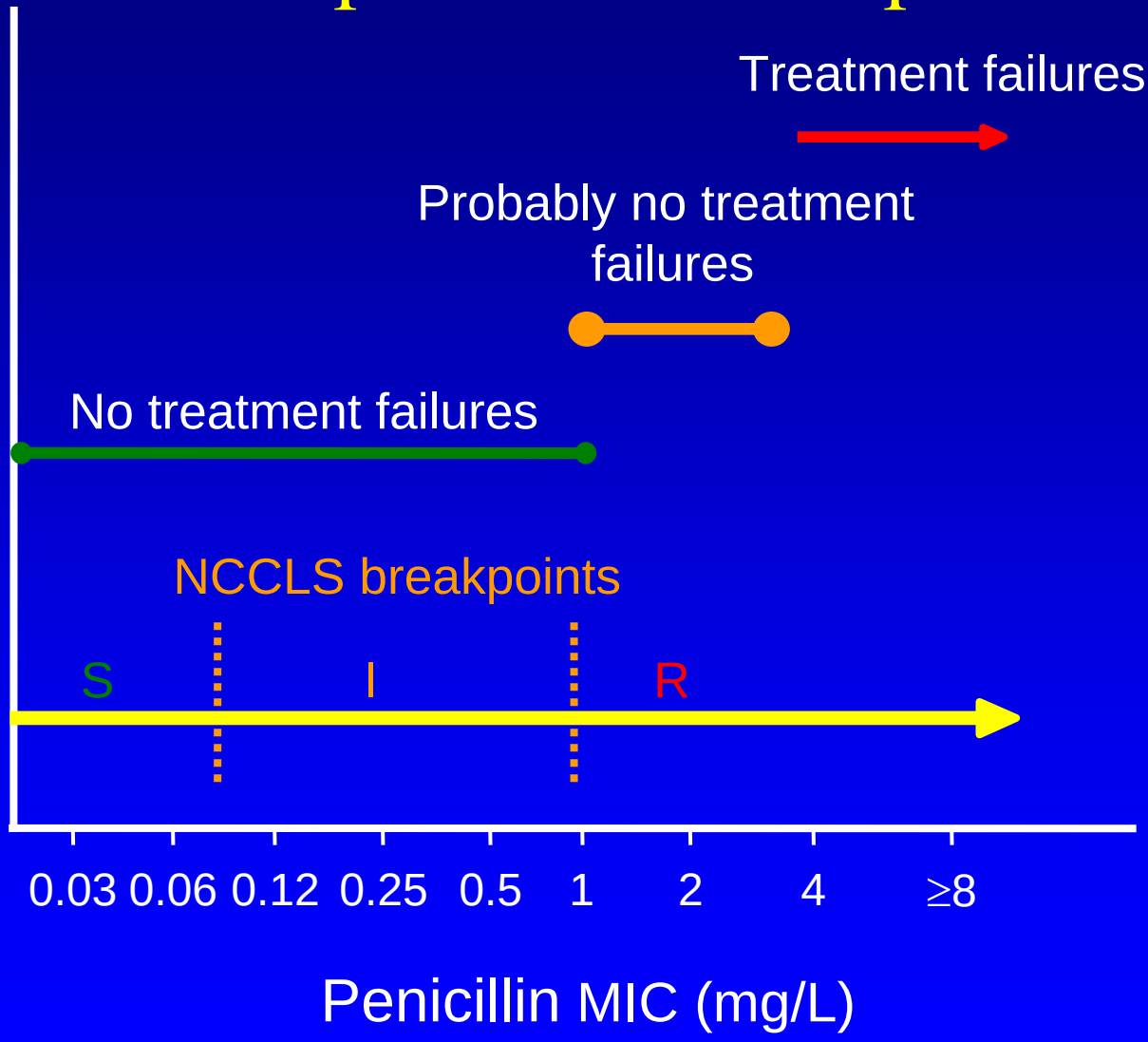
# What is ‘antibiotic resistance’ ?

- Epidemiological resistance
- Clinical microbiology resistance or *in vitro* resistance:
- Clinical resistance or *in vivo* resistance:
  - Increased risk of treatment failures
  - Breakpoints take into account local differences in dosage

# ‘In vivo’ significance of ‘in vitro’ pen-resistance for pneumococci

- pneumococcal pneumonia
  - Antibiotic resistance (breakpoint resistance) is clinically not relevant
    - » Pallares, NEJM, ’95; Siegel, Chest, ’99; Deeks, Ped, ’99; Castillo, Am J Inf Control, 2000; Watanabe, Resp, 2000; Wu, J Formos Med Assoc, 2000; Henriques, JID, 2000; Gomez-Barreto, Arch Med Res, 2000; Amsden, Ann Pharmacother, ’01; Kim, Eur J Clin Microbiol Infect Dis, ’02; Yu, CID, 2003
  - Penicillin-nonsusceptible *S. pneumoniae* pneumonia carries a higher mortality risk
    - » Turett, CID, ’99; Metlay, CID, 2000; Feikin, Am J Public Health, 2000; Yu, CID, 2003

