RESISTANT BACTERIA
and
ANTIBIOTICS

INTRODUCTION
- THE BACTERIAL CELL
- ANTIBIOTICS
- HOW BACTERIA EX-CHANGE DNA
- MECHANISMS OF Ab RESISTANCE
- RESISTANCE TO SPECIFIC Ab
- FUNGAL INFECTIONS
- CONCLUSIONS

BACTERIA and HUMANS
- Before birth
  - Normally no contact with bacteria
- After birth
  - Bacteria as commensals from persons and environment
  - All parts of the body which are in contact with the environment
    - are permanently colonized by bacteria
    - not harmful under normal conditions
  - On incidental penetration of bacteria in tissues,
    - the body reacts by a combined action of
      - blood clotting
      - circulating antibodies
      - fagocytosis

BACTERIA and HUMANS
- Opportunistic pathogens
  - Cannot infect under normal conditions (no penetration into tissues, cannot
    fix to the host cells, cannot multiply, cannot produce toxins…
    - e.g. E. coli )
  - Infect when
    - The host resistance mechanism has been weakened by medication, malnutrition ...
    - Local disruption of the epithelial barriers of the skin and epithelial mucous
      secreting tissues = wounds
- Pathogens are harmful
  - Production of poisonous products
  - Destruction of host cells
  - Excessive growth and depletion of energy reserves of host

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DEVOS Ann 22/02/2005
Mycobacterium tuberculosis are becoming increasingly resistant to antibiotics.

Salmonella enteritidis is a related enteric bacterium related to E. coli.

Endospores of Clostridium and Bacillus species are becoming increasingly resistant to antibiotics.

Shigella is a non-motile enteric bacterium related to E. coli.

E. coli hemolytic type and E. coli hemorrhagic type are becoming increasingly resistant to antibiotics.

Characterization of Bacteria

By shape

- Bacillus anthracis
- Bordetella holmessii
- Clostridium tetanii
- Enterococcus faecalis
- E. coli en
- E. coli hemorrhagic type
- Haemophilus influenzae
- Listeria monocytogenes
- Neisseria meningitidis
- Spirulina spp.
- Staphylococcus aureus
- Streptococcus faecalis
- Strept. pneumoniae
- Salmonella typhii
- Salmonella enteritidis
- Vibrio cholerae

Characterization of Bacteria

By Gram-staining (Hans Christian Gram)

Based on a chemical reaction with the cell wall of bacteria.

Microscopic image of Bacillus anthracis (ATCC 23456), Gram staining, magnification 1,000.

Microscopic image of Staphylococcus aureus (ATCC 25923), Gram staining, magnification 1,000.
Gram +ve and Gram -ve bacteria

Electron Micrograph of a Gram-Positive Cell Wall
Electron Micrograph of a Gram-Negative Cell Wall

Structure of a Gram-Positive Cell Wall
Structure of a Gram-Negative Cell Wall

Transcription and Translation of the insulin protein

1. The DNA coded information (blue helix) in the cell nucleus is copied, or transcribed, to an RNA mirror image (red strand).
2. The ribosome translates the linear pattern described in the RNA to construct a protein strand. The translation is based on the genetic code. Transfer RNAs (cross-like shapes) ferry amino acids to the growing protein chain based on the 3-codon RNA sequence.
3. Finally, the newly made protein strand folds itself into its active form.

RIBOSOMES

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History of Antibiotics

Time line of events

1900
1928, Penicillin discovered
1932, Sulfonamides discovered
1935, Streptomycin discovered
1940, Penicillin becomes commercially available
1941, Erythromycin discovered
1945, Vancomycin introduced
1947, Lincomycin becomes available
1950

1940’s Mass penicillin production
HISTORY OF ANTIBIOTIC RESISTANCE

<table>
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<tr>
<th>Antibiotic</th>
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<th>Used clinically</th>
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INCREASE IN ANTIBIOTIC RESISTANCE

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Penicillin Resistance in the US

