# AmpC β-lactamases

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# β-lactams

- Natural, semisynthetic and synthetic β-lactams
  - Penams (e.g. benzylpenicillin, ampicillin)
  - Cephems, including classical cephalosporins, 2nd (e.g. cefotiam, cefuroxime) and 3rd generation cephalosporins (e.g. cefotaxime, ceftriaxone, ceftazidime)
  - Cephamycins (e.g. cefoxitin, cefotetan)
  - Monobactams (e.g. aztreonams)
  - Penams with a 2,3-double bond in the fused thiazoline ring (e.g. faropenam)
  - Carbapenems (e.g. Imipenem)

# β-lactams

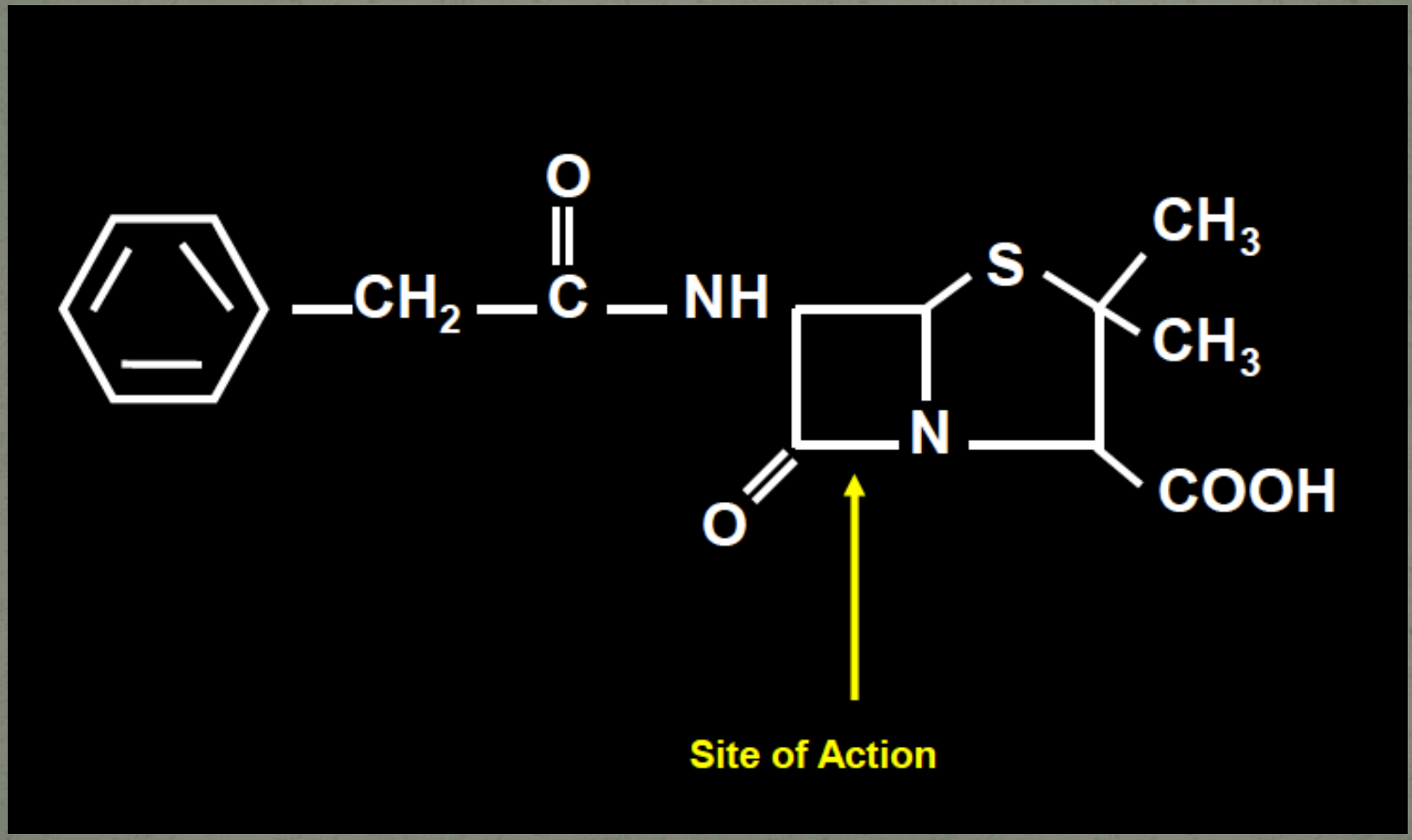
- Bactericidal by
  - Inactivating protein binding proteins → prevents cross-linkage of peptidoglycan → weakening cell wall and causing cell lysis
- In combination of β-lactamase inhibitors, e.g. clavulanic acid
  - Able to overcome the effect of β-lactamase in some β-lactamase producing strains

