Antibiotics 101 for Laboratory Professionals: Part One

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OUTLINE

I. Trying to understand the choice
II. Selected classes of antimicrobials
III. Bacterium-specific examples of resistance
   A. *Streptococcus pneumoniae*
   B.
   C.

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"D#*%it, Jim,
I'm not a physician."

3

…including myself

4

Trying to Understand the Choice

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EARLY EVIDENCE FOR AST

- *Staphylococcus aureus* bacteremia
- Favorable outcome in 60% of patients treated with a drug that inhibited the organism *in vitro*
- No patients responded clinically when treated with a drug that did not inhibit the organism *in vitro*

Antibiot. Ann. **1958-1959:** 748-756; 1959

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NOWADAYS: 90-60 Rule

- Infections due to "susceptible" isolates respond to therapy ~90% of time
- Infections due to "resistant" isolates respond to therapy ~60% of time

Clin. Infect. Dis. **35:** 982-989; 2002

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Laboratory utilizes *in vitro* testing systems to PREDICT antimicrobial effectiveness *in vivo*, independent of confounding factors

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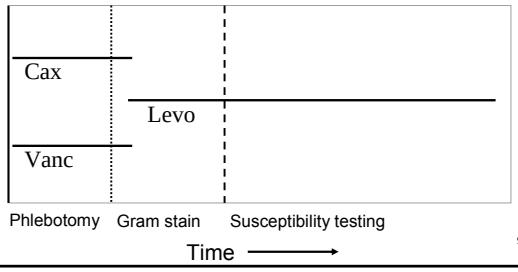
FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)



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INITIATION OF THERAPY

Interval	Cumulative Percentage of Events Occurring Within:	
	6 hours	12 hours
Phlebotomy to Gram stain	63.8†	81.4*
Gram stain to susceptibility testing	45.0†	61.1*
After release of susceptibility results	10.0†	17.4*

† $P < 0.001$; * $P < 0.05$

J. Clin. Microbiol. **41:** 495-497; 2003

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FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability

Cannot Enter Urinary Tract

macrolides
clindamycin
chloramphenicol

Cannot Enter CNS

fluoroquinolones
1st & 2nd generation cephems
clindamycin
macrolides
tetracycline

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FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration

Administration		Example
Medical Lingo	*Colloquial*	
IM	butt	ceftriaxone (also IV)
PO	oral	cephalexin
PO or parenteral	oral or IV	levofloxacin
parenteral	IV	vancomycin

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FACTORS TO CONSIDER

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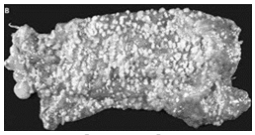
parenteral	IV	vancomycin PO



Pseudomembranous colitis caused by *Clostridium difficile*

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FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion

Fluoroquinolone	Percentage Excretion	
	Renal	Biliary
levofloxacin	+++	-
ciprofloxacin	+++	+++++

Salmonella spp. report
ampicillin
trimethoprim-sulfa
ciprofloxacin

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FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion
- Dosing/half-life

easier for patient
reduced pharmacy co$t

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FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion
- Dosing/half-life
- Synergy

β-lactam/aminoglycoside
rifampin

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FACTORS TO CONSIDER

- Antimicrobial susceptibility testing (AST)
- Spectrum of therapy (empiric therapy)
- Availability
- Route of administration
- Majority of excretion
- Dosing/half-life
- Synergy
- Side effects

hypersensitivity
hematologic
gastrointestinal
renal
otic
pregnancy

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MORE FACTORS TO CONSIDER

- FDA indications

Acute bacterial sinusitis due to *S pneumoniae*, *H influenzae*, or *M catarrhalis*

Community-acquired pneumonia due to methicillin-susceptible *S aureus*, *S pneumoniae* (including multidrug-resistant *S pneumoniae* [MDRSP]), *H influenzae*, *H parainfluenzae*, *K pneumoniae*, *M catarrhalis*, *C pneumoniae*, *L pneumophila*, or *M pneumoniae*. MDRSP isolates are strains resistant to two or more of the following antibacterials: penicillin (MIC ≥ 2 μg/mL), 2nd generation cephalosporins, eg, cefuroxime, macrolides, tetracyclines, and trimethoprim/sulfamethoxazole

