

IV. RESISTANCE TO ANTIBIOTICS

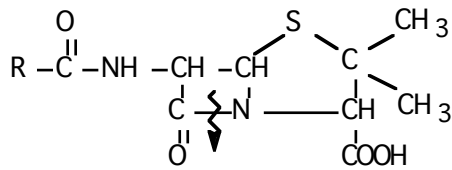
A. Some scary statistics

B. Nature of genotypic alterations → drug resistance

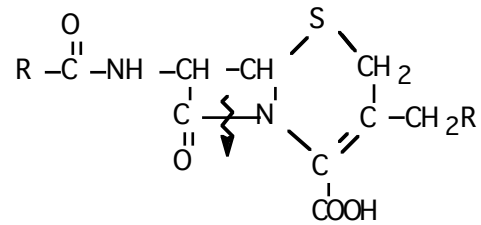
1. Spontaneous mutations
 - a) is the origin of resistance
 - b) occurs in low frequency $1/10^6$ - $1/10^8$ and is unrelated to contact with an antibiotic
 - c) mutations rarely lead to complete resistance with single mutation
 - d) an infection can contain 10^{10} organisms
 - e) selection process is important in increasing the small numbers of drug resistant mutants that appear. This is vertical passage of trait.
2. Transformation
 - a) uptake of genetic material coding for resistance by a sensitive bacterium donated by a resistant bacterium on lysis
 - b) may be how *Strep. pneumoniae* became resistant to penicillins
3. Transduction
 - a) important especially with the gram-positive and especially of clinical importance with the Staph
 - b) during lysogeny a phage can pick up randomly or selectively bits of genetic pieces and/or plasmids coding for resistance which are then donated to a sensitive cell when infected by the phage
4. Conjugation
 - a) resistant determinants located on a plasmid
plasmid = nonessential, self-replicating element composed of a circular piece of double stranded DNA; is independent from chromosomal DNA
R factor = a plasmid coding for resistance and conjugation
Transposon = genes coding for resistance with insertion sequences at either end - can "jump" from plasmid to chromosome and between plasmids
e.g., TEM gene coding for beta-lactamase; Van A gene coding for resistance from *E. fecalis* to *S. aureus*
 - b) R-factor is transferred during mating to drug sensitive recipient cells
 - c) R-factor can be split into two determinants, (1) a resistance transfer factor (RTF) coding for pilus formation and, (2) a resistance determinant; Staph lack RTF and R-factor is transferred mainly by transduction
 - d) dangers of conjugation:
 - i. gram-negative enteric bacteria can transfer R-factor by conjugation to same or even different genera
 - ii. plasmids usually carry genes determining resistance to multiple antimicrobials
 - iii. bacteria can contain multiple plasmids
 - iv. greater than 50% of GI tract inhabitants are capable of transferring R-factor and they may serve as a reservoir of R factors
 - e) modifying factors
 - i. R transfer is under genetic control, however; a repressor for R transfer normally limits the process
 - ii. microbes carrying R-factor may be at a selective disadvantage in an antibiotic free environment which also limits the significance of this process

C. Mechanisms of Ab resistance

- conversion of active drug to inactive derivative - e.g. hydrolysis of penicillins and cephalosporins by beta-lactamases



a penicillin



a cephalosporin

- e.g. beta-lactamases
 - gram-positive bacteria beta-lactamases
 - inducible
 - excreted
 - will lower extracellular antibiotic concentration
 - most gram(+) do not make beta-lactamases
 - Staph. aureus makes a narrow spectrum beta-lactamase with high affinity for most penicillins. It is usually called a "penicillinase."
 - penicillinase has a high K_M for penicillinase resistant penicillins and for some cephalosporins
 - gram-negative bacteria beta-lactamases
 - constitutive or inducible
 - not excreted but located in periplasmic space
 - keep intracellular antibiotic concentrations low
 - don't affect extracellular antibiotic concentrations
 - > 300 known enzymes which vary in K_M for different β -lactam antibiotics; some have broad specificity, e.g. Enterobacter and Klebsiella beta-lactamases
 - plasmid-encoded beta-lactamases
 - produced by S. aureus (gm+), H. influenzae, N. gonorrhoeae, Salmonella, Shigella, E. coli, Klebsiella
 - generally inhibited by the available beta-lactamase inhibitors
 - chromosomally encoded (usually inducible)
 - produced by Enterobacter, Citrobacter, and Pseudomonas, Serratia, Morganella, Providencia
 - not generally inhibited by the available beta-lactamase inhibitors
 - is a cephalosporinase

Table 1. Functional and molecular characteristics of the major groups of β -lactamases.