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Resistance against fluoroquinolones in Hawaiian population

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ANTIBIOTICS: General Concepts

- Selectivity/Toxicity:
  - Toxicity levels for bacteria ≠ from that of host
  - High doses w/toxic effects
  - No antibiotic is completely safe!
- Antibiotics must survive their route of administration
- Broad and narrow spectrum drugs
- Categories of Aβ:
  - Bactericidal: usually Aβ of choice
  - Bacteriostatic: duration of treatment sufficient for host defences
- Combinations:
  - Synergism
  - Antagonism
  - Indifference

ANTIBIOTICS: General Concepts

- Antibiotic susceptibility testing (in vitro)
  - Minimum inhibitory concentration (MIC): Lowest concentration that results in inhibition of visible growth
  - Minimum bactericidal concentration (MBC): Lowest concentration that kills 99.9% of the original inoculum
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ANTIBIOTIC RESISTANCE

• INTRINSIC RESISTANCE
  • Inherent features of bacterial species which prevent antibiotic action
  • Usually expressed by chromosomal genes

• ACQUIRED RESISTANCE
  • MUTATIONS in chromosomal genes
    • The spontaneous mutational frequency is 10^-7
  • TRANSFORMATION
  • CONJUGATION
  • TRANSDUCTION

• MUTATIONS in chromosomal genes
  • The spontaneous mutational frequency is 10^-7
  • Drug-resistant tuberculosis arises this way

• TRANSFORMATION
• CONJUGATION
• TRANSDUCTION
**CONJUGATION**

Bacteria are capable of transferring genes to or acquiring genes from bacteria of a different species or genus.

- Is the transfer of one or more genes from one bacterium to another in a single process that can take as little as an hour to complete.
- Also been called bacterial sex or horizontal gene transfer.
- Allows bacteria to become resistant to antibiotics by acquiring DNA from a bacterium that already has acquired resistance to the antibiotic.
**PLASMIDS**

- Extrachromosomal genetic elements that replicate independently
- Plasmids are mobile by conjugation
- Frequently carry Ab resistance genes
  - Up to 7 different resistance genes on one plasmid
- In the absence of Ab, the plasmid is often lost from the majority of cells
  - Cost of carrying a resistance gene
  - Exposure of the Ab results in all cells having the plasmid(s)
  - Sensitive cells are killed and plasmids are mobilized

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**Conjugation**

- **F-plasmid**
- **F+ bacteria**
- **Mating Bridge**
- **F-bacteria**

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**Range of resistance exchange**

- **Gram +ve Bacteria**
- **YEAST**
- **Non-Conjugative**
- **Conjugative Gram -ve bacteria**
- **Gram +ve Bacteria**
- **Conjugative Gram -ve bacteria**

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References:
TRANSPOSABLE GENETIC ELEMENTS

- Mobile DNA sequences which can move from one location on the DNA molecule onto another molecule
- Not capable of self-replication (no replicon)
- Transposition is mediated by site-specific recombination, mediated by a transposase
- Classified as TransposoN Tn551, Tn4291 etc.
- Central region of transposon often carries Ab resistance gene(s)
  Results in a spread of antibiotic resistance

Transposable Genetic Elements

- Carry genes except those involved in transposition
- Structure:
  - Tn551
  - Tn4291

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  - Transposons or Transposable elements
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BACTERIOPHAGE

- INFECTION
- DESTRUCTION OF THE BACTERIAL DNA
- REPLICATION OF THE VIRAL GENOME
- PRODUCTION OF VIRAL PARTS
- PACKAGING
- LYSIS

Transduction

- INFECTION
- DESTRUCTION OF THE BACTERIAL DNA
- REPLICATION OF THE VIRAL GENOME
- PRODUCTION OF VIRAL PARTS
- PACKAGING
- LYSIS
**GENETIC DETERMINANTS**

- **Intrinsic resistance**
  - Resistance determined by chromosomal genes
  - E.g. Beta-lactamases of gram-ve bacteria inactivate beta-lactam antibiotics

