

DEVELOPMENT OF RESISTANCE IN BACTERIA AGAINST ANTI - MICROBIAL AGENTS: REASONS, THREATS AND ONGOING ENCOUNTER

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The term "Anti Microbial Agents (AMA)" is used to cover a wide variety of drugs. Antibiotics, both natural, semi-synthetic and synthetic, and antibacterial chemo-therapeutic agents come under this term. Unlike many other drugs, anti-microbial drugs are societal drugs, as they not only affect the person or subject being treated, but also the people around that person or subject and the environment.

HISTORY OF DEVELOPMENT OF AMA:

Like most of the great discoveries, a series of developmental steps are lying behind the discovery of antimicrobial agents. These play a great historical roll.

- * Mouldy card used by Chinese on boils.
- * Chalmogra oil used by Hindus in Leprosy.
- * Murcury used by Paracelsus to treat Syphilis.
- * Ehrlich's (1890-1935) dye and chemicals used to treat various diseases ---the term 'Chemotherapy' is given for such drugs of known chemical structure.

- * Domagk (1935) used dye 'Prontosil'-the first Sulfonamide ---to cure pyogenic infection.

- * Though Pasteur's demonstration of 'antibiosis' phenomenon (1877) showed the growth of Anthrax bacilli in urin inhibited by airborne bacteria, Flemming received the recognition for first 'antibiotic' development.

- * Waksman (1944) developed Streptomycin from soil microbe group Actinomycetes ---which latter considered as a very rich source to get many antibiotics (Tripathi 2008).

BASIS OF CHEMOTHERAPY BY AMA:

The antimicrobial chemotherapeutic drugs should possess the following qualities :

- * It should be lethal for invading organisms and almost non-toxic to the host.

- * There should be exploitable biochemical difference between the invading organisms and the host.

Biochemical reactions of bacteria can be classified into three classes, which will be the potential target of AMAs. These are

- i) Class 1: Production of simple carbon compounds by using glucose and other carbon sources.

- ii) Class II: Production of small molecules like amino acids, nucleotides etc. from energy and class 1 compounds.

- iii) Class III: Production of larger molecules like protein, nucleic acids, peptidoglycan etc. from small molecules.

Among these, most of the antimicrobial agents work on Class III and some on Class II reactions of micro organisms (Rang et al 2005).

CLASSIFICATION OF ANTIBIOTICS (Basing on mechanism of action):

- 1. Inhibit cell wall Synthesis: Penicillins, Cephalosporins, Cycloserine, Vancomycin, Bacitracin.

- 2. Cause leakage from cell membranes: Polypeptides--Polymyxins (act with the phospholipids of the membrane), Colistin, Bacitracin. Also Amphotericin-B, Nystatin,

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Hamycin.

3. Inhibit protein synthesis: Tetracyclines, Chloramphenicol, Erythromycin, Clindamycin etc.

4. Cause misreading of m-RNA code and affect permeability : Aminoglycosides-Streptomycin, Gentamycin etc.

5. Interfere with intermediary metabolism: Sulfonamides, Sulfones, Trimethoprim, Pyrimethamin, Ethambutol.

6. Inhibit DNA gyrase : Fluoroquinolones, Ciprofloxacin, Norfloxacin etc.

7. Interfere with DNA function : Rifampicin, Metronidazole etc. (Tripathi 2008).

REASONS OF DEVELOPMENT OF RESISTANCE IN BACTERIA AGAINST AMAS

A. Indiscriminate use of antibiotics:

- a) Low dose use.
- b) Early withdrawal
- c) Unnecessary use
- d) Routine use in Animal Husbandry as growth promoter and also in Food storage industry.

e) Residual effect (fruits, vegetables, agricultural land surroundings, food items and food treated with AMAs at any step).

f) Spread of resistant bacteria in animal and meat handlers.

B. Spread of resistance

a) By transfer of resistant bacteria between people/animals/birds.

b) By transfer of Resistance gene between bacteria (usually on plasmids, vertical & horizontal).

Resistance in bacterial population can be spread from person to person by bacteria, from bacterium to bacterium by plasmids, from plasmid to plasmid or chromosome by transposons (Rang et al 2005).

Subjects related with spread of Resistance:

Plasmid: Plasmids are the extra chromosomal genetic elements of bacterium which can carry genes coding for resistance to antibiotics. These are closed loop of DNA consist of a single gene or up to even more than 500 genes. Plasmid may be single or multi copy and more than one type may exist in a bacterial cell. These are self replicating. Most of the drug resistance are plasmid determined. It is not known how these genes

developed.

Transposons: These are stretches of DNA which can be transferred from one plasmid to another and also from plasmid to chromosome or vice versa. These can also hitch-hike on a plasmid to a new species of bacterium, even if the plasmid is unable to replicate in new host. This probably accounts for the widespread distribution of the resistance genes on different R plasmids among unrelated bacteria.