Functional group	Major subgroups	Molecular class	Attributes of β -lactamases in functional group	Estimated no. of enzymes ^a	
				1995	2000
1		C	Often chromosomal enzymes in gram-negative bacteria but may be plasmid-encoded. Confer resistance to all classes of β -lactams, except carbapenems (unless combined with porin changes). Not inhibited by clavulanic acid.	32	51
2		A, D	Most enzymes responsive to inhibition by clavulanic acid (unless otherwise noted).	136	256
	2a	A	Staphylococcal and enterococcal penicillinases included. Confer high resistance to penicillins.	20	23
	2b	A	Broad-spectrum β -lactamases, including TEM-1 and SHV-1, primarily from gram-negative bacteria.	16	16
	2be	A	Extended-spectrum β -lactamases conferring resistance to oxyimino-cephalosporins and monobactams.	36	119
	2br	A	Inhibitor-resistant TEM (IRT) β -lactamases; one inhibitor-resistant SHV-derived enzyme.	9	24
	2c	A	Carbenicillin-hydrolyzing enzymes.	15	19
	2d	D	Cloxacillin-(oxacillin)-hydrolyzing enzymes; modestly inhibited by clavulanic acid.	18	31
	2e	A	Cephalosporinases inhibited by clavulanic acid.	19	20
	2f	A	Carbapenem-hydrolyzing enzymes with active site serine, inhibited by clavulanic acid.	3	4
3	3a, 3b, 3c	B	Metallo- β -lactamases conferring resistance to carbapenems and all β -lactam classes except monobactams. Not inhibited by clavulanic acid.	13	24
4		? ^b	Miscellaneous unsequenced enzymes that do not fit into other groups.	7	9

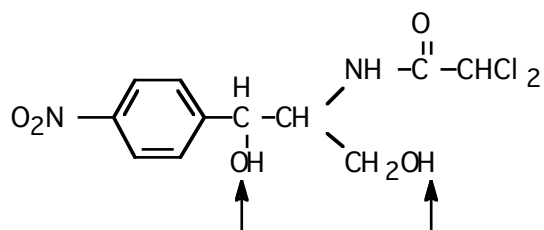
NOTE. Table is adapted from [3, 4].

^a Identified from clinical isolates.

^b Unknown.

Bush, K., Clin. Infect. Dis. 2001; 32: 1085-1089

- b) e.g. acetyl transferases - inactivation of chloramphenicol



acetyltransferase is an inducible enzyme in some gram-positive and constitutive in some gram-negative

- c) other enzymes, e.g. transformation of a aminoglycoside antibiotics; free OH - groups on sugars can be acetylated, phosphorylated, or adenylated; enzymes are coded for on plasmids
2. modification of drug-sensitive site - via mutation; usually seen with drugs affecting protein synthesis
- e.g. streptomycin - subtle changes in protein component of 30s ribosomal subunit may prevent streptomycin from binding
 - e.g. rifamycin - altered DNA dependent RNA polymerase prevents binding
 - e.g. beta-lactams - alteration in penicillin binding protein (PBP) which can result in conformational changes on the binding site thus decreasing antibiotic affinity, e.g. penicillin resistant Strep. Pneumoniae and MRSA. The amount of PBP can also be decreased.

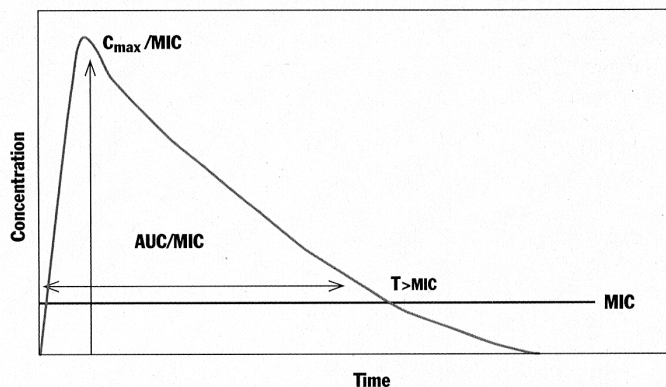
3. loss of bacterial cell permeability
e.g. beta-lactams - change in porin proteins results in decreased permeability;
Pseudomonas is infamous for porin exclusion
4. enhanced efflux e.g. tetracyclines; multidrug efflux pumps are increasingly important in resistance; may be broad spectrum efflux pumps
5. enhancement of alternate metabolic pathway
to avoid inhibited pathway; e.g., sulfa drugs where microbes synthesize enough PABA to overcome block in folate synthesis
6. absence of autolytic enzymes
e.g. beta-lactams - tolerant cells lack autolysins

