Drug Resistance: An Overview

Chulabhorn Mahidol, Ph.D.

It is a great honour for me to be here this evening and to be invited to speak at the opening ceremony of this Congress. Actually, I am a chemist, an organic chemist by practice and training. However, as the first speaker, I would like to take this opportunity to give an overview of the problems of drug resistance as an introduction to the following more specific areas which will be presented by experts.

My interest in many areas of medical science developed from working in the remote rural communities and public health development projects of Their Majesties the King and Queen of Thailand. Every year we travel to these remote areas, spending approximately 2 months in each region. I, myself, am in charge of the Mobile Medical Unit consisting of a number of physicians who have dedicated their free time working with us on voluntary basis. Wherever we visit, hundreds of villagers with health problems will be waiting for treatment and medicine because they live so far away from any health centre.

The diseases we frequently encounter are those common in developing countries, for instance, infectious diseases and in certain regions, malaria.

The problem of drug resistance, which limits the choice of chemotherapeutic agents, has to be taken into serious consideration.

"DRUG RESISTANCE" the theme of this congress, can be viewed differently, depending on whether by scientists or physicians. Scientists will be more concerned with the microorganisms or parasites while physicians would obviously be concerned with the host-environment-microorganism (or parasite) relationship. The definition by the World Health Organization states that:

"Resistance to drug is the ability of the organisms to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance to the host."

In this context, the term "drug resistance" should not be mixed with "drug tolerance" in the sense that drug resistance is an inheritable characteristic that is transmissible while tolerance could be the result of desensitizing the receptors and it is only localized in the host where the tolerance developed.

The first documented phenomenon of drug resistance, to the best of my knowledge, was the discovery by Franke, Roehl and Ehrlich between 1905-1909. When mice infected with trypanosome were treated with drugs in doses too small to cure, a relapse occurred. Renewal of treatment with the normally effective doses also failed. The trypanosomes thus became resistant to the drug. The resistance was hereditary and usually irreversible. Massive dosage was required to kill the resistant strain.

Before the serious problem of drug resistance was recognized, the discovery of various chemotherapeutic agents for bacterial and parasitic infections was considered a great victory. Many authorities thought that these drugs were so good and so efficient that there was a general decline in chemotherapy research. Such optimism was, unfortunately short lived since there was an increase of drug resistance in both bacteria and parasites. The rapidity with which resistance often appeared after introduction of a new drug was astonishing, for example, with Mefloquine, the most promising antimalarial drug which is still under clinical trials there was a report of isolated cases of resistance to this drug in the Thai-Kampuchean border area where it was widely used under the trials. I'll talk about Mefloquine again later on in this lecture.

There are two broad categories of resistance. The first one is the intrinsic resistance which implies that inherent features of the cells are preventing the drug from gaining access to the target site.

The second one is the acquired resistance. In general, there are several mechanisms by which drug resistance can occur:

1. Resistance arises mainly by Natural Selections namely, by the outgrowth of a naturally resistant strain after the drug has killed all the susceptible strains.

Chulabhorn Research Centre, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.
2. Resistance arises by drug-provoked Mutation (chromosomal level).
3. Resistance can be conferred by Gene-transfer.
4. Resistance that occurs by Gene-amplification which results in the overproduction of the gene-products i.e. the protein which could be the enzyme that destroy the effectiveness of the drug.

**TYPES OF RESISTANCE**

Type 1 resistance: Exclusion of drugs from site.
- Uptake of drug into cells is prevented although the ultimate target is still sensitive e.g. in Staphylococcus aureus resistant to tetracycline; the plasma membrane which normally facilitates the uptake of the drug was modified. The organisms are thus protected from the drug’s action even though the drug’s target (the ribosome) is still susceptible.

Type 2 resistance: The changes at enzyme level.
There may be an increase in enzyme production by DNA-amplification.
- There are many cases where drug-destroying enzyme is increased: for example the first case is where Penicillin-resistant strains isolated from patients secrete the enzyme B-lactamase or penicillinase. This enzyme hydrolyses the drug to penicilloic acid, which is inactive. Penicillin itself induces the production of B-lactamase.
- The second case is where the target enzyme is increased. Malarial parasite requires the enzyme dihydrofolate reductase. Antifols such as pyrimethamine are used to kill plasmodium by inhibiting this enzyme. The resistant strain contains about 80 times as much enzyme as the susceptible strain.
- The third case is where the enzyme is altered. In the clinical resistance of Staphylococcus aureus to erythromycin, the 50S ribosome subunits were methylated by a specific enzyme produced by resistant strain.