Gene cassettes and integrons : Gene cassette consists of a resistance gene attached to a small recognition site. Several cassettes may be packaged together in a multi-cassette array, which can be integrated into a larger mobile DNA unit, integron. It can be located on a transposon.

This transposon / integron/multi-resistance cassette array allows rapid and efficient transfer of multidrug resistance between genetic elements within bacterium, and also between bacteria on plasmids (Rang et al 2005).

GENETIC DETERMINANTS OF ANTIBIOTIC RESISTANCE

Chromosomal determinants-mutation. Mutation is of two types - Point and Frame shift mutation. Among these, frame shift mutation is important. Deletion of a base or insertion of an extra base can cause alteration of codon and ultimately a different protein is synthesised, and thus bacterium can acquire resistance power.

Extra-chromosomal determinants --- plasmids. The main method of transfer of resistance gene from one bacterium to another is by conjugative plasmids, which can cause bacterium to make a connecting tube between bacteria through which the plasmid, along with other plasmids, can pass (Pili).

TRANSFER OF RESISTANCE GENES BETWEEN BACTERIA

Conjugation - It is performed through connecting tube - pili. It is very important in horizontal transmission and is common among Gram -ve bacilli of the same or another species. Both chromosomal or plasmid DNA may involve in this process. Resistance factor (R factor) can only be

transferred in presence of RTF (Resistance transfer factor- a small fragment of gene). It occurs mainly in colon part of the intestine.

Chloramphenicol resistance in Typhoid bacilli, Streptomycin resistance in E.coli, Penicillin resistance to Gonococci and Haemophilus, and most of the multidrug resistance bacteria are developed in this way.

Transduction -It may happen through bacteriophage, where bacteriophage act as a carrier of resistance gene. Many Staph. aureus strains acquired resistance by transduction.

Transformation -Gene transfer is performed through naked DNA transfer from donor to recipient bacteria. It is not a very common phenomenon (Rang et al 2005).

Resistance once acquired by any of the above mechanisms becomes prevalent due to the selection pressure of a widely used AMAs i.e. presence of the AMAs provide opportunity for the resistant subpopulation to thrive in preference to the sensitive population.

Both pathogenic bacteria as well as non pathogenic bacteria may get resistant genes in this way and become resistant to antibiotics. The non

pathogenic resistant bacteria can transfer these resistant genes to other pathogenic bacteria and can make them resistant, and this process may be continued for generations among different kinds of bacteria and against different types of antibiotics.

Actual problem lies here.

BIOCHEMICAL MECHANISMS OF RESISTANCE TO ANTIBIOTICS: As a part of struggle for existence, bacteria developed various mechanisms to bypass the effects of AMAs. These can be broadly classified as following.

i) Production of an enzyme that can inactivate the drugs: Inactivation of Beta-lactam antibiotics, Chloramphenicol, Aminoglycosides etc.

ii) Alteration of drug sensitive site or drug binding site: Insensitivity to Erythromycin, Fluoroquinolones, Rifampicin etc.

iii) Decreased drug accumulation in the bacterium (Active efflux): Development of resistance to Tetracyclines, Erythromycin, Fluoroquinolones etc.

iv) Development of a pathway that bypass the reaction inhibited by the antibiotic: Trimethoprim & Sulfonamide resistance (Rang et al 2005, Wikipedia 2010b).