C. Therapeutic Control of Drug Resistance

1. give the proper antibiotic
 - a) is it necessary?
 - b) is pathogen sensitive?
 - c) will drug get to site of infection?
 - d) is antibiotic unnecessarily toxic (risk vs. benefit) - decreases compliance?
 - e) is antibiotic expensive (cost vs. risk of infection) - decreases compliance?
2. give high antibiotic loading doses - e.g. a one-time STAT dose
3. simultaneous therapy with unrelated (MOA) antibiotics
4. use only when necessary - antibiotics in animal feeds?
5. randomly use different antibiotics - rotation
6. patient compliance to complete course of therapy
7. concentration dependent killing vs time dependent killing

a)

■ Figure 1
Antimicrobial pharmacodynamic indices



C_{max} = peak concentration; AUC = area under the curve
MIC = minimum inhibitory concentration; $T_{>MIC}$ = time above the MIC

Formulary/Source: Ref 4,5,41

b)

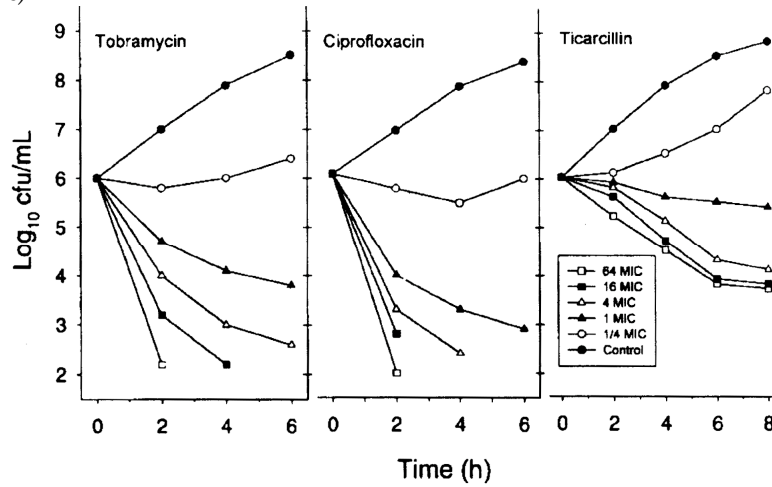
■ Table 1

Pharmacodynamic indices that correlate to efficacy for time-dependent and concentration-dependent killing agents

	Antibiotic class	Pharmacodynamic index
Time-dependent	β-lactams (penicillins, cephalosporins, monobactams, and carbapenems)	$T_{>MIC}$
	Glycopeptide (vancomycin)	$T_{>MIC}$ $C_{max-free}/MIC$ AUC/MIC
Concentration-dependent	Aminoglycosides	C_{max}/MIC AUC/MIC
	Fluoroquinolones	C_{max}/MIC AUC/MIC
	Macrolides (erythromycin, clarithromycin) and Azalide (azithromycin)	C_{max}/MIC AUC/MIC
	Ketolide (telithromycin)	C_{max}/MIC AUC/MIC

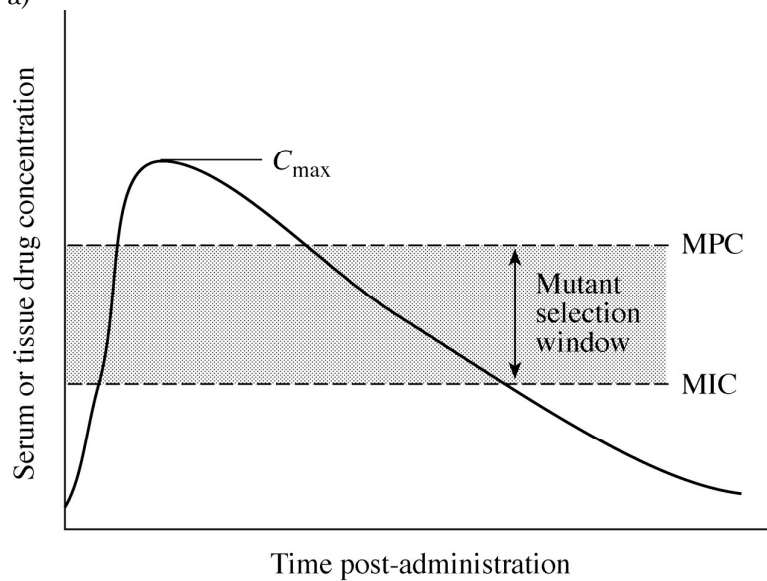
Formulary/Source: Refs 18–22,25–27,29–34,36–39,52–54,56,57,61,62

c)