# Resistance of β-lactam

- 1) Alteration of penicillin-binding protein (PBP) with low affinity for β-lactam
- 2) Presence of efflux pumps which use β-lactam as substrates
- 3) β-lactamase which cleave the amide bond of the β-lactam ring causing inactivation of β-lactam

- *** Different mechanisms of resistance can co-exist within a bacteria

# β-lactamases



# Background of β-lactamase resistance

- First bacterial enzyme reported was produced by a strain Bacillus coli, now named as Escherichia coli, found in 1940 (later known to be AmpC β-lactamase)
- Now > 1000 β-lactamases was discovered in gram negative bacteria and one species can produce different enzymes at the same time
- Clinically important:
  - Extended beta-lactamases (ESBLs)
  - AmpC beta-lactamases (AmpC BLs)
  - Klebsiella pneumoniae carbapenemases (KPC)
  - Metallo-beta-lactamases (MBLs)

# Classifications of β-lactamases

- 1) Ambler Classifications
  - According to the amino acid sequences of enzyme
- 2) Bush-Jacoby Classifications
  - According to hydrolysis and inhibition profiles of the enzyme

# Classifications of β-lactamase

**Table 1**
Modified classification scheme of β-lactamases (according to Ambler, 1980).

| | β-lactamase-class | β-lactamases | Important examples | Preferential occurrence | Important phenotypical resistance traits[a] |
|---|---|---|---|---|---|
| Serine-β-lactamases | A | Broad-spectrum β-lactamases | TEM-1, TEM-2 SHV-1, SHV-11 | Enterobacteriaceae and nonfermenters[b] | ampicillin, cephalotin |
| | | ESBL TEM-type | TEM-3, TEM-52 | | penicillins, 3rd gen. cephalosporins |
| | | ESBL SHV-type | SHV-5, SHV-12 | | |
| | | ESBL CTX-M-type | CTX-M-1, CTX-M-15 | | |
| | | Carbapenemases | KPC, GES, SME | | all β-Lactams[c] |
| | C | AmpC cephamycinases (chromosomal-encoded) | AmpC | Enterobacter spp. Citrobacter spp. | cephamycins (cefoxitin), 3rd gen. cephalosporins |
| | D | AmpC cephamycinases (plasmid-encoded) | CMY, DHA, MOX FOX, ACC, | Enterobacteriaceae | cephamycins (cefoxitin), 3rd gen. cephalosporins |
| | | Broad-spectrum β-lactamases | OXA-1, OXA-9 | Enterobacteriaceae; A. baumannii | oxacillin, ampicillin cephalotin |
| | | ESBL OXA-type | OXA-2, OXA-10 | | penicillins, 3rd gen. cephalosporins |
| | | Carbapenemases; Carbapenemases | OXA-48; OXA-23,-24,-58 | | ampicillin, imipenem; all β-lactams[c] |
| Metallo-β-lactamases | B | Metallo-β-lactamases (Carbapenemases) | VIM IMP | Enterobacteriaceae and nonfermenters | all β-lactams[c] |

[a] Characteristical resistances that are partially used used for diagnostic purposes;
[b] Broad-spectrum β-lactamase TEM-1 frequently occurs in nonfermenters (P. aeruginosa, A. baumannii);
[c] Broad hydrolytic spectrum including carbapenems.

# AmpC β-lactamase

- Class C β-lactamase that hydrolysed
  - Cephems
    - Cephalosporins (except 4$^{th}$ gen)
    - Cephamycins
  - Broad-spectrum penicillins
  - Monobactams
  - β-lactamase inhibitors