LEVAQUIN® (levofloxacin) Tablets/Injection Product Insert (excerpt)

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MORE FACTORS TO CONSIDER

- FDA indications
- Co$t
- Polymicrobial infections
- Cidal vs. static

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Selected Classes of Antimicrobials

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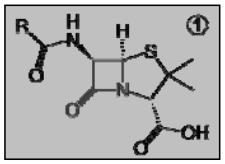
TWO BASIC SUBDIVISIONS

- β-lactam
- Non-β-lactam



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TWO BASIC SUBDIVISIONS

- β-lactam
  - Penicillins — **Cell wall synthesis**
- Non-β-lactam
  - Macrolides — **Protein synthesis**
  - Tetracycline
  - Folate inhibitors — **DNA replication**
  - Fluoroquinolones

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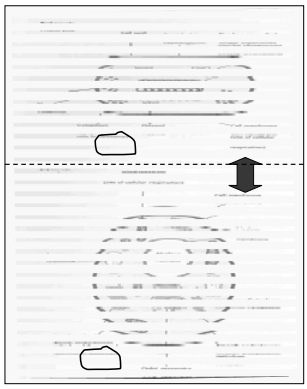

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PENICILLIN CLASS

Subclass (if appropriate)	Agent(s)
penicillin	penicillin
aminopenicillin	amoxicillin
	ampicillin
carboxypenicillin	carbenicillin
	ticarcillin
ureidopenicillin	piperacillin
penicillinase-stable penicillins	dicloxacillin
	methicillin
	nafcillin
	oxacillin

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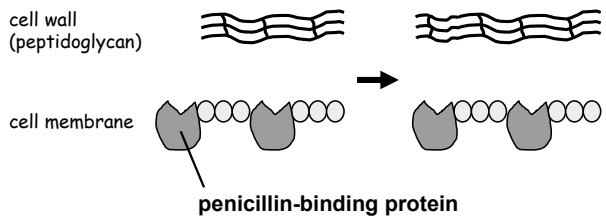
CELL WALL SYNTHESIS



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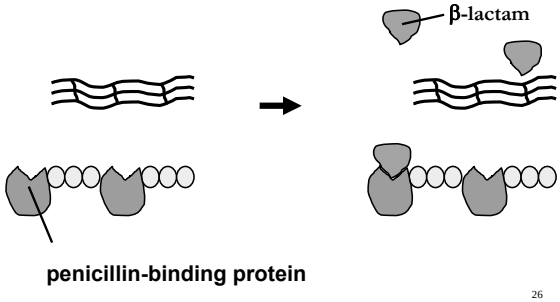
CELL WALL SYNTHESIS



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PENICILLIN CLASS

Parameter	Description
Mechanism of action	1. Bind to bacterial penicillin-binding proteins (PBP), interfering with cell wall synthesis 2. Can trigger membrane-associated autolytic enzymes that destroy cell wall
Activity rendered	Cidal
Route of administration	PO or IV; amoxicillin vs. ampicillin
Distribution	Well; CNS penetration
Half-life	0.5 to 1.5 hours → q4h or q6h
Excretion	Mostly renal; ampicillin with great biliary
Adverse effects	Allergic skin rash, drug fever, diarrhea, severe anaphylaxis is rare

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PENICILLIN CLASS

Parameter	Description
Spectrum of activity	Penicillins: streptococci, anaerobes, *Neisseria*, agent of syphilis Aminopenicillins: (similar to penicillin PLUS) *Listeria*, enterococci, *Haemophilus*, some enteric GNR Carboxypenicillins: better enteric GNR coverage, some *Pseudomonas aeruginosa*, anaerobes Ureidopenicillins: even better enteric GNR coverage, better *Pseudomonas aeruginosa*, anaerobes Penicillinase-stable penicillins: Staph w/o *mecA*
Interesting stuff	Otitis media (stay tuned)

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MACROLIDE CLASS

Subclass (if appropriate)	Agent(s)
NONE	erythromycin
	azithromycin
	clarithromycin