- **Acquired resistance**
  - Resistance determined by chromosomal genes
  - E.g. Nalidixic acid resistance in E.coli is caused by the mutation at the N-terminus of the GyrA protein which reduces nalidixic acid binding

**ACQUIRED DETERMINANTS**

- Resistance determined by plasmids
  - Extrachromosomal genetic elements that replicate independently of the chromosome

- Resistance determined by transposons
  - Mobile genetic elements capable of transferring (transposing) themselves from one DNA molecule to another

**ANTIBIOTIC RESISTANCE MECHANISMS**

- Antibiotic inactivation
- Reduced antibiotic accumulation
  - Impaired uptake
  - Enhanced efflux
- Bypass antibiotic-sensitive step
- Altered antibiotic target/Overproduction of target

**SULFONAMIDES**

Chemical similarity between the sulfa drugs and PABA
**SULFONAMIDES: Mechanism of action**

FA is synthesized in two steps in bacteria.

**RESISTANCE TO SULPHONAMIDES**

- **Chromosomal-encoded**
  - Hyperproduction of PABA
  - Mutation of dihydropteroate synthetase (DHPS) lowers affinity for sulphonamides

- **Plasmid-encoded**
  - Duplication of DHPS enzyme
  - At least 2 types (I and II) of the DHPS enzymes have been found which are only 50% homologous in sequence.
  - Fluid sulphonamides 10,000-fold less efficiently.

**Quinolones**

- Quinolone antibacterial drugs:
  - Nalidixic acid
  - Norfloxacin
  - Ofloxacin
  - Ciprofloxacin

- Broad-spectrum agents
- Rapidly kill bacteria
- Act by inhibiting the activity of the bacterial DNA gyrase, preventing the normal functioning of DNA.

- Bacterial DNA exists in a supercoiled form.
- The enzyme DNA gyrase, a topoisomerase, is responsible for introducing negative supercoils into the structure.

- Humans do possess DNA gyrase but it is structurally distinct from the bacterial enzyme and remains unaffected by the activity of quinolones.

**RESISTANCE TO QUINOLONES**

- Only chromosomal mutations
  - gyrase mutations confer nalidixic acid resistance only
  - N-terminal point mutation in DNA gyrase which reduce affinity of binding of quinolones

- Only chromosomal mutations confer resistance to nalidixic acid and to ciprofloxacin
- Amino acid substitutions which reduce affinity of binding

**RIFAMPICIN**

- The bacterial DNA-dependent RNA polymerase is inhibited
- Used as an antamycolytic
- Bactericidal
- Little effect on eukaryotic cells.
- It is active against the mitochondrial (and chloroplast) RNA polymerase but its penetration into mitochondria is so poor that it displays very little activity in intact eukaryotic cells.
- Used in treating tuberculosis and against meningococcal meningitis.
Eukaryotic and Prokaryotic RNA Polymerase

- 3 classes of RNA polymerase, I, II, and III, binds to promoters to initiate transcription.
- Nuclear RNAP is complex enzyme containing 2 LSU + several SSU, 5 are common.
- Prokaryotic RNAP consist of four Core subunits (2α; β; β') and one regulatory subunit (σ).

The bacterial RNA polymerase complexed with DNA

- The bacterial RNAP bound to a short piece of DNA containing a promoter sequence.
- The closed complex - because the DNA is not melted.
- The open complex - where the RNAP actually melts DNA to expose one of the double-helical strands to the core enzyme which will use it as the template to synthesize RNA.

The bacterial RNA polymerase complexed with rifampicin

- Rifampicin binds to the beta-subunit of the RNA polymerase and inhibits transcription.

RESISTANCE TO RIFAMPICIN

- Only chromosomal mutations.
- Altered DNA-dependent RNA polymerase.
- Beta sub-unit of the RNA polymerase does no longer bind rifampicin.