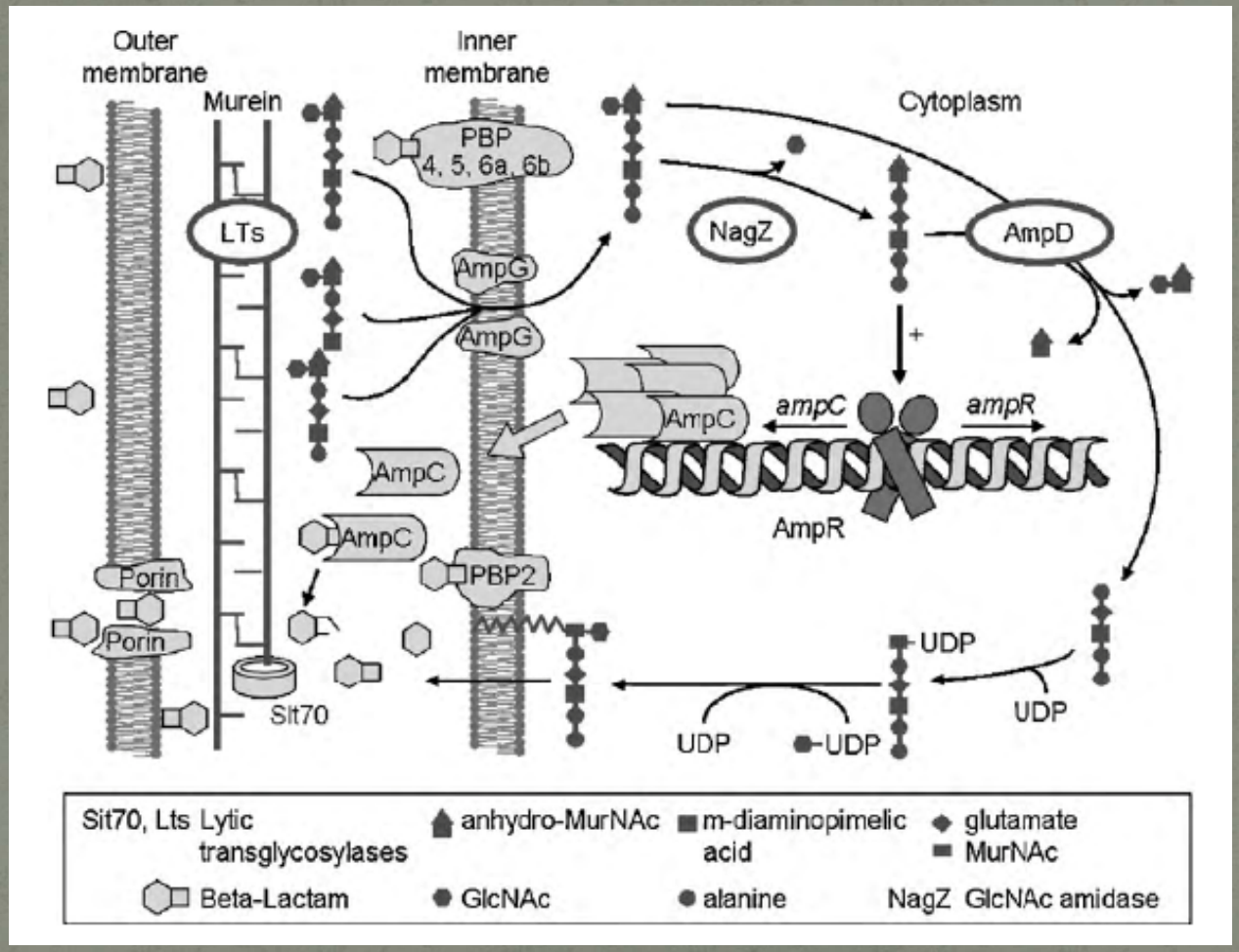
# Type of AmpC β-lactamase

- 1) Chromosomal
  - Constitutive
    - Enterobacter sp.
    - Citrobacter freundii
  - Indicible
    - SPICE organisms
      - Serratia sp
      - Pseudomonas aeruginosa
      - Indole positive Proteae: Proteus vulgaris, Providencia sp., Morganella morganii
      - Citrobacter sp.
      - Enterobacter cloacae