Type 3 resistance: Is the case of increased metabolite production e.g. increased in the production of p-aminobenzoic acid in sulfonamide resistant bacteria (Staphylococci, pneumococci).

**GENETIC DETERMINANTS OF ANTIBiotic RESISTANCE**

Genetic and biochemical mechanisms of acquired antibiotic resistance can be summarized as follows:

- **Resistance determined by chromosomal genes** Spontaneous mutation in chromosomal genes encoding the target site or affecting access to that site can seldom be found in mutant bacteria because in some case, mutation may reduce the survival rate in their normal environments e.g. gentamicin-resistant Staphylococcus aureus isolated from patients grow more slowly than the wild type. However, chromosomal mutation, on the other hand, can also increase the resistance of bacteria without reducing their ability to survive, e.g., in gonococci resistance to B-lactams, there was no reduction in virulence.

b) **Plasmid-determined resistance** “Plasmids” are extrachromosomal genetic elements which replicate independently of the chromosome. In this category, resistance in bacteria results from the acquisition of new DNA encoding genes determining a variety of specific proteins. This is more common than chromosomal resistance. The resistant genes themselves are acquired as new DNA by their plasmid vectors. They are frequently found in “TRANSPOSONS” which are sometimes called the “jumping genes.”

“Transposons” are translocatable genetic elements capable of transferring or transposing themselves from one DNA molecule to another. Unlike plasmids, transposons are unable to replicate independently. They must be maintained as part of a functional replicon such as a plasmid, bacteriophage or host chromosome. It is possible for bacteria to acquire many different resistant genes by insertions of a series of transposons into sites on plasmids.

A variety of drug resistant genes or the r-genes can be linked together on R-factor plasmid. The larger of these plasmids also contain a resistance transfer factor or RTF, in addition to several r-genes.
- R factors with an RTF region can be transmitted between different bacterial species.
- Small R factor plasmids lacking an RTF region cannot be transmitted by conjugation and usually confer resistance to a single antibiotic.
- r-gene can integrate into another plasmid that contains an RTF region, forming a transmissible R plasmid.

**COUNTERACTING ANTIBiotic RESISTANCE:**

Microbial resistance that occurs to all classes of antibiotic currently in clinical use has stimulated research and development of new drugs. Over the past 40 years, a number of strategies have been employed in an attempt to overcome this problem. The approaches fall within 3 major categories encompassing the search for natural products, semisynthesis and total synthesis of new drugs. In all cases, understanding of the resistance mechanisms has proved essential for the rational development of new antibiotics.

To demonstrate the strategies used to fight against antibiotic resistance, I have chosen the B-lactam antibiotic group in which penicillin is the progenitor as an example. Tremendous progress made in the B-lactam antibiotic research may set a very good example of approaches to overcome resistance.

Knowledge that resistance to penicillin was the
result of the drug-inactivating enzyme, B-lactamases or penicillinases led to the development of new concepts in antibiotic research.

Several approaches were made to overcome resistance problems.
1. The search for novel B-lactams from nature
2. Chemical modification of existing antibiotic molecules
3. The search for B-lactamase inhibitors
4. The use of combined therapy with two or more unrelated drugs to prevent drug resistance.

1. Searching for novel B-lactams
The search for novel agents with stability to B-lactamases resulted in the discovery of CEPHALOSPORIN C in the mid 1950s. The antibacterial activity of this agent was not as impressive as penicillin but its resistance to staphylococcal penicillinase and the structural similarities to penicillin made it a compound of interest to the pharmaceutical industry.

The cephalosporins contain the 7-aminocephalosporanic acid nucleus which consists of a B-lactam ring fused with a six-membered dihydrothiazine ring. Alteration of various groups at R1 and R2 of the 7-aminocephalosporanic acid nucleus resulted in a variety of semisynthetic derivatives. The antimicrobial activity of the cephalosporins is dependent on the structural integrity of the B-lactam ring, the structural configuration of the molecule and the chemical binding site.