8. the concept of mutant selection window

a)



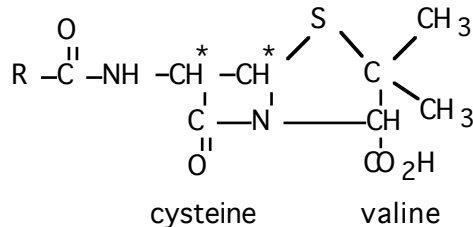
V. ANTIBIOTICS INHIBITORY BACTERIAL CELL WALL BIOSYNTHESIS

A. PENICILLINS

1. Introduction

- still a widely prescribed and useful group of antibiotics
- are potent, nontoxic, and have fairly broad spectrum with use of beta-lactamase inhibitors

2. Chemistry



- Structure/Activity Relationships considerations
 - need conformation, intact β -lactam ring
 - can modify 5-membered ring and replace R groups

3. Production

1928 - Fleming, *P. notatum*

1938 - Flory and Chain

1941-49 - international cooperation with U.S. and England mainly using *P. chrysogenum*

1959 - England - Beecham developed process for forming 6-APA which lead to commercial development of semisynthetic derivatives

4. General Properties of Penicillins

- stability in stomach acid
varies; Pen G is labile and has low bioavailability so is only rarely used orally; amoxicillin has high stability and bioavailability and is only used orally.
- susceptibility to Gram + β -lactamses (penicillinase) varies; Pen G, Pen V, amox, ampi are very susceptible but modification of side chain can lead to penicillinase resistance, e.g. dicloxacillin (a "penicillinase resistant penicillin")
- susceptibility to Gram (-) β -lactamases – highly susceptible except ticarcillin and piperacillin
- bacteriocidal

e) allergy

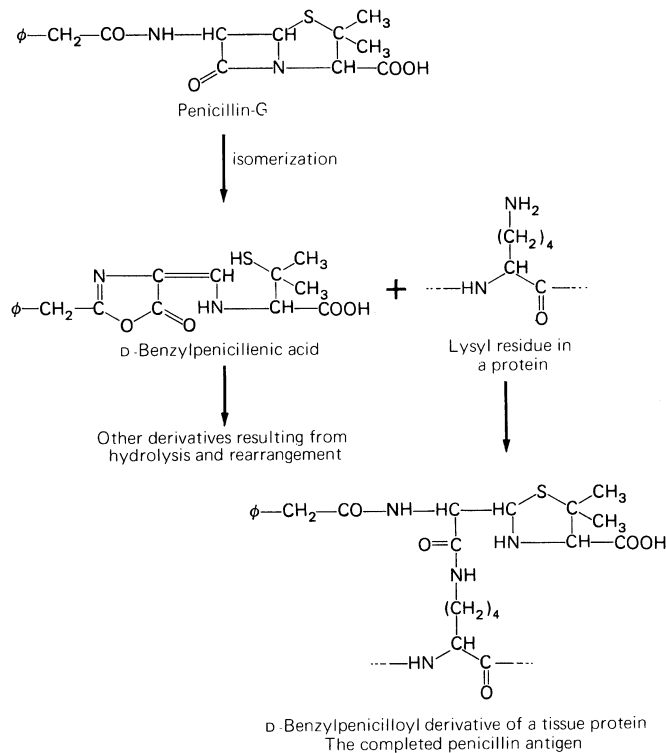


Figure 4-5. Proposed chemical pathway for the formation of the penicillin antigen. A very small percentage of penicillin G is isomerized at physiological pH to D-benzylpenicillenic acid, which then reacts with the ε-amino group of lysyl residues in proteins to form the complete penicillin antigen. (From Levine.⁸⁵)

This is considered the "major determinant" of penicillin allergy. The "minor determinants" are a variety of other penicillin breakdown products.