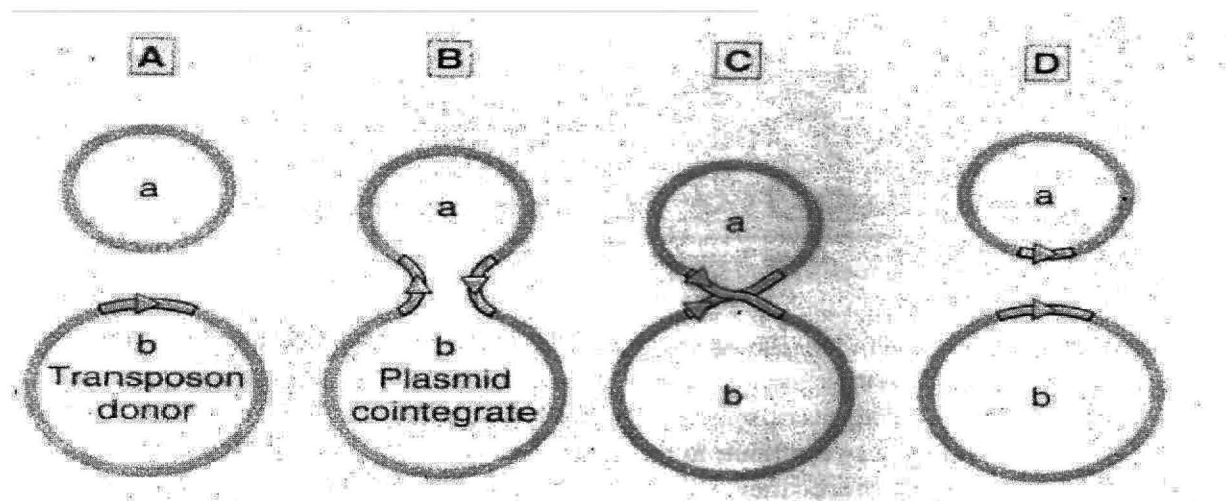
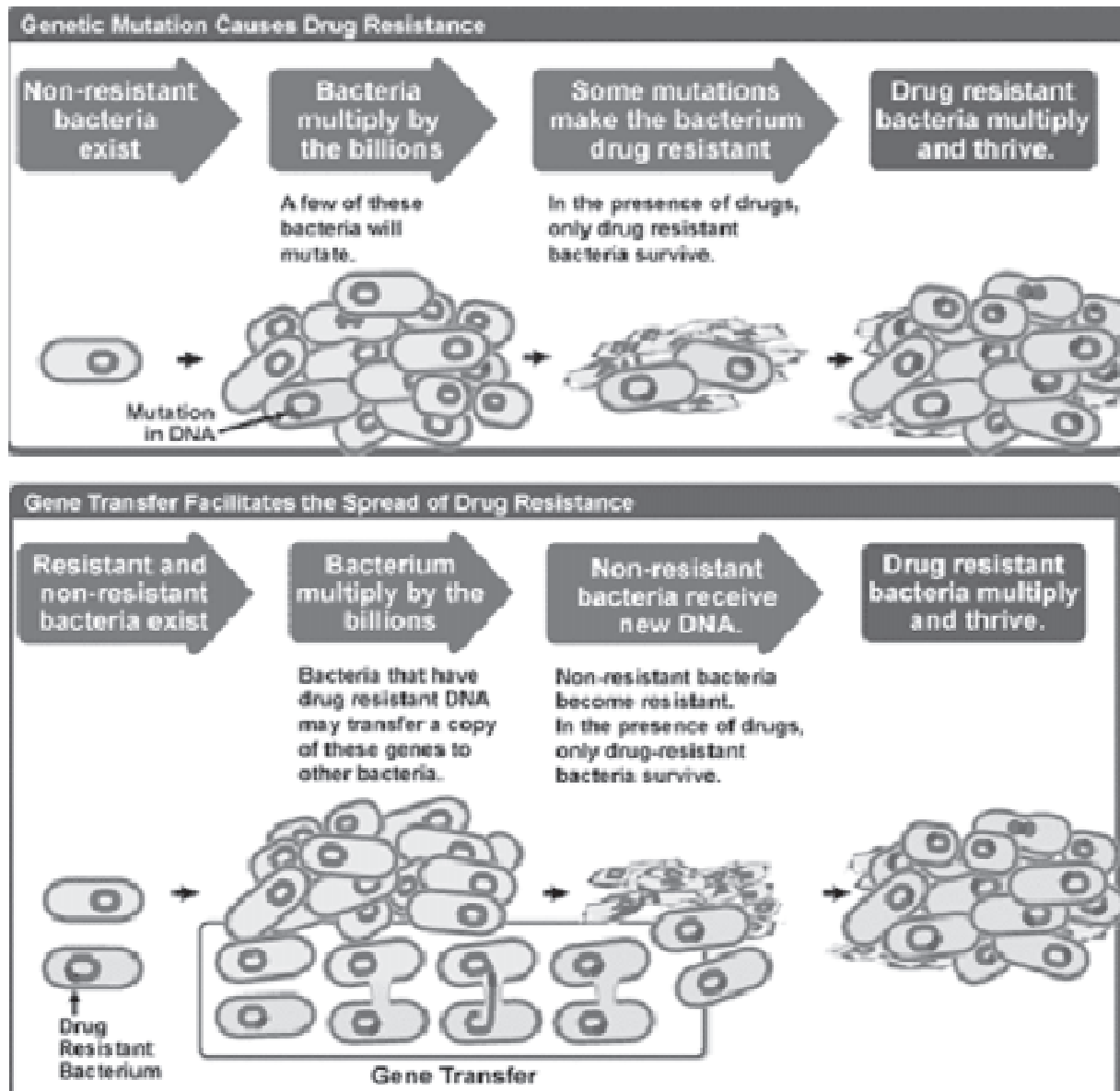


Fig: Transfer and replication of a transposon carrying antibiotic resistance gene: a and b two plasmids, b containing a transposon. An enzyme encoded by the transposon cuts DNA of both donor and recipient plasmids and form a cointegrate. During this process transposone replicates. Then another transposon encoded enzyme resolve the cointegrate and both plasmids now contain the transposon DNA (Rang et al 2005).



MULTIDRUG RESISTANCE: The following facts can serve a hint to understand the actual condition.

*Antibiotic-resistant microorganisms, sometimes referred to as "super bugs", may contribute to the re-emergence of diseases which are currently well-controlled.

*Some strains of Staphylococci and Enterococci are resistant to virtually all current antibiotics, infection of these organisms can cause serious and virtually untreatable **nosocomial infection**

(infection gathered from Hospital/Nursing home).