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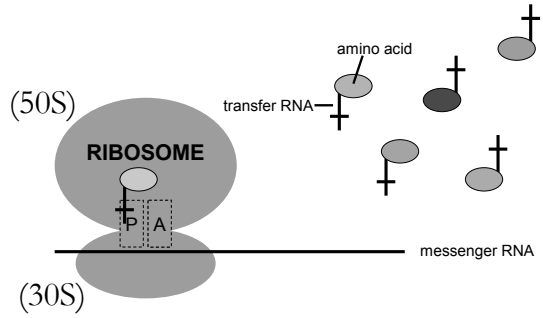
PROTEIN SYNTHESIS



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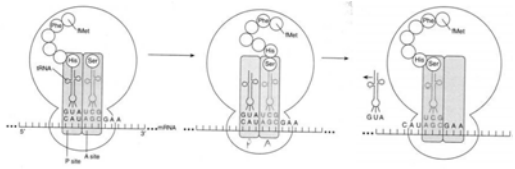
MACROLIDE CLASS



- Reversible binding to 50S ribosomal subunit
- Blocks translocation reaction of peptide elongation (no A to P)

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MACROLIDE CLASS

Parameter	Description
Mechanism of action	Bind reversibly to 50S ribosomal subunits, blocking the translocation reaction of polypeptide chain elongation
Activity rendered	Static
Route of administration	PO or IV
Distribution	Well, especially tissue and intracellular; no CNS
Half-life	1.5-48 hours; azithromycin 2-4 days in tissue
Excretion	Renal and biliary
Adverse effects	Nausea, vomit, diarrhea, hypersensitivity; reversible hearing loss with high dose + renal insufficiency

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MACROLIDE CLASS

Parameter	Description
Spectrum of activity	Mostly Gram positive; anaerobes Atypical pneumonia: *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila* URT: *S. pneumoniae*, *Bordetella pertussis*, *H. influenzae*, *Moraxella catarrhalis* STD: *C. trachomatis*, *N. gonorrhoeae*, syphilis
Interesting stuff	~50% resistance rate in β-hemolytic streptococci; emerging problem in penicillin-allergic patients (moms with group B)

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TETRACYCLINE CLASS

Subclass (if appropriate)	Agent(s)
NONE	tetracycline
	doxycycline
	minocycline

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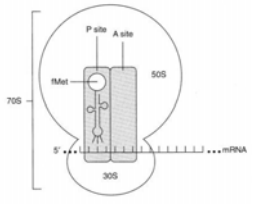
TETRACYCLINE MECHANISM

- Energy-dependent entry
- Inhibit attachment of aminoacyl-tRNA to ribosome acceptor site



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TETRACYCLINE CLASS

Parameter	Description
Mechanism of action	Bind reversibly to 30S ribosomal subunit, preventing attachment of aminoacyl~tRNA to the A site in the RNA-ribosome complex; energy-dependent penetration of cell membrane
Activity rendered	Static
Route of administration	PO or IV
Distribution	Well, especially in tissue and human milk; no CNS
Half-life	8-22 hours → q6h or q12h
Excretion	Renal and biliary
Adverse effects	Nausea, vomit, esophageal ulcerations; permanent tooth discoloration when given during development

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TETRACYCLINE CLASS

Parameter	Description
Spectrum of activity	Broad spectrum (including anaerobes and MRSA) Tick drug: *Rickettsia* spp. *Borrelia burgdorferi* *Coxiella burnetii* typhus Skin & soft tissue infections; intraabdominal infections STD and pelvic inflammatory disease
Interesting stuff	Absorption improved in fasting state (antacids, food impair absorption); avoided in pregnancy & in kids under 8

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FOLATE PATHWAY INHIBITORS

Subclass (if appropriate)	Agent(s)
NONE	sulfonamides
	trimethoprim
	trimethoprim-sulfamethoxazole

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FOLATE SYNTHESIS

- Overall goal: pyrimidine synthesis (DNA)
- Two-step process

para-aminobenzoic acid $\xrightarrow{\textbf{DHPS}}$ dihydrofolate

dihydrofolate $\xrightarrow{\textbf{DHFR}}$ tetrahydrofolate

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FOLATE PATHWAY INHIBITORS

Parameter	Description
Mechanism of action	Sulfonamides: competitive inhibition of PABA conversion into dihydrofolate Trimethoprim: inhibition of dihydrofolate reductase (DHFR)
Activity rendered	Cidal
Route of administration	PO or IV (for trimethoprim-sulfamethoxazole)
Distribution	Well; CNS penetration
Half-life	10-12 hours → q6h to q12h
Excretion	Renal
Adverse effects	(more commonly due to sulfonamide component) Mild GI, allergic skin rash (3%); hematopoietic changes