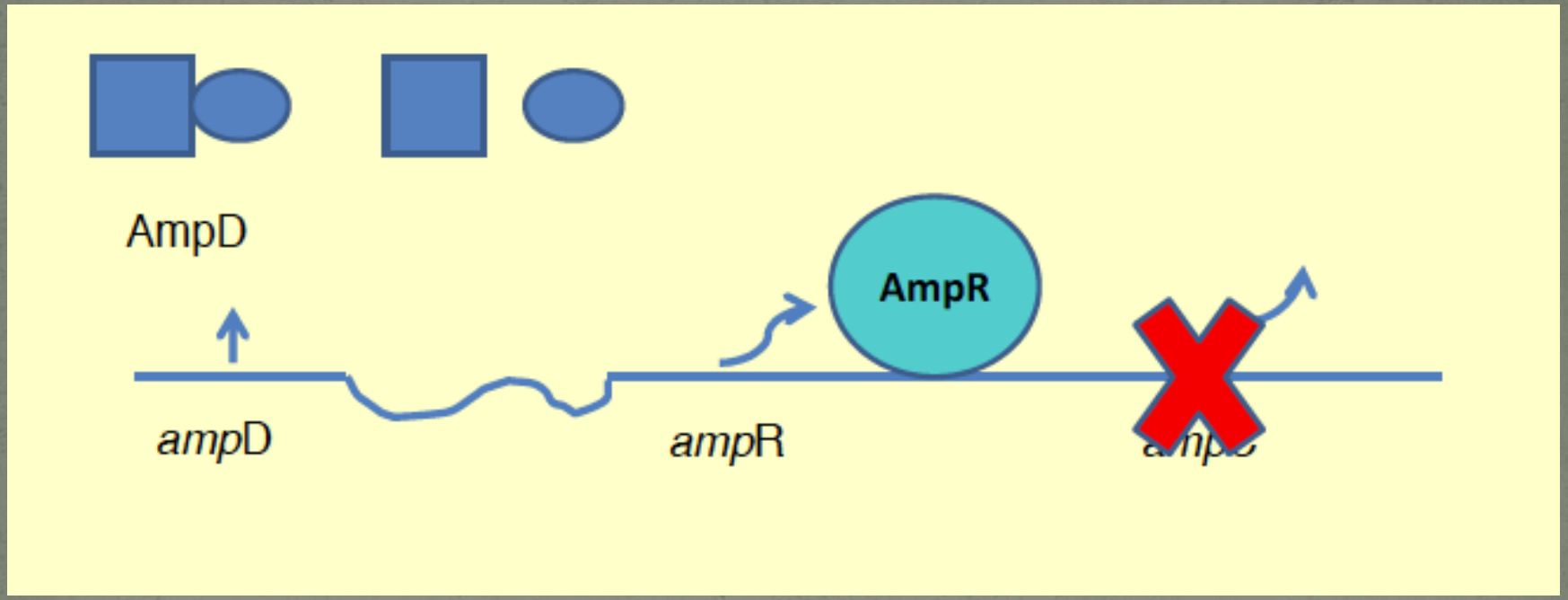
# Type of AmpC β-lactamases

- 2) Plasmid-mediated
  - Transmissible
  - Mostly detected in organisms without intrinsic ampC gene
    - E. coli
    - K. pneumoniae
    - Proteus mirabilis
    - Salmonella sp.