*Some strains of Mycobacterium tuberculosis have become resistant to most anti-tuberculous drugs.

*United Kingdom Health Protection Agency has stated that most isolates of bacteria with NDM-1 enzyme (New Delhi Metallo beta lactamase -1 enzyme, isolated from patient supposed to gather the infection from New Delhi) are resistant to all standard intravenous antibiotics. (Todar 2009).

Table : Action of AMAS and mechanism resistance developed by bacteria against them.

Mechanism of action of Antibiotics	Name and action of Antibiotics	Resistance developed by Bacteria
A. Inhibition of cell wall synthesis	Penicillins, Cephalosporins, Carbapenems, Monobactams etc. beta lactams	a) Drug inactivation b) insensitivity of target c) Decreased permeability by altered gram - ve outer membrane porins .d) Active efflux.
	Vancomycin	Alteration of binding site of Vancomycin by substitution of terminal amino acid of peptidoglycan subunit.
	Bacitracin	Not properly known.
B. Inhibition of protein synthesis	1. Aminoglycosides (Gentamycin, Kanamycin, Tobramycin, Streptomycin, Neomycin, Amikacin etc.): Attach with 30 S Ribosome and hamper it's work in protein synthesis.	a) Drug inactivation by aminoglycoside modifying enzymes. b) Decreased permeability through gram-ve outer membrane. c) Active efflux.
	2. Macrolides (Erythromycin, Azithromycin etc.): Attach with 50 S Ribosome and hamper it's work in protein synthesis.	a) Alteration of target of antibiotics by Ribosomal methylation and mutation of 23 S rRNA. b) Active efflux.
	3. Lincosamides (Lincomycin, Clindamycin etc.): Attach with 50 S Ribosome and hamper it's work in protein synthesis.	Alteration of target of antibiotics by Ribosomal methylation.
	4. Chloramphenicol: Attach with 50 S Ribosome and hamper it's work in protein synthesis.	a) Drug inactivation by Plasmid derived Chloramphenicol acetyl transferase enzyme. b) Active efflux.
	5. Tetracyclines (All Tetracyclines, Doxycycline, Minocycline etc.): Attach with 30 S Ribosome and hamper it's work in protein synthesis.	a) Active efflux b) Insensitivity of target.
	6. Mupirocin : Hamper the protein synthesis by hampering the work of bacterial enzyme Isoleucine tRNA synthetase. 7. Quinupristin/Dalfopristin (act on 50S Ribosome). 8. Linezolid (act on 50S Ribosome).	Mutation in genes code for Isoleucine tRNA synthetase . a) Alteration of target. b) Active efflux. c) Drug inactivation. Alteration of target by mutation of 23S rRNA.

C. Inhibition of Nucleic acid synthesis or activity	1.Quinolones (Nalidixic acid and it's fluorinated derivatives: Norfloxacin, Ciprofloxacin, Ofloxacin, Levofloxacin etc. all synthetic chemicals) Hamper the action of 'A' subunit of DNA gyrase and Topoisomerase IV enzyme and hamper Nucleic acid synthesis of bacteria.	a) Mutation of gyrase genes b) Decreased permeability of cell membrane by changing the structure of Porins. c) Active Efflux.
	2. Nitrofurantoin derivatives (Nitrofurantoin, Furazolidone etc. – all synthetic chemicals): Act after breakdown by bacterial enzymes – short lasting chemicals produced – act on bacterial DNAs	Resistant bacteria found, but mechanism not properly known
	3. Novobiocin: Hamper the action of 'B' subunit of DNA gyrase enzyme and hamper Nucleic acid synthesis of bacteria	Resistant bacteria found, but mechanism not properly known.
	4. Nitroimidazoles (Metronidazole, Tinidazole etc.): Act after production of some short lasting chemicals inside Anaerobic bacteria – act on bacterial DNAs	Resistant bacteria found, but mechanism not properly known
	5. Rifampin: Act on DNA dependent RNA polymerase and inhibit transcription	Insensitivity of target (mutation of Polimerase gene).
D. Inhibition of Bacterial metabolism	Sulfonamides and Trimethoprim :Inhibition of bacterial metabolism by hampering the folic acid synthesis of bacteria	Production of insensitive targets (dihydro folate reductase for trimethoprim and an altered dihydro pterate synthetase for Sulfo namides) to bypass metabolic block.
E. Alteration of cell membrane permeability	1. Polymyxins (Polymyxin B, Colistin, Polymyxin E): Act by changing permeability of membranes by charge alteration.	Resistance mechanism not properly known.
	2. Gramicidin A : Forming pores in lipid layers of bacterial membrane.	Resistance mechanism not properly known.
	3. Daptomycin : From channels that disrupt membrane potential.	Resistance mechanism not properly known

(As per Harrison, 2008).