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FOLATE PATHWAY INHIBITORS

Parameter	Description
Spectrum of activity	Broad spectrum (except *Pseudomonas aeruginosa*) UTI etiologies except *Enterococcus* spp. Enteric pathogens Acute otitis media, sinusitis, acute bronchitis, pneumonia (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*)
Interesting stuff	Fungal therapy & prophylaxis *(Pneumocystis carinii*) AIDS patients have higher frequency of adverse reactions (70%)

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QUINOLONE CLASS

Subclass (if appropriate)	Agent(s)
quinolone	nalidixic acid
fluoroquinolone	ofloxacin
	norfloxacin
	ciprofloxacin
	levofloxacin
	moxifloxacin
	gatifloxacin
	trovafloxacin
	temafloxacin, grepafloxacin

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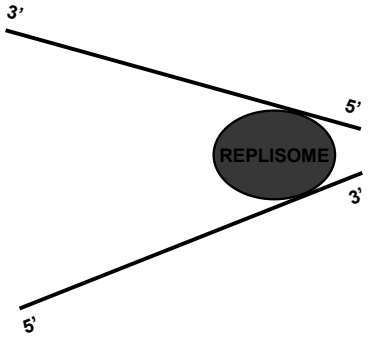
DNA REPLICATION



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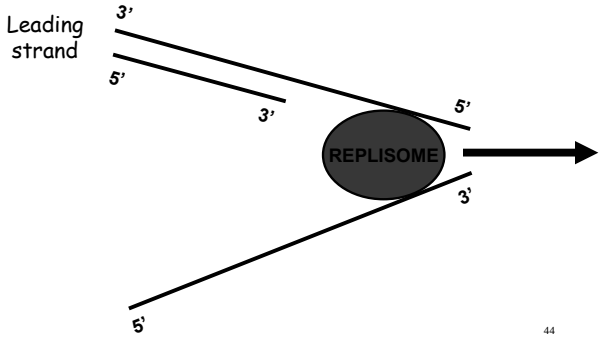
DNA REPLICATION



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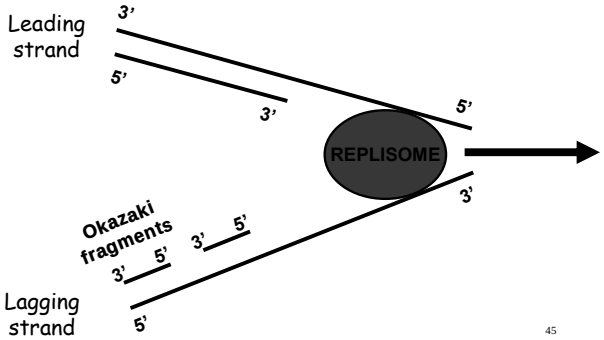
DNA REPLICATION



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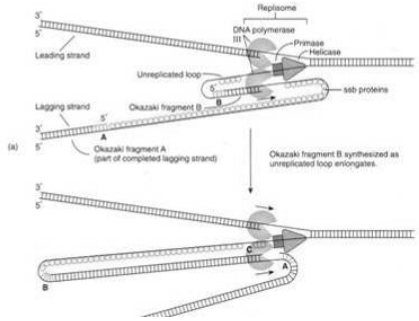
DNA REPLICATION



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RELAXING/RECOVERY ENZYMES

- DNA topoisomerase IV
  - *parC* ⟶ Two C subunits
  - *parE* ⟶ Two E subunits
- DNA gyrase
  - *gyrA* ⟶ Two GyrA subunits
  - *gyrB* ⟶ Two GyrB subunits

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QUINOLONE CLASS

Parameter	Description
Mechanism of action	1. Binds DNA gyrase (primarily in Gram negatives) 2. Newer agents bind DNA topoisomerase (primarily in Gram positives)
Activity rendered	Cidal
Route of administration	PO or IV
Distribution	Well; levo, gati, moxi CNS; also intracellular delivery
Half-life	3.5 to 20 hours → q12h or q24h
Excretion	Renal; ciprofloxacin also with strong biliary excretion
Adverse effects	Growth plate development; cipro: tendonitis, rupture Nausea, vomit, diarrhea, pseudomembranous colitis?