# Regulation of ampC in Enterobacteriaceae



# Constitutive AmpC



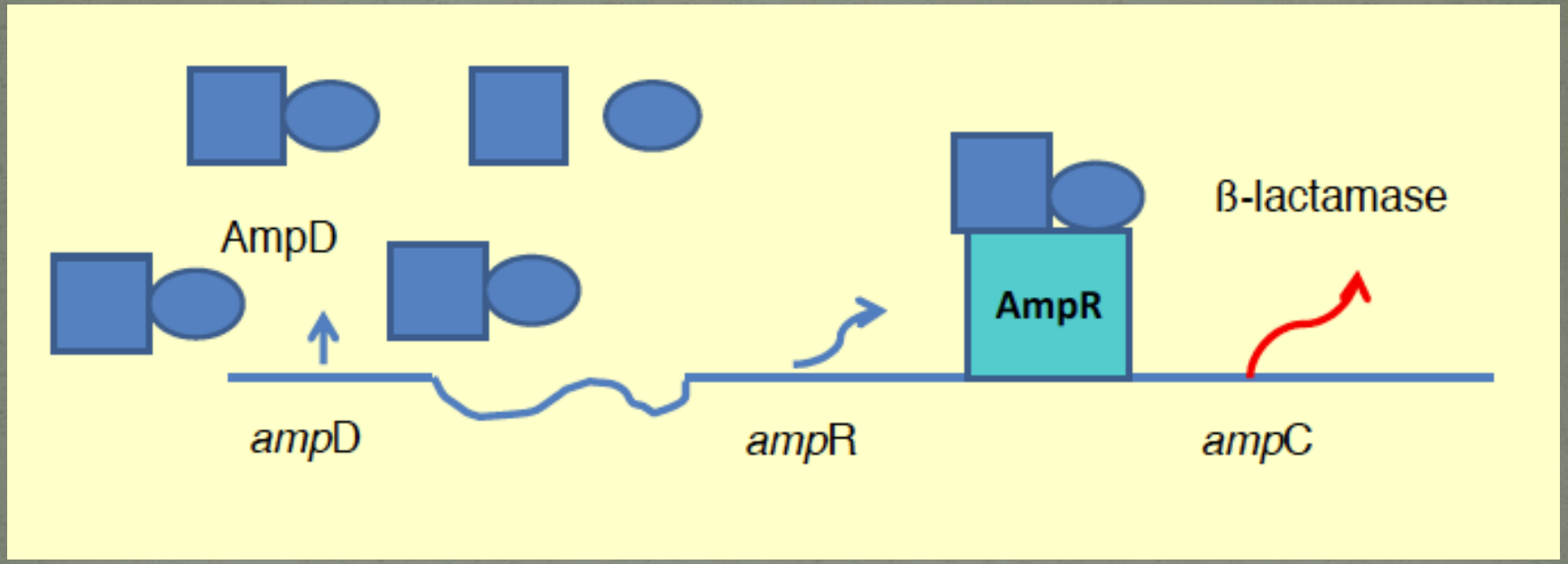

Courtesy of Dr Naomi Cheng, QEH

# Constitutive AmpC

- AmpR: Transcriptional regulator
- AmpD: Amidase which can remove degradation products / wall fragments

- Upon β-lactam exposure → accumulation of wall fragments → conformational change in AmpR → over-expression of AmpC
- Presence of AmpD → recycles wall fragments → reducing the conc. and preventing over-expression of AmpC

# Induced AmpC



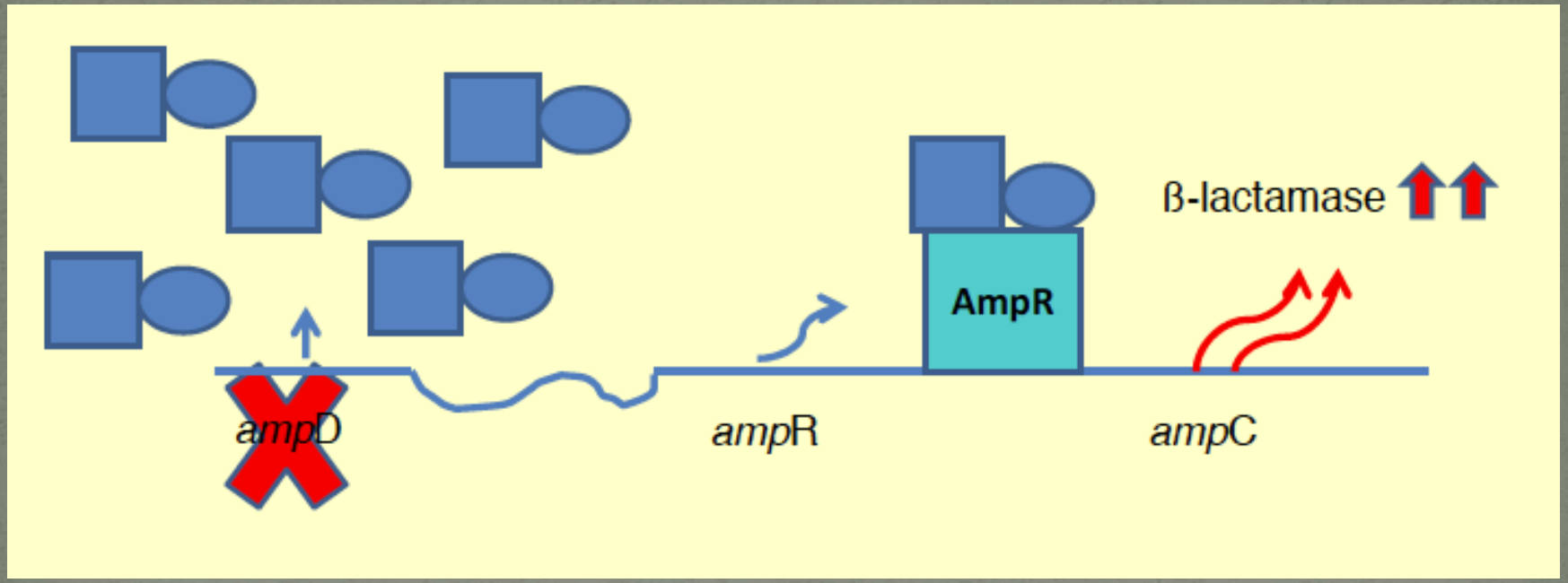

Courtesy of Dr Naomi Cheng, QEH

# Induced AmpC

- Overproduction of wall fragments
- AmpD overwhelmed
- Activated AmpR
- Expression of AmpC