SOME RECENT FACTS RELATED WITH NEW DELHI METALLO-BETA-LACTAMASE-1 (NDM-1):

*NDM-1 was first detected in a *Klebsiella pneumoniae* isolate from a Swedish patient of Indian origin in 2008. It was detected later in bacteria in India, Pakistan, the United Kingdom, the United States, Canada, Japan and Brazil. The most common bacteria that make this enzyme are Gram-negative such as *Klebsiella pneumoniae* and *Escherichia coli*, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer.

*NDM-1 is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. These include the antibiotics of the carbapenem family, which are kept for the treatment of antibiotic-resistant bacterial infections. The gene for NDM-1 is one member of a large gene family that encodes beta-lactamase enzymes called carbapenemases. Bacteria that can produce carbapenemases are often referred to in the news media as “superbugs” because infections caused by them are very difficult to treat. Carbapenemases are particularly dangerous resistance mechanisms, since they can inactivate a wide range of different antibiotics.

*A study by a multi-national team was published in the August 2010 issue of the journal *The Lancet Infectious Diseases*. This examined the emergence and spread of bacteria carrying the *bla*_{NDM-1} gene. This reported on 37 cases in the United Kingdom, 44 isolates with NDM-1 in Chennai, 26 in Haryana, and 73 in various other sites in Pakistan and India. The authors’ analysis of the strains showed that many carried *bla*_{NDM-1} on plasmids, which will allow the gene to be readily transferred between different strains of bacteria by horizontal gene transfer. All the isolates were resistant to multiple different classes of antibiotics, including beta-lactam antibiotics, aminoglycosides and fluoroquinolones,

*An environmental point prevalence study conducted between September 26 to October 10, 2010 found bacteria with the NDM-1 gene in drinking water and seepage samples in New Delhi. 50 tap

water samples and 171 seepage samples were collected from sites within 12 km of central New Delhi. Of these samples, 20 strains of bacteria were found to contain NDM-1 gene in 51 out of 171 seepage samples and 2 out of 50 tap water samples (Wikipedia 2011).

ROLE OF ANIMAL HUSBANDRY IN DEVELOPMENT AMR

*Drugs are used in animals that are used as human food, such as cows, pigs, chickens, fish, etc., and these drugs can affect the safety of the meat, milk, and eggs produced from those animals and can be the source of super bugs.

For example, farm animals, particularly pigs, are believed to be able to infect people with MRSA (Methicillin Resistant *Staphylococcus aureus*).

*The resistant bacteria in animals due to antibiotic exposure can be transmitted to humans via three pathways, those being through the consumption of meat, from close or direct contact with animals, or through the environment.

*In 2001, the Union of Concerned Scientists estimated that greater than 70% of the antibiotics used in the US are given to food animals (e.g. chickens, pigs and cattle) in the absence of disease.

Antibiotic use in food animal production has been associated with the emergence of antibiotic-resistant strains of bacteria including *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Enterococcus* spp. (The Pew campaign 2009).

Evidence of Human Health Impacts (from animals and birds)

*During the late 1990s, resistant strains of *Campylobacter* bacteria, one of the most common causes of diarrhea in humans, were discovered in chickens and people. These kinds of bacteria were resistant to fluoroquinolones, a class of antibiotics of important use in human medicine.

*Prior to 2005, farmers also used fluoroquinolones on chicken flocks for prevention and treatment of respiratory disease. Often, whole flocks received the antibiotics indiscriminately through drinking water, which quickly led to the

development of resistant bacteria. Through molecular sub typing, researchers were able to trace the resistant bacteria found in humans back to poultry (The Pew campaign 2009).

ROLE OF ANTIBIOTIC GROWTH PROMOTERS IN DEVELOPMENT OF RESISTANCE: SOME FACTS

The four types of bacteria most commonly associated with resistance due to use of antibiotics as growth promoter are *Salmonella*, *Campylobacter*, *Escherichia coli* and the enterococci; these bacteria are likely to be transmitted frequently from animals to humans (Hughes *et al* 2001).

Salmonella species

* It was reported that, in 1983, an outbreak of food poisoning caused by a resistant strain of salmonella was linked to hamburgers made from cattle fed with chlortetracycline (Bonner 1998). A chloramphenicol-resistant strain of *Salmonella enterica* var. Newport was traced from beef burgers to herds that had been dosed with chloramphenicol (Spika *et al.* 1987). An outbreak of multi-drug resistant *Salmonella enterica* var. Enteritidis was reported following consumption of raw milk (Tacket *et al.* 1985).

*Using medically important drugs, such as chloramphenicol and tetracycline, as growth promoters would seem to be the most obvious route towards resistant strains that pose a threat to human health, but the selection of resistance is not necessarily that simple. A significant correlation between the use of the aminoglycoside apramycin as a growth promoter and the isolation of resistant *Salmonella*, especially *Salmonella enterica* var. Typhimurium DT104, in cattle was reported. Aminoglycoside resistance in these bacteria is due to the acquisition of an acetylating enzyme. This enzyme also confers resistance to gentamicin, an important drug in human medicine (JETACAR 1999).