- 1) immediate - urticaria, pruritus, shock; occurs within 60 min – rare but highly feared; IgE mediated and directed against minor determinants
- 2) accelerated - rash; occurs within 72 h - common (5-10% of treated); Ig M and Ig E directed against major determinant
- 3) late - fever, urticaria; occurs > 72 h – rare; Ig M and Ig E
- 4) incidence -
 - 5% show (+) reaction with skin test using penicilloyl polylysine (Prepen®)
 - 50-70% of (+) have clinical Sx on amin. of penicillins - mostly rash
 - <1% of (+) have serious reaction; overall incidence is about <.02%, <.0014% → death
 - semisynthetic show ↓ reactions; oral show ↓ reactions relative to parenteral doses but an allergy to one penicillin means that the patient would be assumed to have a problem with any penicillin
 - allergy to penicillin does not necessarily mean allergy to cephalosporins but cross reactivity does occur (~10%); rule: if patient has serious reaction to a penicillin, don't give any beta lactam except aztreonam
 - ampicillin → 9.5% allergy; this is due, in part, to the chemical nature of ampicillin, i.e. not all ampicillin induced rashes are true penicillin allergies. Amoxicillin has a somewhat lower incidence
 - desensitization protocols can be employed if infection requires penicillin therapy for an allergic patient

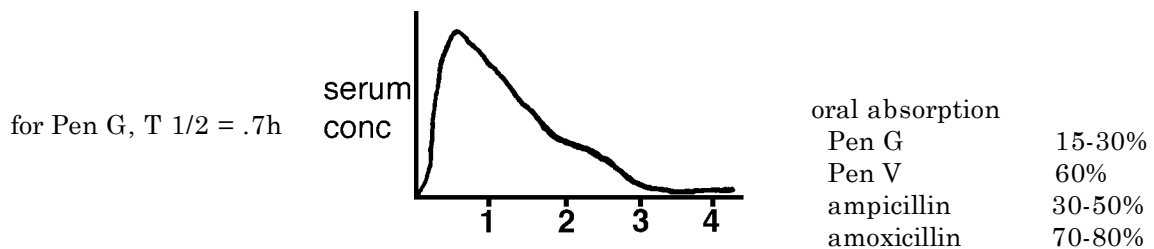
a rare interstitial nephritis is sometimes seen which may be a type of allergic reaction

5) skin testing - is useful but an imperfect test (see above) commercial PrePen® is not now marketed but Allerquest claims approval is due in late 2007.
a pharmacist can make up a mixture of "minor determinants" that will better predict more serious reactions but this is rarely done

- 6) precautions on penicillin allergy
- ask about prior reactions
 - ask about the type of allergic reaction (i.e. symptoms of anaphylaxis)
 - don't give a cephalosporin to a patient who has had an anaphylactic or other serious reaction to a penicillin
 - skin testing may be helpful if a penicillin is clearly needed
 - a desensitization protocol (small repetitive increasing doses of a penicillin) can be used if a penicillin is clearly needed. Go to the literature to find a good protocol. Patient probably needs to be hospitalized.

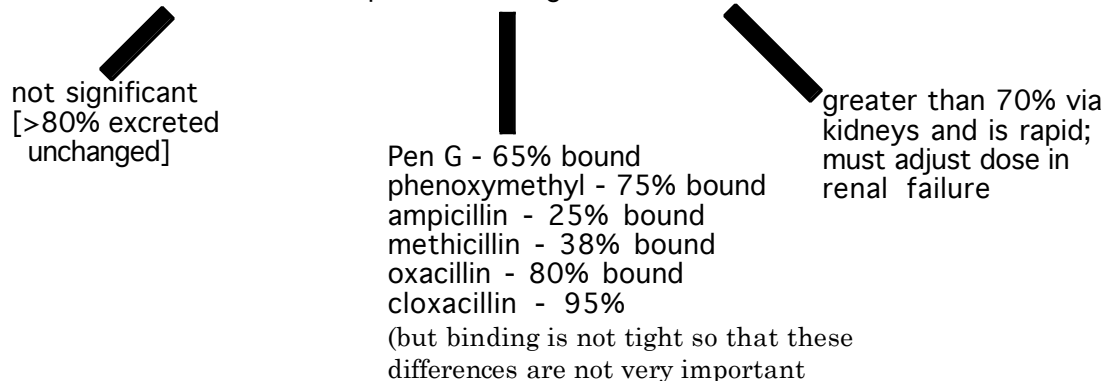
f) toxicity – some rare toxicity with high dose IV-therapy; diarrhea (ampicillin); neurotox; seizures; nephrotox; hemolytic anemia pseudomembranous colitis and bacterial overgrowth with the broad spectrum agents; generally the safest group of antibiotics, however

g) absorption
orally - varies with the drug used



h) distribution - high in kidney (>70%), low in cerebrospinal fluid (except when inflammation is present)

i) metabolism and serum protein binding and excretion



j) Probenecid - inhibits tubular secretion of organic acids (most pens and cephs) which results in increased β -lactam blood levels (~2 x).