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QUINOLONE CLASS

Parameter	Description
Spectrum of activity	Gram positive and Gram negative coverage--losing activity versus MRSA Otitis media agents *Neisseria gonorrhoeae*, *Chlamydia trachomatis* ciprofloxacin: enteric pathogens, *P. aeruginosa* levofloxacin, moxifloxacin: *S. pneumoniae* gatifloxacin, moxifloxacin: Anaerobes Some activity versus *Mycobacterium* spp.
Interesting stuff	Losing them versus common enteric GNR Canadian studies predict loss versus *S. pneumoniae*

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Mechanisms of Resistance

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BACTERIUM-MEDIATED RESISTANCE

- Altered target
- Enzymatic inactivation
- Diminished penetration
- Efflux
- Altered physiology

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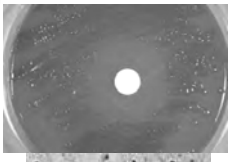
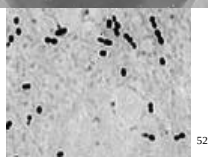
Streptococcus pneumoniae

- Community-acquired pneumonia
- Bacteremia
- Otitis media
- Sinusitis
- Meningitis
- Endocarditis





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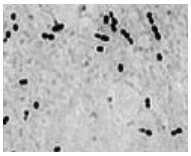
PENICILLIN SUSCEPTIBILITY

Year	% Susceptible
2002	75.9
2003	80.2
2004	74.4
2005	71.0
2006	73.5
2007	62.2



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PENICILLIN vs. *S. pneumoniae*

Specimen Source	% Intermediate	% Resistant
Upper respiratory tract	14.1	31.3
Lower respiratory tract	14.0	20.9
Blood	10.7	14.9
CSF/sterile fluid	6.1	24.2

Age (years)	% Intermediate	% Resistant
0-5	13.7	28.9
6-20	10.3	25.3
21-64	11.2	18.8
65 or older	14.2	15.9

Antimicrob. Agents. Chemother. **45:** 1721-1729; 2001 54

Direct Detection of Bacterial Biofilms on the Middle-Ear Mucosa of Children With Chronic Otitis Media

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BIOFILM FREQUENCY

- 93% of ears with recurrent otitis media
- 91% of ears with otitis media with effusion

JAMA. **296:** 202-211; 2006

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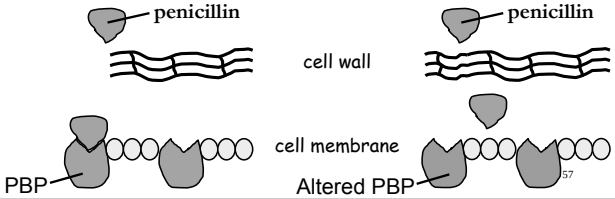
RECOMBINATION W/ FOREIGN DNA

- PBP of less-susceptible species recombine with native, susceptible species
- Transformation: uptake of "naked" DNA



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NON-β-LACTAM ANTIMICROBIALS

Year	Percentage Susceptible		
	Erythromycin	Tetracycline	Trimethoprim-sulfamethoxazole
2004	75	81	80
2005	69	83	73
2006	80	85	76
2007	58	76	63

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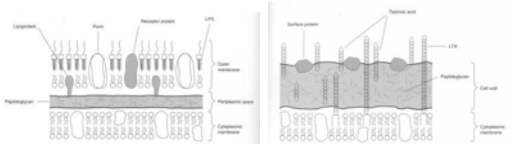
MACROLIDE RESISTANCE

- Size matters



Gram negative **Gram positive**

- Methylation of ribosome

  ermA → erythromycin ribosomal methylase

- Expression of efflux pumps

  Resistance to macrolides, not clindamycin

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TETRACYCLINE RESISTANCE

- Efflux mechanism by which bacterium exchanges tetracycline-cation complex for a proton
- Ribosome protection proteins

  Bind to ribosome
  Change ribosome configuration
  Inhibit binding of tetracycline

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TRIMETH-SULFA RESISTANCE

- Intrinsic resistance

  Due to permeability barrier or active efflux, decreased access to target enzymes that assist in manufacture of tetrahydrofolate

  Low affinity for organism-specific target enzymes that assist in manufacture of tetrahydrofolate