*Some scientists believe that antibiotic growth-promoters may contribute indirectly to the spread of salmonella by allowing animals to be kept in unhygienic conditions. Growth promoters may act as masking agents for proper sanitation by reducing

the pathogen load. The fundamentally unhygienic conditions of intensive broiler chicken production have been criticized. Broilers are reared in confined housing; this allows any pathogen to spread through the cohort rapidly (Hughes *et al* 2001).

Campylobacter species

**Campylobacter*, particularly *Campylobacter jejuni* and *C. coli*, is the most common cause of bacterial food poisoning in developed countries, such as the UK and USA. Gastrointestinal disease caused by *Campylobacter* shares many of the clinical symptoms of salmonella infection. Unusually for a Gram-negative bacterium, the agent of choice in infections requiring therapy is the macrolide erythromycin, which is also used as a growth promoter for pigs in America. The widespread use of macrolides in the food industry is of concern because of the clinical importance of this family of antibiotics (Hughes *et al* 2001).

*The therapeutic use of fluoroquinolones has had been associated with increased resistance. Fluoroquinolone-resistant strains are emerging around the world. Engberg *et al.* (2001) reviewed *in vitro* macrolide and quinolone resistance prevalence and trends in campylobacter isolated from humans, showing a temporal relationship between use of quinolones in food animals and resistant isolates in humans. It was also reported that the use of fluoroquinolones to treat respiratory diseases in poultry seems to have led to the development of fluoroquinolone-resistant *Campylobacter* in the gut of treated birds (Endtz *et al.* 1991).

Escherichia coli

*When they are located in the gut, *Escherichia coli* strains are regarded as non-pathogenic, Gram-negative members of the commensal flora of humans and animals. But they are capable of causing problems at almost any site of the body (Hughes *et al* 2001).

*Some strains, the most notorious being *E. coli* O157, produce Vero cytotoxins and are referred to as Vero-Toxigenic *Escherichia coli* (VTEC) strains. These bacteria may cause haemorrhagic

colitis and about 5 per cent of cases progress to the haemolytic uraemic syndrome in humans, with a case fatality rate of about 10 per cent. This is the major cause of acute renal failure for children in the UK. In adults, symptoms of haemolytic uraemic syndrome are seen along with neurological complications (Hughes *et al* 2001).

The natural reservoir for VTEC strains is the gastrointestinal tract of cattle and possibly other domesticated animals and so these bacteria may be subject to selection pressure from antibiotic growth promoters (Hughes *et al* 2001).

Enterococci

*The enterococci, a group of Gram-positive organism, are of increasing concern, since they cause illness and death, especially in severely compromised patients in hospitals. Improper use of antibiotics in a clinical setting has resulted in the selection of multi-resistant enterococci, which are resistant to all conventional systemic antimicrobial therapies (Hughes *et al* 2001).

*It has been suggested that the use of antibiotic growth-promoters, in particular the drug avoparcin, has contributed to the emergence of vancomycin-resistant enterococci (VRE). Both drugs are glycopeptides and the *van* genes found in enterococci confer resistance to both drugs (Hughes *et al* 2001).

*Following molecular genetic analysis of vancomycin-resistant enterococci from the faeces of poultry, pigs and humans, it was found that there was evidence of transmission of the Tn1546 transposon, which codes for resistance to glycopeptides, between farm animals and humans (McDonald *et al.* 1997). Similar analyses were performed to examine the prevalence of vancomycin-resistant *Enterococcus faecium* in faeces from turkeys and those involved in their cultivation (Van den Bogaard *et al.* 1997).

*The European Commission banned the use of avoparcin as a growth promoter on the grounds of unknown risk. It was found that, after the ban, a decrease was observed in contamination of meat products by vancomycin-resistant enterococci. The reduction was statistically significant in poultry (from 18.8 per cent to 9.6 per cent) allowing the

conclusion that avoparcin withdrawal has been successful in reducing VRE contamination in meat products (Del Grosso *et al.* 2000).

ENCOUNTER AGAINST BACTERIAL RESISTANCE

A. Combinational use of AMAs:

1. Streptopenicillin
2. Trimethoprim and Sulfonamides
3. Combination of Beta lactum and Aminoglycoside antibiotics – better result found in *Pseudomonas* infection.
4. In Tuberculosis, combinational use of Isoniazid+Rifampicin+Pyrazinamide/Ethambutol is in practice.

B. Rejuvenating of ineffective AMAs :

- a) Use of Efflux inhibition agent (as use of Phe-Arg-β-naphthylamide with antibiotics).
- b) Use of Beta-Lactamase inhibitors - Including Clavulanic acid and Sulbactam /Tazobactam with antibiotics.
- c) Use of analogue of antibiotics: Antibiotic Vancomycin acted against bacterial cell wall synthesis. Bacteria developed resistance against it by bringing about a slight change in the protein chain's binding site for Vancomycin, so it was easily dislodged by the transpeptidase enzyme of the bacteria.