# ‘In vivo’ significance of ‘in vitro’ pen-resistance for pneumococcal pneumonia



# ‘In vivo’ significance of ‘in vitro’ ery- resistance in pneumococcal infections

- In vivo-in vitro paradox:
  - ‘In vivo’ success of macrolides/azalides despite in vitro resistance
    - Lynch ’02, Amsden ’99, Bisahi ’02
  - Treatment failure, breakthrough bacteremia
    - Leclerq ’02, Hyde ’01, Kelley ’00, Musher ’02, Kays ’02, Lonks ’02, Van Kerkhoven ’03, Butler ’03,
  - Failure to eradicate ery-R strains in acute otitis media :

# ‘In vivo’ significance of ‘in vitro’ ery-resistance in pneumococcal infections

- In vitro susceptibility testing may overestimate resistance levels
  - Does not take into account
    - Tissue penetration
    - Additional non-antimicrobial effects
    - Host response
    - Low-level resistance (efflux) may be overcome by antibiotics

# ‘In vivo’ & ‘in vitro’ FQ- resistance in pneumococci

**TABLE 1.** MICROBIOLOGIC CHARACTERISTICS OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED BEFORE, DURING, OR AFTER THERAPY WITH ORAL LEVOFLOXACIN FROM FOUR PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA.*

| PATIENT NO. | SOURCE AND TIME OF CULTURE | SEROTYPE | PFGE PATTERN† | SUSCEPTIBILITY TO LEVOFLOXACIN‡ | MINIMAL INHIBITORY CONCENTRATION§ | | | AMINO ACID SUBSTITUTION | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | LEVOFLOXACIN | MOXIFLOXACIN | GATIFLOXACIN | IN PARC | IN GYRA |
| | | | | | μg/ml | | | | |
| 1 | Sputum, before treatment | 23F | A | S | 1 (S) | 0.12 (S) | 0.25 (S) | — | — |
| | Sputum, after treatment | 23F | A | R | 8 (R) | 1 (S) | 2 (I) | S79F | S81F |
| 2 | Sputum, before treatment | 6A | B | S | 4 (I) | 0.25 (S) | 0.5 (S) | S79F | — |
| | Sputum, during treatment | 6A | B | R | 16 (R) | 4 (R) | 4 (R) | S79F | S81F |
| 3 | Blood, before treatment | 14 | C | R | 16 (R) | 4 (R) | 2 (I) | S79F | S81Y |
| | Pleural fluid, during treatment | 14 | C | R | 16 (R) | 4 (R) | 2 (I) | S79F and D83Y | S81Y |
| 4 | Sputum, during treatment | ND | ND | R | 16 (R) | 4 (R) | 8 (R) | S79Y | E85K |

*PFGE denotes pulsed-field gel electrophoresis, S susceptible, R resistant, I having intermediate susceptibility, and ND not done. Dashes indicate that no mutation was found.

†Unique PFGE patterns are designated by arbitrary single letters.

‡Susceptibility was tested by the disk-diffusion method.

§The degree of susceptibility is indicated in parentheses.

Davidson, N Eng J Med, ’02

# ‘In vivo’ significance of ‘in vitro’ FQ-resistance for pneumococcal pneumonia

- Increased risk of selecting FQ-resistant *S. pneu* during treatment if
  - *S. pneu* with reduced susceptibility (first step mutants)
  - Use of insufficiently active FQ’s (peak/MIC)
- Increased risk of clinical failure with FQ-resistant *S. pneu*
  - » Perez-Trallero, Eur J Clin Microbiol Infect Dis, ’90; Davidson, NEJM, ’02, de la Campa, AAC, ’03, Perez-Trallero, EID, ‘03

# Why is breakpoint resistance often a bad predictor of clinical outcome

- Microbiological (breakpoint) definition of resistance is imprecise
- Underlying host factors impact on infection outcome
- Dosage, target organ not taken into account

# Why is breakpoint resistance often a bad predictor of clinical outcome

- In vitro susceptibility determination differs from in vivo situation
  - Small 'in vitro' inoculum size
    - $10^4$ CFU - $5.10^5$ CFU/ml or $mm^2$ vs $10^5$-$10^9$/ml or $mm^2$ in vivo
  - Constant antibiotic concentration for 16-18 hrs vs changing concentrations in vivo

# conclusions

- Mix of criteria (clinical, pharmacological, epidemiological) or single criterion for establishing breakpoints?
- Patient / organ / organism dependent breakpoints ?
- Regional breakpoints or internationally comparable breakpoints ?
- Regular reassessment of breakpoints ?

# conclusions

- Other ‘in vitro’ methods to determine susceptibility that resemble more the ‘in vivo’ situation ?
- Switch to MIC determination ?