  Bacterium with ability to absorb exogenous folate or thymine

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TRIMETH-SULFA RESISTANCE

- Intrinsic resistance
- Acquired resistance trimethoprim

  Promoter mutations → enzyme overproduction

  Point mutations → low affinity of enzyme for drug

- Acquired resistance sulfonamides

  Point mutations → low affinity of enzyme for drug

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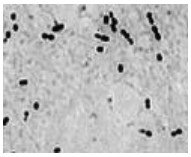
LEVOFLOXACIN SUSCEPTIBILITY

Year	% Susceptible
2002	97.2
2003	100.0
2004	99.5
2005	100.0
2006	97.3
2007	99.3



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LEVOFLOXACIN vs. *S. pneumoniae*

Antimicrobial Susceptibility Breakpoints and First-Step *parC* Mutations in *Streptococcus pneumoniae*: Redefining Fluoroquinolone Resistance

Sue Lim,*† Darrin Bast,*† Allison McGeer,*† Joyce de Azavedo,*† and Donald E. Low*†

Emerg. Infect. Dis. **9:** 833-837; 2003

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METHODS

- Clinical MIC breakpoints (CLSI)

  Levofloxacin: ≤ 2 susceptible
  4 intermediate
  ≥ 8 resistant

- Micro/molecular MIC breakpoints

  Sequenced *parC*, *gyrA*

Emerg. Infect. Dis. **9:** 833-837; 2003

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ROLE OF *parC* AND *gyrA*

Table 2. Number of isolates with ParC and GyrA amino acid substitutions and their corresponding levofloxacin MICs

MIC (μg/mL)	No. strains with amino acid substitutions in ParC (%)	ParC and GyrA (%)
8	0/10 (0)	10/10 (100)
≥16	0/15 (0)	15/15 (100)

*29/82 isolates were randomly examined for GyrA mutations.

Emerg. Infect. Dis. **9:** 833-837; 2003

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FREQUENCY OF *parC*

Table 1. ParC amino acid substitutions found in 115 *Streptococcus pneumoniae* isolates with levofloxacin MICs ≥2 µg/mL and corresponding levofloxacin MICs

ParC amino acid substitution	No. isolates inhibited by levofloxacin MIC (µg/mL) of					Total no. of strains
	2	4	8	16	≥32	
Ser79→Phe	28	4	3	9	3	47
Ser79→Tyr	7	1	3	2	1	14
Ser79→Ala	1	0	0	0	0	1
Asp83→Asn	7	0	3	0	0	10
Asp83→Gly	1	0	0	0	0	1
Asp83→Tyr	3	0	0a	0	0	3
Asp83→Val	1	0	0	0	0	1
Asp83→Ala	0	0	1	0	0	1
No. isolates/total with amino acid substitutions	48/82 (59%)	5/8* (63%)	10/10	11/11	4/4	78/115 (69%)

*One isolate with no ParC amino acid substitution found to have active efflux; two isolates had ParC amino acid substitutions at sites other than Ser79 or Asp83.

Emerg. Infect. Dis. **9:** 833-837; 2003

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CONCLUSIONS

- *parC* is first-step mutation in reducing fluoroquinolone susceptibility
- MIC breakpoints do not always predict levofloxacin resistance

Emerg. Infect. Dis. **9:** 833-837; 2003

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Why is this important?

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CLINICAL FAILURE

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

N. Engl. J. Med. **346:** 747-750; 2002

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FLUOROQUINOLONE RESISTANCE

- Spain: 5.3%

  Antimicrob. Agents. Chemother. **44:** 3481-3482; 2000

- Hong Kong: 12.1%

  Antimicrob. Agents. Chemother. **43:** 1310-1313; 1999

- Northern Ireland: 15.2%

  J. Antimicrob. Chemother. **41:** 420-421; 1998

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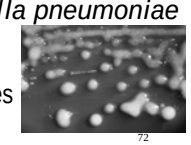
THE END

- Next time (Part Two): *Staphylococcus aureus*

  penicillin
  oxacillin/nafcillin
  vancomycin
  clindamycin
  synercid
  linezolid

Klebsiella pneumoniae

  cephems
  monobactams
  β-lactamase inhibitors
  carbapenems
  aminoglycosides
  polymyxins



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