Protein Synthesis Inhibitors

- Bactericidal
  - Aminoglycosides
    - Streptomycin, Kanamycin, Neomycin
    - Gentamicin, Tobramycin, Amikacin, Netilmicin
    - Oxazolidone (Linezolid)
- Bacteriostatic
  - Chloramphenicol
  - Tetracyclines
    - Doxycycline, Minocycline
  - Macrolides
    - Erythromycin, Azithromycin, Clarithromycin
    - Clindamycin
  - Streptogramins
    - Quinupristin/Dalfopristin (Synercid)
Inhibition of protein synthesis by Ab

TETRACYCLINS

- Tetracyclins include: 
  - Aureomycin
  - Terramycin
  - Panmycin
- Isolated from Streptomyces spp.
- Bacteriostatic
- Have the broadest spectrum of antimicrobial activity.
- Inhibit the codon-anticodon interaction.
- Tetracyclines can also inhibit protein synthesis in the host, but are less likely to reach the concentration required because eukaryotic cells do not have a tetracycline uptake mechanism.

TETRACYCLINS blocks translation during bacterial protein synthesis

The tetracyclins (Tc, doxycycline, demedecycline, minocycline, etc.) block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA.

MECHANISM OF TETRACYCLINE RESISTANCE

Plasmid/transposon-encoded
- Membrane proteins are encoded by the R-genes
- Mechanism involves energy-dependent efflux
- Decreased accumulation of the antibiotic

CHLORAMPHENICOL

- Chloramphenicol member: Chloromycetin
- Broad spectrum antibiotic with similar to the tetracyclines.
- Bacteriostatic
- At present, it is the only antibiotic prepared synthetically.
- Reserved for treatment of serious infections because it is potentially highly toxic to bone marrow cells.
- It inhibits protein synthesis by attaching to the ribosome
- Interferes with the formation of peptide bonds between amino acids.
- It behaves as an antimetabolite for the essential amino acid phenylalanine at ribosomal binding sites.

RESISTANCE TO CHLORAMPHENICOL

Plasmid/transposon-encoded
- Enzymatic inactivation
- Plasmid-encoded chloramphenicol acetyl transferase (cat)
- Impaired uptake
- Plasmid-encoded cat genes encode a protein which reduces uptake of the antibiotic

Ribosome of procaryote

Cat

Chloramphenicol

Chloramphenicol monoacetate

INACTIVE

Protein synthesis

Cat leads to the acetylation of chloramphenicol that prevents binding to the ribosome.
**MACROLIDES**

- Macrolides are: Erythromycin, Clarithromycin, Azithromycin
- Discovered in 1952
- Metabolic products of a strain of Actinomyces (Soil bacteria, Streptomyces erythreus), originally obtained from a soil sample.
- Erythromycin is an orally effective antibiotic with bacteriostatic activity.
- Used in penicillin-allergic patients against Staphylococcus, Streptococcus, Pneumococcus

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**ERYTHROMYCIN: mechanism of action**

- Erythromycin (and other macrolides) inhibit protein synthesis
- Bind to the 23S rRNA molecule of the bacterial ribosome
- The association between erythromycin and the ribosome is reversible.
- Gram +ve bacteria accumulate about 100 times more erythromycin than do gram -ve micro-organisms.
- Certain resistant microorganisms with mutational changes in components of the 30S subunit fail to bind the drug.

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**MACROLIDES blocking translation during bacterial protein synthesis**

Mode of action of Macrolides:
- The macrolides (erythromycin, azithromycin, clarithromycin, dirithromycin, troleandomycin, etc.)
  - Bind reversibly to the 50S subunit.
  - They appear to inhibit elongation of the protein by preventing the enzyme peptidyltransferase from forming peptide bonds between the amino acids (macresp).
  - They may also prevent the transfer of the peptidyl RNA from the A-site to the P-site as shown here (macresr).

http://www.cat.co.md/antibiotics/biotics/erythromycin.html

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**ERYTHROMYCIN--Resistance Methylases**