- *** It is a reversible process once the inducing agent is removed

# Derepressed / mutant AmpC




Courtesy of Dr Naomi Cheng, QEH

# Derepressed / mutant AmpC

- Mutation of AmpD (most common) / Mutation of AmpR (less common)
- Causing AmpC hyperinducibility or constitutive hyperproduction

# β-lactamase inducer

| Good | Poor |
|---|---|
| Benzylpenicillin | Piperacillin |
| Ampicillin, anoxicillin | Aztreonams |
| Cefazolin, cephalothin, cefoxitin | 2nd generation cephalosporin |
| Imipenem, Meropenem | 3rd generation cephalosporin |
| Clavulanic acid | 4th generation cephalosporin |

# Substrates for AmpC β-lactamase

| Good (=unstable) | Variable (*) | Poor (=stable) |
|---|---|---|
| Penicillin | 2nd, 3rd generation cephalosporin | Cefoxitin |
| Cefazolin, cephalothin | Aztreonam | Imipenem |
| | Piperacillin | 4th generation cephalosporin |

(*) Labeled as weak substrates, but can still be hydrolyzed if enough enzyme is made

# Different scenerios

- Good inducer + good substrate
  - Aminopenicillin
  - 1st generation cephalosporin
  - Clavulanic acid
    - Esp in Pesudomonas aeruginosa which the clinically achieved concentration of clavulanic will antagonize the activity of Ticarcillin
- Good inducer but poor substrate
  - Imipenem
  - Remains active as long as other resistant mechanism (porin loss or efflux pump) are absent

# Different scenerios

- Non-inducer + "good" substrate
  - Carboxy-ureidopenicillin (Ticarcillin, piperacillin)
  - 2nd and 3rd generation cephalopsorin
  - Aztreonam
  - *** Remained active against SPICE organisms in the absence of another inducer β-lactam
- Non-inducer + poor substrate
  - Cefpirome
  - Cefepime
  - *** Not as stable as Imipenem and can be affected by hyperproduction or derepressed mutant

# Laboratory Detection

- Not routinely performed in laboratory setting
- No CLSI recommendations on detection of AmpC β-lactamases
  - Phenotypic methods
    - Broth dilution
    - Disk diffusion
    - Commercial tests
  - Multiplex PCR
  - Mass spectrometry to detect BL-degraded fragments of antibiotics

# When to suspect?

- R: 3rd gen cephalosporin but NOT to cefepime
- R: Cefoxitin
- I/R: Augmentin
- Serratia sp. “S” to ceftazidime
- Providencia sp, Morganella sp., & Serratia sp. S/I to cefoxitin

# Does AmpC β-lactamase bother?

- 1) Misidentifications as ESBLs
- 2) Induction / transmission of AmpC
- 3) Treatment issue

# ESBL ? AmpC β-lactamase

- Phenotypically ESBL and AmpC β-lactamase produces positive ESBL screening test
- ESBL screening positive isolates will go over a confirmatory test (Ceftazidime + Clavulanic acid / cefotaxime + Clavulanic acid)
  - True ESBL → Synergism +ve in confirmatory test
  - False +ve: Often in Acinetobacter sp.

# ESBL ? AmpC β-lactamases

- Confirmatory test –ve ≠ Non-ESBL producing strains
  - AmpC β-lactamases producers
  - GNB which can produce both ESBL and AmpC β-lactamase
- Cefoxitin resistance or synergism with AmpC inhibitor (e.g. Boronic acid / Cloxacillin)

# Induction of AmpC

- Chow et al (1991)
- 129 patients with Enterobacter bacteremia
  - Treatment with cefotaxime / ceftazidime / ceftizoxime
    - 6/31 (19.3%) developed resistance to cephalosporin anf augmented β-lactamase production
  - Treatment with aminoglycosides → 1/89 (1%)
  - Treatment with other β-lactams (extended spectrum penicillins, imipenem and Aztreonam) → 0/50 (0%)

# Induction of AmpC

- Kaye KS et al (2001)
- 477 initially susceptible Enterobacter sp infection
  - 19% receving broad-spectrum cephalosporins developed resistance
  - Resistance is more likely to appeared if the original isolates came from blood (29%) then from urine / tissue (7%), p 0.04

# Induction of AmpC

Emergence of Antibiotic Resistance during Therapy for Infections Caused by *Enterobacteriaceae* Producing AmpC β-Lactamase: Implications for Antibiotic Use[▽]