Penicillins and cephalosporins were the sole representatives of naturally occurring B-lactam antibiotics until the 1970’s when another group of B-lactam antibiotic represented by the CEPHAMYCINS, and CARBAPENEMS were found. These drugs are produced by members of actinomycetes.

The carabapenems, products of streptomycetes are presently under study. They seem to be one of the most powerful broad-spectrum antibiotics being developed, with high intrinsic activity against a wide range of bacteria and stability to B-lactamases. Lastly, the discovery of bacterial-produced monocyclic B-lactam antibiotics by Squibb and Takeda is another "promising hope" in B-lactam research. These monobactams can be prepared by total synthesis. AZTREONAM, a synthetic monobactam was found to be highly potent against aerobic gram negative bacteria and it is also stable to B-lactamases.

2. Chemical modification of the existing antibiotic molecules
The basic structure of penicillin consists of a thiazolidine ring (A) connected to a B-lactam ring (B) to which a side chain R is attached. The whole penicillin nucleus is the chief structural requirement for its biological activity. Enzymatic cleavage by B-lactamase results in penicillionic acid which is devoid of antibacterial activity.

Modification of the side chain R will alter both the biological activity as well as the susceptibility of the resultant derivatives to B-lactamases.

Penicillins

Development of new penicillins over the past decades have been restricted to modification of the 6-B-side chain on the B-lactam nucleus.
Preparation of semisynthetic B-lactamase-stable penicillin such as methicillin, cloxacillin, oxacillin and dicloxacillin was a breakthrough and a relief against penicillinase producing staphylococci. Following the introduction of these new drugs, staphylococci...
once again succumbed to the action of B-lactam until, within the last decade, the new breed of antibiotic resistant strain of *Staphylococci* emerged. Mutation in certain penicillin-binding proteins were suggested to be responsible for methicillin resistant *Staphylococci*. However, the detailed mechanisms by which these organisms resist are not fully understood at present. Elucidation of these mechanisms therefore, must be a priority for the future.

![Methicillin](image)

Chemical modification of the cephalosporin structure has also been achieved, resulting in a number of cephalosporin derivatives designated as second and third generations. The "second generation" includes drugs such as cefoxitin, cefaclor, cefuroxime, etc. The second generation cephalosporins have somewhat increased activity against gram-negative organisms. The third generation cephalosporins such as cefotaxime and moxalactam are generally less active than first generation agents against gram-positive cocci, but they are much more active against Enterobacteriaceae.

![Cefotaxime](image)

![Moxalactam](image)

3. B-Lactamase inhibitors Although the discovery of many semisynthetic B-lactam antibiotics stable against a wide range of B-lactamases were considered a breakthrough, there are some limitations. Most of these new drugs are narrow spectrum. The new broad spectrum penicillins such as amoxycillin, carbenicillin and the ureidopenicillins are readily inactivated by plasmid mediated B-lactamases.

The search for novel B-lactamase inhibitors has been fulfilled not so long ago with the isolation of the naturally-occurring CLAVULANIC ACID from *Streptomyces clavuligerus* in the mid 1970s. Clavulanic acid is used in combination with amoxycillin, resulting in the drug called AUGMENTIN. In addition to clavulanic acid, other B-lactamase inhibitors include olivanic acid, izumenolide, penasailin and penicillanic acid sulfones.

B-lactamase inhibitors are also being developed for use with enzyme-susceptible cephalosporins.

![Clavulanic Acid](image)

![Amoxycillin](image)

**PARASITIC DRUG RESISTANCE**

Drug resistance in malaria is considered to be one of the major problems in many tropical countries.