Different classes of genes involved:

- **ermA**: Staphylococcus aureus, Tn554
- **ermAM**: Streptococcus sanguis, pAM17
- **ermC**: Staphylococcus aureus, pE194
- **ermD**: Bacillus licheniformis, Chromosome
- **ermE**: Streptomyces erythreus, Chromosome
- **ermA':** Arthrobacter sp., Chromosome
- **ermF**: Bacteroides fragilis, pBF4
- **ermG**: Bacillus subtilis, Chromosome

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**AMINOGLYCOSIDES**

- Aminoglycoside family includes: streptomycin, gentamicin, kanamycin, tobramycin, kanamycin, amikacin, etc.
- Clinically important group of antibiotics
- Broad-spectrum of activity
- Are bactericidal in action.
- Have a variety of effects within the bacterial cell
  - Principally inhibit protein synthesis by binding to the 30S ribosomal subunit to prevent the formation of an initiation complex with messenger RNA.
  - They also cause misreading of the messenger RNA message, leading to the production of nonsense peptides.
  - Aminoglycosides increase membrane leakage at high doses.
- Antibiotics such as gentamicin and kanamycin exist as mixtures of several closely related structural compounds.
STREPTOMYCIN

- Effective against gram-ve bacteria
- Also used in the treatment of tuberculosis.
- Binds to the 30S ribosome
- Changes its shape
- Inhibits protein synthesis by causing a misreading of messenger RNA information.

Agricultural use of streptomycin leads to resistance

In regions of dense apple and pear production, streptomycin is applied by air-blast spray equipment to hundreds of hectares of nearly contiguous orchards. While growers strive to minimize drift by spraying during calm weather, non-target organisms on plants, in the soil, and in water are exposed to low doses of streptomycin. Low doses of antibiotics applied to large areas over long periods of time contribute to the build-up of resistance in clinical bacteria. Might a similar scenario be played out in apple and pear orchards?

RESISTANCE TO AMINOGLYCOSIDES

Plasmid/transposon-encoded enzymes

- Effect antibiotic uptake
- Enzymatic modifications of the antibiotic
  - Three classes of enzyme
    - Acetyltransferases (AAC)
    - Adenylyltransferases (AAD)
    - Phosphotransferases (APH)
  - Enzymes divided into sub-types on the basis of the sites they modify in the antibiotics (> 30 in total)

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  - Quinolones
  - Rifampicin
- IMPAIRED RNA SYNTHESIS
  - Sulfonamides
  - Chloramphenicol
- IMPAIRED PROTEIN SYNTHESIS
  - Macrolides (Erythromycin, Clarithromycin)
  - Aminoglycosides (Streptomycin, Gentamycin, Tobramycin, Kanamycin)
  - Beta-lactams (Penicillin, Ampicillin,..)
  - Monobactams
  - Carbapenems
- IMPAIRED DNA SYNTHESIS
- IMPAIRED RNA SYNTHESIS
- IMPAIRED PROTEIN SYNTHESIS

BETA LACTAM ANTIBIOTICS

- Penicillins
  - Penicillin G
  - Ampicillin
- Cephalosporins
  - Cefuroxime
  - Cefotaxime
- Carbapenems
  - Imipenem
- Monobactams
  - Aztreonam

Structure of Penicillin

Penicillin V

Ampicillin

Penicillin G
Similarity between the Gram +ve cell wall and Penicillin

Image courtesy of the University of Texas-Houston Medical School

β-LACTAMS

- Are bactericidal
- Interfere with cell wall synthesis by binding to penicillin-binding proteins (PBPs) which are located in bacterial cell walls
- Inhibition of PBPs leads to inhibition of peptidoglycan synthesis
  - Penicillin works outside the cell
  - Inhibits the D-Ala-D-Ala tail of the peptidoglycan
  - Blocks transpeptidase

RESISTANCE TO β-LACTAMS

- Production of β-lactamase enzymes
  - Most important and common mechanism of resistance in clinical isolates
  - Hydrolyses the β-lactam ring causing inactivation
- Alteration in PBPs leading to decreased binding affinity
  - Major cause of resistance in Staphylococcus aureus (VRSA) and Streptococcus species
- Alteration of outer membrane, leading to decreased penetration
  - Major cause of resistance in Pseudomonas species
  - Major cause of resistance in Staphylococcus species
  - Other pathogenic gram –ve species

RESISTANCE TO BETA LACTAMS

Enzymatic inactivation

VRSA?