TABLE 1 - Properties of Penicillins

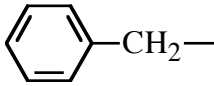
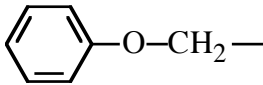
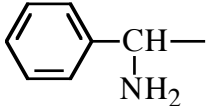
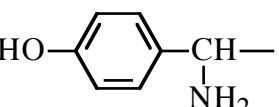
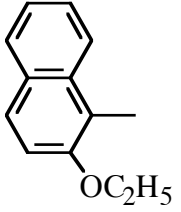
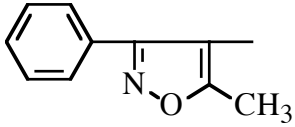
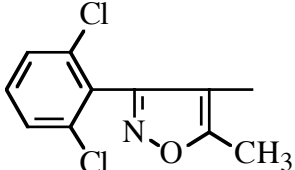
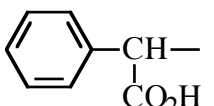
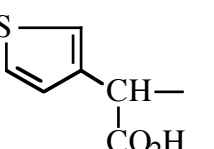
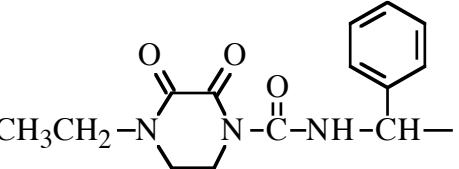
Type of penicillin	Structure (R group)	Oral absorption	Routes of admin.
1. "Natural: Penicillin G		poor	IV, IM
Penicillin V		60-70%	PO
2. Amino Ampicillin		30-50%	PO, IV, IM
Amoxicillin		70-90%	PO
3. Penicillinase resistant Nafcillin		poor	IV, IM
Oxacillin		poor	IV, IM
Dicloxacillin		good	PO
4. Extended spectrum Carbenicillin (indanyl)		OK	PO
Ticarcillin		poor	IV, IM
Piperacillin		poor	IV, IM

TABLE 2 - Antimicrobial Spectrum of Penicillins

Penicillin Type	Staph β -lactamase (penicillinase) resistance	Gram(-) β -lactamase resistance	spectrum	use
Penicillin G	no	no	Gm(+) and some neisseria	IV
Penicillin V	no	no	" "	mild GM(=) infections e.g. strep throat
Ampicillin	no	no	Gm(+) and some gm(-)	IV, IM
Amoxicillin	no	no	" "	oral use for mild infections
Nafcillin	yes	no	staph	IV, IM
Oxacillin	yes	no	"	IV, IM
Dicloxacillin	yes	no	"	PO
Carbenicillin (indanyl)	no	\pm	mild UTI involving pseudomonas	PO
Ticarcillin	no	\pm	Pseudomonas* and also some Enterobacteriaceae	IV, IM
Piperacillin	no	better than ticar	Pseudomonas* and also some Enterobacteriaceae	IV, IM

*Pseudomonas often elaborate group 1 beta lactamases (cephalosporinase) which will not hydrolyze ticar- and piperacillin (i.e. ticar and pip can be effective for pseudomonas infections).

TABLE 3 - Combination Products with β -lactamase inhibitors

	resistance to Staph β -lactamase	resistance to Gram(-) β -lactamase *	spectrum	use
amoxicillin/clavulanic acid (Agumentin®)	yes	yes	broad	PO
ampicillin/sulbactam (Unisyn®)	yes	yes	broad	IM, IV
ticarcillin/clavulonic acid (Timentin®)	yes	yes	broad	IM, IV
piperacillin/tazobactam (Zosyn®)	yes	yes	broad	IM, IV

*note: the type 1 β -lactamase of Pseudomonas is not inhibited by the available β -lactamase inhibitors but ticar and pip are not usually hydrolyzed so they can be used.