From this table, one can see that malaria is once again on the increase in many geographical regions. The failure to reduce the transmission was due to the resistance of parasites to drugs and vector resistance to insecticides.
Table 1 Numbers (in Thousands) of Malaria Cases Reported.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa*</td>
<td>5,120</td>
<td>4,209</td>
<td>5,390</td>
<td>4,477</td>
<td>6,682</td>
<td>5,847</td>
<td>2,039</td>
<td>1,119</td>
</tr>
<tr>
<td>Americas</td>
<td>269</td>
<td>357</td>
<td>379</td>
<td>399</td>
<td>469</td>
<td>515</td>
<td>603</td>
<td>638</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>4,162</td>
<td>6,105</td>
<td>7,304</td>
<td>5,540</td>
<td>4,790</td>
<td>3,658</td>
<td>3,561</td>
<td>3,351</td>
</tr>
<tr>
<td>Europe</td>
<td>7</td>
<td>13</td>
<td>41</td>
<td>119</td>
<td>93</td>
<td>34</td>
<td>38</td>
<td>60</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>480</td>
<td>429</td>
<td>348</td>
<td>227</td>
<td>162</td>
<td>125</td>
<td>137</td>
<td>144</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>173**</td>
<td>188**</td>
<td>211**</td>
<td>4,457</td>
<td>3,422</td>
<td>2,706</td>
<td>3,654</td>
<td>3,450</td>
</tr>
<tr>
<td>Total (excl. Africa)</td>
<td>5,091</td>
<td>7,092</td>
<td>8,283</td>
<td>10,742</td>
<td>8,936</td>
<td>7,038</td>
<td>7,993</td>
<td>7,643</td>
</tr>
</tbody>
</table>

*Mainly clinically diagnosed cases; does not include the majority of chronic infections
**Excluding China.

---

### Anopheles

- **Sporozoites**
- **Sporogony**
- **Oocyst**

### Human Host (Blood)

- **Sporozoites**
- **Trophozoites**
- **Erythrocytic Schizogony**
- **Merozoites**

- **Causal Prophylactic Drugs**
- **Erythrocytic (Tissue) Schizogony**

### Human Host (Tissue)

- **Sporontocidal Drugs**
- **Gametocytocidal Drugs**

- **E.E. Merozoites**
- **Latent Exo. Erythrocytic (Tissue) Schizogony**

- **Antirelapse Drugs**

This simplified diagram illustrates the life cycle of malarial parasites (*P. vivax* and *P. ovale*) and the sites of action of various antimalarial drugs currently used.

**Fig. 1** This diagram demonstrates the life cycle of Plasmodium and the sites of action of various antimalarial drugs currently used.

The development of chloroquine, pyrimethamine, primaquine and other antimalarial drugs around the 1960s gave a false sense of security and the impression that these drugs, together with the effective new insecticides were the ultimate tools for eradicating malaria. Such hope disappeared in the early 1960s when Harinasuta reported the chloroquine resistant strains of *Plasmodium falciparum* in Southeast Asia. Fortunately, resistance to chloroquine, so far, is limited to *P. falciparum*. Elucidation of the mechanism of drug resistance in this case is difficult since the mode of its action is still unclear at present.
Resistance to antimalarial drug, in general, is attributable to selection of mutants which survive by utilizing alternative metabolic pathways to those inhibited by the particular drug. The chloroquine-resistant *P. falciparum* is more stable both in the vector and the human host than the drug-sensitive strains. Pyrimethamine-resistant strain, on the other hand, tends to recede and in absence of the drug, the pyrimethamine-sensitive strains may outgrow.

The emergence of drug-resistant malarial parasites prompted the United States Army Medical Research and Development Command to launch a major program in development of new antimalarial drug in 1963. Under this program, more than a quarter of a million compounds have been synthesized and tested for antimalarial activity. But only about 3 per cent or 7,500 compounds showed significant activity in the primary test systems. Among these compounds, the most promising one seemed to be from the quinoline methanol group WR 142490, the "MEFLOQUINE." Some members of drugs in each group have been tested in man. The development of these antimalarial drugs is extremely costly and time consuming, but there seems to be no other alternatives for combating malaria in the foreseeable future.

![Chemical Group General Structure](image)

**Chemical Group**
1) The 9-Phenanthrene methanols group

**General Structure**

HOCHR

2) The 4-Quinoline methanols group

HOCHR

3) The 4-Pyridine methanols group

4) The (Arylthio) quinazolines group

5) The Phenylphenol group

6) The Dihydro triazines group

7) The Sesquiterpene Lactones group (Qinghaosu)

**Fig. 2** Relevant classes of antimalarial drugs under the development programs now can be summarized as follows.

For prevention, drug combinations have been employed to delay the rate of emergence of parasite resistance to each drug in the combination. For example, FANSIDAR, a very useful antimalarial drug is the combination of pyrimethamine and sulfadoxine. This drug exerts a more rapid and potent action than either of the individual compound. Unfortunately, over the past few years, fansidar seems to have lost its effectiveness in the treatment of multiple-drug-resistant *P. falciparum* malaria in Southeast Asia. A single dose therapy is no longer effective in Thailand especially in the population of the refugee camp along the Kampuchean border.