Scientists are now bringing out an analogue of Vancomycin called LY333328 with hydrophobic chain attached to it and which prefer to be surrounded by other hydrophobic molecules such as those that make up the cell membrane which is hidden behind the protective peptidoglycan shield. Thus, they help the antibiotic to anchor better and giving more power to Vancomycin.

- d) Jamming of pump: Tetracycline disrupt the ribosomes and interfere with protein synthesis of bacteria. Bacteria developed resistance to Tetracycline by developing an efflux pump. Scientists at Tufts University developed a compound to jam the efflux pump, so that Tetracycline can work again effectively

C. Development of new antibiotics:

New antibiotics of the following three classes are developed : Cyclic lipopeptides (daptomycin), glycyclines (tigecycline), and oxazolidinones (linezolid). Tigecycline is a broad-spectrum antibi-

otic, whereas the two others are used for Gram-positive infections. These developments show promise as a means to counteract the bacterial resistance to existing antibiotic Resistance-modifying agents.

D. Phage therapy :

Phage viruses are commonly a part of the ecology surrounding bacteria and provide substantial population control of bacteria in the intestine, the ocean, the soil, and other environments.

Bacteriophage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. Phages were used topically, orally or systemically in Polish and Soviet studies. The success rate found in these studies was 80–95% with few gastrointestinal or allergic side effects. British studies also demonstrated significant efficacy of phages against *Escherichia coli*, *Acinetobacter spp.*, *Pseudomonas spp* and *Staphylococcus aureus*

E. Bacteriocins : They are also a growing alternative to the classic small-molecule antibiotics. Because bacteriocins are peptides, they are more readily engineered than small molecules. This may permit the generation of cocktails and dynamically improved antibiotics that are modified to overcome resistance.

Different classes of bacteriocins have different potential as therapeutic agents. Small molecule bacteriocins (microcins, for example, and lantibiotics) may be similar to the classic antibiotics. Colicin-like bacteriocins are more likely to be narrow-spectrum, demanding new molecular diagnostics prior to therapy but also not raising the spectrum of resistance to the same degree.

F. Nutrient withdrawal

Nutrient withdrawal is a potential strategy for replacing or supplementing antibiotics. The restriction of iron availability is one way the human body limits bacterial proliferation. Mechanisms for freeing iron from the body (such as toxins and siderophores) are common among pathogens. Building on this dynamic, various research groups are attempting to produce novel chelators that would

withdraw iron otherwise available to pathogens.

G.Vaccination : Vaccination against common pathogens are also given trials. While theoretically promising, anti-staphylococcal vaccines have shown limited efficacy, because of immunological variation between *Staphylococcus* species, and the limited duration of effectiveness of the antibodies produced. Development and testing of more effective vaccines is under way.

H. The alternatives to antibiotic growth promoters in animal/bird feed.

i) In-feed enzymes

In-feed enzymes are routinely added to pig and poultry feeds and work by helping to break down certain components of the feed, such as glucans, proteins and phytates, that the animal may have problems digesting. They are produced as fermentation products from fungi and bacteria and seem to only have a positive effect on the animal (Hughes *et al* 2001).

ii) Competitive exclusion products

Competitive exclusion products are in-feed microbes consisting of a variety of species of bacteria that are marketed as being “friendly”. The mechanism of action is believed to be that, by allowing such bacteria to colonise the gastrointestinal tract, potential pathogens are prevented from colonising the gut and thus causing infection. These products are often administered to newborn animals, especially poultry, to colonise the gastrointestinal tract and prevent *Salmonella* and *Campylobacter* infections. These products are also given to animals that have been treated therapeutically with antibiotics, to re-colonise a gut that may have been depopulated by the antimicrobial action of the drugs (Hughes *et al* 2001).

iii) Probiotics

Probiotics are similar to competitive exclusion products. They are believed to improve the overall health of an animal by improving the microbial balance in its gut. The way they work has not been established, although it has been hypothesized that their action can be summarised in three ways. The first proposal is a reiteration of the competitive ex-

clusion principle: by colonising the gut in large numbers, the probiotic bacteria exclude pathogens and thus prevent them from causing infection. The second possibility is that they act as a stimulus for the immune system. As the immune system is engaged following exposure to probiotic bacteria, any hostile bacteria are also noticed, following increased surveillance by leukocytes, and thus potential pathogens are eliminated. The third suggestion proposes that probiotics have a strong, positive influence on intestinal metabolic activities, such as increased production of vitamin B12, bacteriocins, and propionic acid.