5. More detail on penicillins on UW formulary

a) Penicillin G - (mainly IV use)

1) spectrum

a. gram-positive cocci

- i. *Strep. pyogenes* (Group A, beta hemolytic strep) causes strep throat, scarlet fever, erysipelas and necrotizing fasciitis. *S. pyogenes* generally sens. - usually don't even test.
- ii. *Streptococcus pneumoniae* - now resistance to penicillins is a problem, resistance is due to altered PBPs. (UW = ~ 20% resistant)
The most common agent in otitis media (middle ear infection) (~ 35%) and community acquired pneumonia. (~ 50-80%)
A very important pathogen.
- iii. *Anaerobic strep.* - sensitive
- iv. *S. viridans* - usually sens.
- v. *Enterococcus faecalis*
Enterococcus faecium } often resistant and need heroic treatments
(*E. faecium* is the most difficult to treat. At UW most strains are resistant to pen G and vanco.)
- vi. Staph - almost all are now resistant

b. gram-positive rods

- i. Clostridium - usually sens.
e.g. *C. tetani*, *C. perfringens* but *C. difficile* is resistant

c. gram-negative cocci

- i. *Neisseria meningitidis* - often sensitive
- ii. *Neisseria gonorrhoeae* - no longer agent of choice for this infection due to acquisition of plasmids coding for TEM-1 beta lactamases.

d. gram-negative rods - mostly resistant

- i. Enterobacteriaceae - *E. Coli*, *Salmonella*, *Shigella*, *Serratia*, *Enterobacter*, *Klebsiella*, *Proteus* - all resistant
- ii. Pseudomonas - resistant
- iii. Hemophilus - resistant

e. *Treponema pallidum* - sens.

2) Indications - penicillin G (IV)

- a. meningococcal meningitis and septicemia
- b. clostridial infections (not *C. difficile*)
- c. listeria infections
- d. serious streptococcal infections (but strep pneumoniae can be resistant)
- e. neurosyphilis

b) Procaine Pen G

- 1) equimolar mix of Pen G and Procaine = insol salt.
- 2) peak blood levels in about 2 h. and persist for about 24 h.
- 3) use: gonorrhea, 4.8 MU 1 M plus 1 g Probenecid (this is now not a regimen of first choice due to increased resistance)

c) Benzathine Pen G

- 1) N-N-dibenzylethylenediamine pen G; longest acting of all penicillins
- 2) levels persist for 14-20 days but are low ∴ not good for gonorrhea but OK for syphilis
- 3) good for rheumatic fever prophylaxis and strep. throat treatment

d) Pen V (PO use) - 60% absorbed, acid stable, but food still interferes with absorption somewhat ∴ best given 1/2h ac or 2-3h pc

use: mild infections, especially “strep throat;” not useful for gonorrhea, Neisseria, or syphilis

indications: for mild to moderate URI caused by strep, for scarlet fever, for erysipelas, for strep pyogenes (strep throat)

patient counseling:

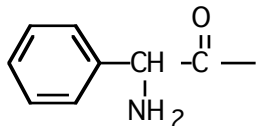
- take complete course of therapy
- use nonhormonal contraceptive if taking OC (??evidence for problem)
- best taken on an empty stomach, 1h ac or 2h pc but there is not uniform agreement on this need
- caution on allergy
- caution on diarrhea; consider use of probiotic
- if diarrhea persists, contact prescriber

e) Penicillinase - resistant penicillins -- semisynthetic

- 1) nafcillin and dicloxacillin
 - a. should be reserved for Staph infections
 - b. dicloxacillin is the PO drug of choice here
 - c. methicillin resistant staph aureus (MRSA) are resistant due to altered penicillin binding proteins (PBPs). Hospital MRSA will be resistant to all beta lactams; vancomycin will be needed or other combination; community strains will often respond to trimethoprim-sulfa PO or Xyvox PO can be used.
 - d. counseling – see Pen V but need for taking on an empty stomach is better defined

6. Amino Penicillins

a) Ampicillin (mainly used IV now)



- 1) A minor chemical modification → broader spectrum; still P'ase sens.; penetrates porins in gram-negative bacteria because it is a relatively polar molecule
- 2) Spectrum
 - a. Gram-positive - similar to Pen G but sl. less active. Exception = *Enterococcus faecalis* which is generally more sensitive to ampicillin, nevertheless is often used in combination with an aminoglycoside for *E. faecalis*
 - b. Gram-negative - active here where Pen G and Pen V are not - these agents penetrate Gm (-) cell wall. For example, the following are often sensitive:

E. coli - causes >90% of all acute, uncomplicated urinary tract infections - resistance is on the increase due to β -lactamase production

Proteus mirabilis - causes 60-70% of all proteus infections - is urinary tract invader

Proteus vulgaris, *Morganella morganii*, *Providencia sp.* and *Citrobacter* are resistant to ampicillin and are more difficult to treat

Salmonella - typhoid, gastroenteritis, bacteremia (resistance to ampicillin is \uparrow)

Shigella - alternate to TMP-SMX

Neisseria meningitidis sens. unless penicillinase producer

Neisseria gonorrhoeae 3.5 g stat dose is convenient, but effectiveness is \downarrow due to penicillinase producers. No longer agent of first choice here.