Sang-Ho Choi,[1,3] Jung Eun Lee,[1,3] Su Jin Park,[1,3] Seong-Ho Choi,[1,3] Sang-Oh Lee,[1,3] Jin-Yong Jeong,[1,3] Mi-Na Kim,[2] Jun Hee Woo,[1,3] and Yang Soo Kim[1,3]*

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732 patients with infections due to Enterobacter sp., S marcescens, C freundii or Morganella morganii

# Induction of AmpC

TABLE 5. Emergence of resistance during broad-spectrum cephalosporin therapy

| Characteristic | No. of patients with emergence of resistance to the therapy/total no. of patients in the group (%) | |
|---|---|---|
| | All patients | Patients with bacteremia |
| Overall | 11/218 (5.0) | 4/54 (7.4) |
| Organism | | |
| *Enterobacter* spp. | 10/121 (8.3) | 4/30 (13.3) |
| *E. cloacae* | 8/65 (12.3) | 2/18 (11.1) |
| *E. aerogenes* | 2/51 (3.9) | 2/10 (20.0) |
| *E. agglomerans* | 0/4 (0) | 0/2 (0) |
| *E. asburiae* | 0/1 (0) | 0 |
| *C. freundii* | 1/39 (2.6) | 0/8 (0) |
| *S. marcescens* | 0/37 (0) | 0/10 (0) |
| *M. morganii* | 0/21 (0) | 0/6 (0) |
| Type of infection | | |
| Biliary tract infection | 6/52 (11.5) | 2/22 (9.1) |
| Urinary tract infection | 0/46 (0) | 0/6 (0) |
| Pneumonia | 0/43 (0) | 0/2 (0) |
| Skin and soft tissue infection | 4/29 (13.8) | 1/1 (100) |
| Intra-abdominal infection | 1/18 (5.6) | 1/5 (20.0) |
| Primary bacteremia | 0/17 (0) | 0/17 (0) |
| Catheter-associated infection | 0/1 (0) | 0/1 (0) |
| Other | 0/12 (0) | |
| Monotherapy vs combination therapy | | |
| Monotherapy | 10/181 (5.5) | 3/41 (7.3) |
| Combination therapy | 1/37 (2.7) | 1/13 (7.7) |
| Concomitant bacteremia | | |
| Bacteremia positive | 4/54 (7.4) | 4/54 (7.4) |
| Bacteremia negative | 7/164 (4.3) | |
| ESBL production | | |
| ESBL positive | 1/5 (20.0) | 0 |
| ESBL negative | 10/213 (4.7) | 4/54 (7.4) |

- Factors associated
  - Enterobacter sp 8.3% (10/121) than other species
  - Biliary tract infection 11.5% (6/52), p 0.024
  - Combination therapy did not prevent resistance

# Treatment

- 1) 3rd generation cephalosporin should be avoided in severe infections (bacteremia / biliary tract infections) due to
  - Enterobacter spp
  - C. freundii
  - Serratia sp.
  - Morganella sp.

# Treatment

- 2) 4th generation cephalosporins
  - Poor inducer of AmpC
  - Zwitterionic cephalosporins which can penetrates rapidly through outer membrane, and is little hydrolyzed by the enzyme
  - Kang et al (2004): High inoculum of bacteria → cefepime MICs increase dramatically for some AmpC producers

# Treatment

- 3) Carbapenems
  - Generally susceptible
  - Imipenem
    - More rapidly bactericidal than 3rd generation cephalosporin
  - Studies have demonstrated increase MIC to carbepenems in some isolates which are porin-deficient

# Treatment

- 4) Non β-lactams: Aminoglycosides / FQ
  - Survey of 4 most common GNB isolates from ICU from 1994-2000

**Table 1.** Antimicrobial Susceptibility Rates for All and for the 4 Most Common Species of Gram-Negative Bacilli, Intensive Care Unit Surveillance, 1994-2000*

