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# Resistance to chemotherapeutic agents

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# Two types

- Intrinsic
- acquired



# Clinical significance of resistance

1. Parasites tolerate normally used doses of chemotherapeutic agents
    - Higher doses are required to clear the parasites
    - Increased toxicity to the host with the use of higher doses
    - Necessitate change of drugs (may be expensive; may run out of options)
    - Agents of choice today may not be useful after sometime .
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# Nosocomial infections

- Are usually by resistant organisms e.g. Staphylococcal and Pseudomonas infections
  - Increase days of hospitalization
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# Origin of resistance

## Human related factors

- Irrational combination therapies
  - Use of antibiotics for prophylaxis in hospitals makes them perfect breeding grounds for resistant bacteria
  - Under dosing
  - over prescription of antibiotics, non-prescription availability of antibiotics
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- Inclusion of antibiotics in livestock feed
  - Meat can contain traces of resistant nonpathogenic enterobacteria that can transfer resistance to pathogenic bacteria, either directly, or via human enterobacteria or dermal flora
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## **Parasite related factors:**

### Simple adaptation:

- When exposed to chemotherapeutic agents parasites undergo both qualitative & quantitative changes
  - These are adaptive changes that enable them to survive in the harsh environment presented by the exposure to chemotherapeutic agents.
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- Resistance phenotypes involve cellular proteins (enzymes, binding proteins, active transport proteins)
  - Basis of resistance is mutation or acquisition of gene(s) coding for critical proteins
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## Mutations:

- Spontaneous mutations of chromosomal DNA (1 in  $10^6$  –  $10^7$  cell divisions)
    - (a) single gene mutations leading to resistance to one or more drugs
    - (b) several gene mutations leading to resistance to different drugs, e.g. multidrug resistance in *Mycobacteria*
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# Spread of resistance

- Cross infection
  - Transfer of genetic material (transduction, transformation, conjugation)
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# Mechanisms of resistance

- (1) Enzymatic inactivation
- Inactivation of penicillins and cephalosporins by  $\beta$ -lactamases
  - Acetylation of chloramphenicol to 1-acetoxy, 3-acetoxy & 1,3-diacetoxy derivatives which are inactive (*S. pneumoniae*)
  - Acylation, phosphorylation & adenylation of aminoglycosides
  - Inactivation of rifampicin by ribosylation via an ADP-ribosylated intermediate in *Mycobacteria*

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## (2) Loss of uptake process

- Tetracyclines
  - Chloroquine
  - Amino glycosides (plasmid mediated resistance): outer membrane undergoes changes which reduce active transport into the cells.
  - Decreased uptake of metronidazole (nitroimidazoles)
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(3) Increased production of a metabolite that competes with the drug.

- resistance to sulphonamides
  - resistance to pyrimethamine
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#### (4) Reduced affinity of drug sensitive site

- Penicillins
  - Sulphonamides (due to repetitions of 1 or 2 amino acids in DHF synthetase)
  - Trimethoprim (single amino acid substitution in dihydrofolate reductase)
    - (a) substitution of Ser 108 with Asp 108 in the DHFR raised pyrimethamine resistance 500 times
    - (b) Ile 164-Met 164 raised pyrimethamine resistance 10 times
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- Rifampicin (caused by alterations on the RNA polymerase beta subunit gene (rpoB) which cluster in the 23 amino acids between amino acid 511 and 533
    - (a) Substitution of codon 531; Ser (TCG) –Leu (TTG)
    - (b) Substitution of codon 516; aspartic acid (GAC) – Tyrosine (TAC)
    - (c) Codon 531; Serine (TCG) – phenylalanine (TTT)
  - Quinolone – changes in the DNA gyrase A subunit (gyr A) gene [*Bacteroids fragilis*; *E.coli*]
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(5) Increased/reduced production of a drug sensitive enzyme

These are quantitative changes

- Resistance to pyrimethamine, proguanil
  - Resistance to nitroimidazoles (metronidazole, tinidazole) due to decreased nitroreductase activity/decreased pyruvate:ferrodoxin oxidoreductase activity
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## (6) Loss/modification of drug binding site

- Tetracycline resistance in some G+ve bacteria (occurs through ribosomal protection and is encoded by 2 genes, tet(M) and tet(O))
  - Erythromycin (methylation of the ribosomal binding site encoded by a methylase enzyme)
  - Aminoglycosides (deletion of the receptor protein on the 30S subunit). It is a chromosomal mediated resistance.
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## (7) Efflux of drug from the cell

- Resistance of *Streptococcus pneumoniae* to erythromycin
  - Active efflux of the tetracyclines present in some tetracycline resistant G-ve bacteria
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# Resistance in neoplastic cells

- (1) Reduced intracellular concentration
    - Increased drug efflux: doxorubicin, actinomycin D, vinca alkaloids, etoposide
    - Decreased uptake (methotrexate)
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(2) Decreased conversion to active form of drug

- Antimetabolites (5 FU)

(3) Increased production of target enzyme

- Methotrexate

(4) Decreased affinity of target enzyme to a drug

- Methotrexate, hydroxyurea, etoposide
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(5) Increase repair of drug-induced DNA damage

- Alkylating agents

(6) Increased detoxification of drug

- Alkylating agents
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(7) Decreased activity of enzyme required for drug-induced cell death

- Doxorubicin, etoposide

(8) Suppression of drug-induced cell death by apoptosis

- Most anticancer drugs
  - Mutations in the P<sub>53</sub> gene and over expression of the Bcl-2 gene family
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■ knowing the mechanisms by which resistance develops could help in identifying new molecular targets for antiparasitic chemotherapy

Important points to note

■ Failing of chemotherapy is likely to occur very early with drugs for which a single point mutation confers a marked reduction in susceptibility

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- Drugs which have a low clearance and a shallow concentration/dose-effect relationship increase the chance of selecting for resistance
  - Use of combination of drugs that do not share the mechanisms of resistance will reduce the chances of selection
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