Problem caused by the use of live bacterial products is that there may be potential dangers concerning antibiotic resistance and cryptic virulence factors. A report from the Scientific Committee for Animal Nutrition (2001) concerning the safety of a probiotic product found that two of the principal strains within the product, *Pediococcus acidilactici* and *Lactobacillus plantarum*, were resistant to tetracyclines. Resistance was found to be coded for by the *tet(S)* gene, which is often located on highly mobile genetic elements. As a result, it was concluded that because of the possible dissemination of tetracycline resistance genes in animal bacterial populations, the food chain and the environment, the use of that product poses a risk when used in animal nutrition(Hughes *et al* 2001).

iv) Infection control measures

The use of antimicrobials as growth promoting agents rests on their role in controlling infection in growing animals. Similarly, many of the alternatives are aimed at controlling infection, often indirectly.

The Australian Pig Farming Industry pioneered the “all-in-all-out” method of pig production. This is a new system, used to replace the older technique of having a constant stream of pigs moving through the farm. Instead of having a range of ages, all the pigs weaned within a week are designated into a single cohort and are housed together in one shed. They are not allowed to mix with pigs from other cohorts and so cross-infection between groups is prevented. “Segregated early weaning” takes note of the observation that the sow is an important

source of pathogens. If piglets are weaned early, they are less likely to come into contact with pathogens from their mothers. Care must be taken not to create welfare problems by weaning animals too early, however. Vaccination may be used to offer protection against certain pathogens, such as enterotoxigenic *E. coli* and various mycoplasma infections(Hughes *et al* 2001).

V) Use of cytokines in animal feed :

The Australian Commonwealth Scientific and Industrial Research Organization (CSIRO), has been working on addition of cytokines instead of antibiotics to animal feed. These proteins are made in the animal body “naturally” after a disease and are not antibiotics so they do not contribute to the antibiotic resistance problem. Furthermore, studies on using cytokines have shown that they also enhance the growth of animals like the antibiotics now used, but without the drawbacks of non-therapeutic antibiotic use. Cytokines have the potential to achieve the animal growth rates traditionally sought by the use of antibiotics without the contribution of antibiotic resistance associated with the widespread non-therapeutic uses of antibiotics currently utilized in the food animal production industries(The Pew campaign 2009).

vi) The Swedish model

Sweden posed the question of suitable alternatives to antibiotics in 1985, when its Parliament passed the Feeding stuffs Act and banned the use of antibiotics for growth promotion. Calves, turkeys and fattening pigs did not appear to be affected significantly by the ban as efforts were made to establish new feeds and housing for those housed animals and birds and, after an initial “unsettled” period of outbreaks of necrotic enteritis, were considered successful. Sweden has shown the rest of the world that it is possible to have modern farming without the use of antibiotics as growth promoters(Hughes *et al* 2001).

I Bioactive phytochemicals.

* Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Most are secondary metabolites, of

which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds including those having antibacterial activity.

*Traditional healers have long used plants to prevent or cure infectious conditions. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. For example the combination of 5'-methoxyhydrnocarpine and berberine in herbs like *Hydrastis canadensis* and *Berberis vulgaris* can block the MDR-pumps that cause multidrug resistance. This has been shown for *Staphylococcus aureus*.

J. ABC transporter blockers :

One of the major causes of antibiotic resistance is the decrease of effective drug concentrations at their target place, due to the increased action of ABC transporters of bacteria. Since ABC transporter blockers can be used in combination with current drugs to increase their effective intracellular concentration, the possible impact of ABC transporter inhibitors is of great clinical interest. ABC transporter blockers that may be useful to increase the efficacy of current drugs have entered clinical trials and are available to be used in therapeutically

K. Use of microbial counter intelligence :

a)Design of new antibiotics—Information about bacterial genes and their proteins has allowed the scientists to go beyond the enemy lines and use the inside information against the organism itself in the

way of developing new types of AMAs.

b) Researchers are looking for the essential genes that can be targeted, such as ,the genes that are required for it to live, or the genes required by the bacterium to infect organism, or the genes that help the bacterium to develop resistance .Each gene can be experimentally disrupted and its effect determined and then the target gene may be selected .Then these genes can be inhibited from producing a single protein ,disrupting the bacterium's ability to infect an organism or develop resistance.

*By comparing a potential target's genetic sequence with the genes found in human/animals, researchers are trying to identify the genes that are unique to bacteria and want to focus on them.

*By comparing target's genetic sequence with those of other bacteria, they are trying to evaluate the selectivity of the drug,i.e.,whether it would be active against many of them (Broad spectrum) or will be specific for few bacterial genomes(Narrow spectrum).

c) Interfering with bacterial RNA and protein synthesis :

*By binding small organic molecules to a part of a specific mRNA sequence,mRNA can be prevented from synthesizing a protein .

*By using an antisense RNA ,they can stop the translation of a specific mRNA.

d) By construction and manipulation of plasmid:

Antibiotic resistance is an important tool for genetic engineering. By constructing a plasmid which contains an antibiotic resistance gene as well as the gene being engineered or expressed, a researcher can ensure that when bacteria replicate, only the copies which carry along the plasmid survive. This ensures that the gene being manipulated passes along when the bacteria replicates (Harrison 2008,Wikipedia 2010a, Wikipedia 2010b,).

So, there may not be easily available resistance gene for the bacteria to acquire.

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