| Antimicrobials | All Isolates | | *Pseudomonas aeruginosa* | *Enterobacter* Species | *Klebsiella pneumoniae* | *Escherichia coli* |
|---|---|---|---|---|---|---|
| | All Isolates (n = 35 790) | Absolute Change in % Susceptible, 1994-2000 | (n = 8244) | (n = 4999) | (n = 4877) | (n = 4027) |
| Group 1, ≥90% susceptibility | | | | | | |
| Amikacin | 90 | –1 | 90 | 98 | 94 | 100 |
| Group 2, 80%-90% susceptibility | | | | | | |
| Imipenem | 89 | –1 | 83 | 99 | 99 | 100 |
| Tobramycin | 83 | –3 | 87 | 92 | 85 | 96 |
| Ciprofloxacin | 81 | –10 | 76 | 90 | 88 | 97 |
| Group 3, ≤79% susceptibility | | | | | | |
| Cefepime | 78 | 2† | 71 | 84 | 90 | 98 |
| Ceftazidime | 78 | –3 | 80 | 63 | 87 | 97 |
| Gentamicin | 78 | –6 | 68 | 92 | 85 | 95 |
| Piperacillin/tazobactam | 78 | 0‡ | 78 | 73 | 87 | 95 |
| Aztreonam | 71 | –4 | 67 | 68 | 86 | 97 |
| Ticarcillin/clavulanate | 65 | –2 | 42 | 56 | 82 | 84 |
| Piperacillin | 59 | –4 | 74 | 60 | 39 | 64 |
| Ceftriaxone | 59 | –2 | 17 | 63 | 86 | 98 |
| Cefotaxime | 58 | –2 | 13 | 64 | 88 | 98 |
| Ticarcillin | 44 | –5 | 43 | 52 | 5 | 62 |
| Ampicillin/sulbactam | 35 | –3 | 2 | 21 | 65 | 63 |

*Data are presented as percentages.
†Based on % change, 1998-2000.
‡Based on % change, 1997-2000.

# Treatment

- 5) Colistin
  - Cyclic polypeptide which alter cell membrane permeability → causing content leakage and cell death
  - Bactericidal against most GNB
  - Side effects: Nephrotoxicity and neurotoxicity

# Treatment

- 6) Tigecyclines
  - Parental semisynthetic glycylcycline agent that is bacteriostatic
  - Binds to 30S ribosomonal subunit → Inhibition of bacterial protein translation
- 7) Fosfomycins
  - Against cell wall synthesis
  - Approved for treatment of uncomplicated UTI in adults due to E coli and Enterococcus faecalis

# Treatment

- 8) Temocillin
  - A 6-α-methoxy derivative of ticarcillin
  - Active in vitro against many AmpC-producing Enterobacteriaceae (both Chromosomal / plasmid-mediated) and some ESBL producers
  - Limited clinical experience

# Extended spectrum AmpC β-lactamases

- Nordmann and Mammeri (2007) described a novel mechanism of resistance due to production of variant cephalosporinase – namely extended spectrum AmpC (ESAC) β-lactamase
  - Structurally related to wild-type cephalosporinase by insertions, deletions or substitution
  - Broader spectrum of resistance, esp against carbapenems
- Has been found in E coli (Mammeri 2008), Pseudomonas aeruginosa (Rodriguez-Martinez JM, 2009) and Acinetobacter baumannii (Rodriguez-Martinez JM, 2010)

# Bring home message

- 1) AmpC β-lactamases are clinically important mechanism of resistance and is an emerging threat
- 2) AmpC can be inbred, induced or mutated into existence
- 3) AmpC β-lactamases producing strains pose diagnostic and therapeutic difficulties for clinical microbiologists

# What should be asked…?

Organism 1 : Enterobacter cloacae (from aerobic and anaerobic broth)

| ANTIBIOTICS | ORGANISM 1 |
|---|---|
| Ampicillin | R |
| Gentamicin | S |
| Cefuroxime (I.V.) | R |
| Cefuroxime (Oral) | R |
| Levofloxacin | S |
| Timentin | S |
| Augmentin | R |
| Netilmicin | S |
| Amikacin | S |
| Piperacillin/Tazobactam | S |
| Cefepime | S |
| Meropenem | S |

# What should be asked…?

Organism 1 : Proteus mirabilis (greater than 100,000 CFU/ml)

| ANTIBIOTICS | ORGANISM 1 |
|---|---|
| Ampicillin | S |
| Gentamicin | S |
| Cefuroxime (I.V.) | S |
| Cefuroxime (Oral) | S |
| Ciprofloxacin | S |
| Cotrimoxazole | S |
| Augmentin | S |

# What should be asked…?

Organism 1 : Klebsiella species (greater than 100,000 CFU/ml)

| ANTIBIOTICS | ORGANISM 1 |
|---|---|
| Ampicillin | R |
| Gentamicin | S |
| Cefuroxime (I.V.) | R |
| Cefuroxime (Oral) | R |
| Ciprofloxacin | R |
| Cotrimoxazole | R |
| Augmentin | R |
| Amikacin | S |
| Sulperazone | S |
| Cefotaxime | I |
| Ceftazidime | I |
| Ertapenem | S |

# The end