H. influenza - common cause of otitis media in children, and bacterial meningitis in children, other = pneumonia, etc., epiglottitis; effectiveness of ampicillin is decreasing due to β -lactamases

Not effective for most other Gm (-) pathogens, e.g.

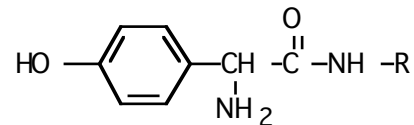
- Pseudomonas - sepsis, particularly a problem with burn and immunosuppressed patients; UTI
- Enterobacter - occasional cause of UTI and sepsis; difficult to treat
- Klebsiella - pneumonia, UTI
- Serratia - pneumonia, UTI, sepsis, endocarditis

- 3) Distribution - similar to Pen G, but larger amount secreted via bile and is conc. in intestine \therefore have incidence of diarrhea and problems with super infection in G.I. tract. (Note: high gut concentrations may be an advantage for sensitive Shigella, Salmonella infections.)
- 4) Adverse effects - secreted in bile and diarrhea and GI overgrowth with Candida is a problem; risk of pseudomembranous colitis with ampicillin and all β -lactams having a broad spectrum.
- 5) Allergy - 9-10% get some type of allergic reaction.

Question - Does reaction to ampi. mean allergic to penicillin?

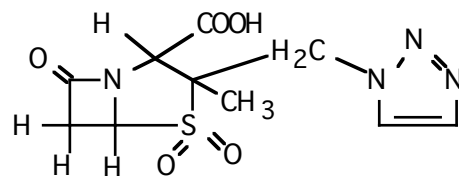
Answer - maybe not but is difficult to distinguish true Pen. allergy from ampicillin allergy.

b) Amoxicillin



- 1) oral only
- 2) better oral drug than ampicillin - \uparrow T_{1/2}, \downarrow flow in bile \therefore less diarrhea, food doesn't affect absorption much
- 3) TID dosing or high dose BID
- 4) not as good for gut infections as ampicillin, e.g. *Shigellosis*
- 5) indications
 - a. ear, nose, throat - *Strep. pneumoniae*, *Strep. pyogenes*, *H. flu* (res. is problem)
 - b. genitourinary - *E. coli*, *Proteus*, *Enterococcus faecalis*
 - c. soft tissue - *Strep.*, *H. flu* (resistance is a problem)
 - d. prophylaxis of endocarditis in dental patients
 - e. still the best selling antimicrobial

- c. combination of these anti pseudomonas penicillins and clavulanic acid or tazobactam (e.g. Timentin or Zosyn) may be useful for mixed infections involving Pseudomonas and one or more plasmid mediated beta lactamase producing pathogens.
 - d. these agents will still be inactivated by penicillinases and some plasmid mediated beta lactamases unless an inhibition is present
- 2) Ticarcillin plus clavulanic acid, Timentin ®. This is better for mixed infections involving Pseudomonas.
- Indications:
- a. septicemia – β -lactamase producing Klebsiella, Staph., Strep., Pseudomonas
 - b. lower respiratory – β -lactamase producing Staph., H. flu., Klebsiella
 - c. bone – Staph.
 - d. skin – Klebsiella, E. coli, Staph.
 - e. UTI – E. coli, Klebsiella, Pseudomonas, Citrobacter, Enterobacter, Senatia, Staph.
 - f. genitourinary – Enterobacter, Staph., E. coli, Staph. epi
 - g. intra abdominal – Bacteriodes, E. coli, Klebsiella
 - h. Pseudomonas – in combination with aminoglycoside
- 3) Piperacillin plus Tazobactam Zosyn ® Lederle
- a. more potent against Gm (-) bacteria than Ticarcillin. Active against Klebsiella.
 - b. may be more potent against Pseudomonas
 - c. dose 4-20 g/day IV
 - d. used for mixed infections, presumptive therapy, and for Pseudomonas infections and mixed infections involving Pseudomonas.
 - e. bile and renal excretion, therefore no dose adjustment needed in renal failure



Tazobactam

- 4) Carbenicillin indanyl ester (Geocillin®)– orally absorbed and used for mild Pseudomonas UTI.