- 1992: Laboratory transmission of resistance from VRE to S. aureus showed VRSA is possible
- Late 1990’s: First reports of vancomycin insensitive Staph
- Proved to be bugs with vastly thickened cell wall
- Thick cell wall of VRSA reduces virulence
- These isolates retained other antibiotic sensitivities

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    - Chloramphenicol
    - Erythromycin
    - Streptomycin
    - Aminoglycosides (Kanamycin)
- ACTING ON THE CELL WALL
  - Beta-lactams (Penicillin, Ampicillin,..)
- ACTING ON THE CELL MEMBRANE
- FUNGAL INFECTIONS
- CONCLUSIONS

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Cell membrane

- Antibiotics including the polymixins and gramicidin act by interfering with the functioning of the bacterial cell membrane by increasing its permeability.
- Gramicidin is one of a family of cyclic decapeptides active against Gram +ve bacteria.
- Polymixins have a smaller peptide ring attached to a peptide chain ending with a branched fatty acid.
- They act specifically against Gram +ve bacteria, although chemically modified derivatives do have a broader spectrum of activity.
- These antibiotics are toxic to humans and are now rarely used in clinical practice.

Gating of gramicidin channels

Gating (opening & closing) of a gramicidin channel is thought to involve reversible dimerization.

An open channel forms when two gramicidin molecules join end to end to span the membrane.

This model is consistent with the finding that, at high concentrations of gramicidin, the overall transport rate depends on [gramicidin]$^2$.

INTRODUCTION

- ANTIBIOTICS
- THE BACTERIAL CELL
- HOW BACTERIA EX-CHANGE DNA
- MECHANISMS OF Ab RESISTANCE
- RESISTANCE TO SPECIFIC Ab
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FUNGAL INFECTIONS

- Caused by eukaryotic organisms
- For that reason they generally present more difficult therapeutic problems than do bacterial infections.
- There are relatively few agents that can be used to treat fungal infections.

YEAST and FUNGAL INFECTIONS

- The fungal cell wall may be considered to be a prime target for selectively toxic antifungal agents
- It consists of a chitin structure, absent from human cells.
- No clinically available inhibitor of chitin synthesis analogous to the β-lactams exists at present.
- Much effort is being directed towards developing such agents.
- Other targets are currently being exploited.

Polyene AB: Nystatin

- Polyene antibiotics bind to sterols (ergosterols) within the fungal membrane, disrupting its integrity.
- This makes the membrane leaky, leading to a loss of small molecules from the fungal cell.
- Polyene antibiotics include nystatin, used topically for candida infections and amphotericin B.
Candida species have adapted several mechanisms of fluconazole resistance. The most important of these consisting of:

- an alteration in the target enzyme 14 alpha-demethylase (change in binding site or overexpression of the enzyme).
- from enhanced drug efflux caused by plasma membrane transporters. The net effect of the efflux pump is to decrease the intracellular concentration of fluconazole and thus reduce the concentration of drug that reaches the active site of the enzyme 14 alpha-demethylase.

**Mechanisms of resistance with Fluconazole-Resistant Candidiasis**

**CONCLUSION**

The widespread development of antibiotic resistance by bacteria results in an increasing interest for alternative therapies in the treatment of infected wounds.

**INTRODUCTION**

**THE BACTERIAL CELL**

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**RESISTANCE TO SPECIFIC Ab**

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**CONCLUSION**