**SUMMARY**

I would like to conclude that the emergence and spread of drug-resistant bacteria and parasites have a profound effect on clinical practice and impose limitations on the options available for treatment.

Advances in the knowledge of the mechanisms of resistance are of direct importance in the development of new drugs, but, unfortunately, they have not shown the way towards prevention. This is well illustrated in the case of the spread of bacterial transposons. The emergence of resistance may be prevented, from the scientific point of view, by the use of combined therapy with two or more unrelated drugs on the one hand, and careful usage of drugs which may imply strict government control, on the other. The most important step is to minimize further spread of resistance to the existing drugs as well as the newly developed ones.
Lastly, the development of drug resistance should be viewed as a battle between microorganisms and man. It is in fact a race between the speed with which man can invent new drugs to kill the microorganisms and the speed with which the microorganisms can modify themselves to overcome drug effects. Antibiotic-resistant bacteria can be induced experimentally within 6 months, but development of any NEW drug requires about 10 years and at the approximate cost of US$ 60 million before it can be released for use. One could envisage that if drug research and development does not progress at the maximum capacity and if the government of each country does not pay enough attention to science and technology, man could easily be the loser in this battle.
CURRICULUM VITAE

Her Royal Highness Princess Chulabhorn Ph.D.


EDUCATION
1979: Bachelor of Science (Organic Chemistry) First Class Honours, Faculty of Sciences and Arts, Kasetsart University.
1985: Honorary Doctorate Degree, Thammasat University; Honorary Doctorate Degree, King Mongkut Institute of Technology.
1986: Honorary Doctorate Degree, Mae Jo Agricultural College, Chiangmai.

ACADEMIC DISTINCTION
1976: Professor Tab Nilanidhi Foundation Prize for being first in Chemistry and Biology among Freshmen Class of Kasetsart University.
1978: Kasetsart University Prize for outstanding general proficiency.
1979: Professor Tab Nilanidhi Foundation Prize for outstanding results in Chemistry and Scholarship to pursue Ph.D. work in organic chemistry.
1986: Elected as a Member of the Society; a Chartered Chemist and Honorary Fellow of the Royal Society of Chemistry, London;
   UNESCO “Einstein Gold Medal Award”

PUBLICATIONS

LECTURES AND SEMINARS
1978: Eucalyptus oil in Thailand.
1979: Oxidative phenolic coupling.
1980: Nitroaliphatic compounds as intermediates in synthesis.
1982: Recent work on Thai Zingiberaceous Plants.
1985: Pro and Con of Chemical Compounds.
   Current Status of Chemical Warfare.
   Primary Speaker at the Annual Meeting of the Science Society of Thailand.
1986: Keynote Speaker at the International Meeting on Environmental Toxicology, Bangkok, Thailand.
   Recent Work on Thai Zingiberaceous Family at Upsala University, Sweden and Heidelberg University, Germany.
   Recent Work on a-Halosulfynyl Carbanions, at Notre Dame de la Paix, Belgium.
   New Trend for Drug Development on Natural Products; Ulm University, Germany.

RESEARCH PROJECTS
1. Chemical Constituents of Eucalyptus oil and other essential oils in Thailand.
2. Synthesis of some nitroaliphatic compounds.
3. Chemistry of some a-Halosulfynyl Carbanions.
4. Chemistry and Pharmacology of Thai Zingiberaceous Plants.
5. Scientific investigation of Medicinal Plants for National Development.

POSITIONS:
- Wing Commander of the Royal Thai Air Force.
- Staffmember, Mahidol University.
- Consultant to Petroleum Authority of Thailand.
- Director of Chulabhorn Research Center, Mahidol University.
- Visiting Professor; Upsala University, Sweden.
- Visiting Professor, Ulm University, Germany.
- Executive Board of Director of International Organization for Chemical Sciences Development.