IJPSR (2012), Vol. 3, Issue 03 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 November, 2011; received in revised form 20 December, 2011; accepted 17 February, 2012

REDUCTION OF ANTIBIOTIC RESISTANCE IN BACTERIA: A REVIEW

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Keywords:
Antibiotic Drug Resistance,
Curing,
Plasmids,
Yeast Cell Wall Preparation (YCWP)

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ABSTRACT

Drug resistant bacteria have been posing a major challenge to the effective control of bacterial infections for quite some time. One of the main causes of antibiotics drug resistance is antibiotic overuse, abuse, and in some cases, misuse, due to incorrect diagnosis. Bacterial antibiotic resistance is a significant issues faced by various industries, including the food and agricultural industries, the medical and veterinary profession and others. The potential for transfer of antibiotics resistance, or of potentially lethal antibiotic resistant bacteria, for example from a food animal to human consumer, is of particular concern. A method of controlling development and spread of antibiotic-resistant bacteria include changes in antibiotic usage and pattern of usage of different antibiotics. However, the ability of bacteria to adapt to antibiotic usage and to acquire resistance to existing and new antibiotics usage overcomes such conventional measures, and requires the continued development of alternative means of control of antibiotic resistance bacteria. Alternative means for overcoming the tendency of bacteria to acquire resistance to antibiotic control measures have taken various forms. This article explains one method evaluated for control, that is reducing or removing antibiotic resistance is so called "curing" of antibiotic resistance. Antibiotic resistance is formed in the chromosomal elements. Thus elimination of such drug-resistance plasmids results in loss of antibiotics resistance by the bacterial cell. "Curing" of a microorganism refers to the ability of the organism to spontaneously lose a resistance plasmid under the effect of particular compounds and environmental conditions, thus recovering the antibiotic sensitive state.

INTRODUCTION: Today, there are about 4000 compounds with antibiotic properties. Antibiotics are used to treat and prevent infections, and to promote growth in animals. Antibiotics are derived from three sources: moulds or fungi; bacteria; or synthetic or semi-synthetic compounds. They can be used either internally or topically, and their function is to either inhibit the growth of pathogens or to kill them [1]. Antibiotics can thus be divided into Bacteriostatic drugs, which merely inhibit the growth of the pathogen, and Bactericidal drugs, which actually kill the bacteria. Antibiotics can also be divided into broad-spectrum and narrow-spectrum antibiotics. For example, Tetracycline, a broad spectrum antibiotic, is active against G+ve bacteria, G-ve bacteria [11, 13], and even against mycobacteria; whereas penicillin, which has a relatively narrow spectrum [4, 7], can be used mainly against G+ve bacteria. Other antibiotics, such as Pyrazinamide, have an even narrower spectrum, and can be used merely against *Mycobacterium*

tuberculosis. Antibiotics fight against bacteria by inhibiting certain vital processes of bacterial cells or metabolism. Based on these processes, we can divide antibiotics into five major classes:

1. Cell wall inhibitors, such as Penicillin and Vancomycin.
2. Inhibitors of nucleic acid synthesis, such as Fluoroquinolones, which inhibits DNA synthesis, and Rifampin, which inhibits RNA synthesis.
3. Protein synthesis inhibitors, such as Aminoglycoside.
4. Anti-metabolites, such as the sulfa drugs.
5. Antibiotics that can damage the membrane of the cell, such as Polymyxin B, Gramicidin.

Mechanisms of Antibiotic Resistance in Bacteria: The many mechanisms that bacteria exhibit to protect themselves from antibiotics can be classified into four basic types (**fig. 1**). Antibiotic modification is the best known: the resistant bacteria retain the same sensitive target as antibiotic sensitive strains, but the antibiotic is prevented from reaching it [2, 3]. This happens, for example, with β-lactamases- the β-lactamase enzymatically antibiotics in Gram negative bacteria gain access to the cell that depends on the antibiotic, through water filled hollow membrane protein known as a porin (**fig. 2**).

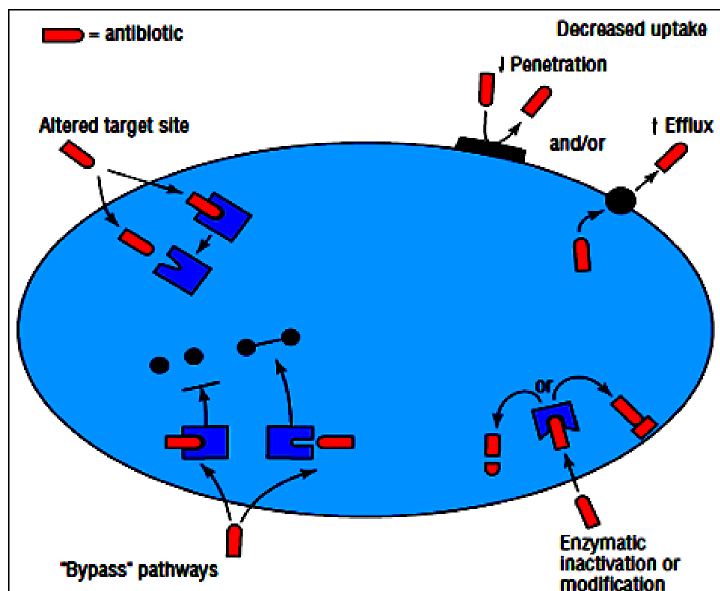

FIG. 1: FOUR MAJOR BIOCHEMICAL MECHANISMS OF ANTIBIOTIC RESISTANCE

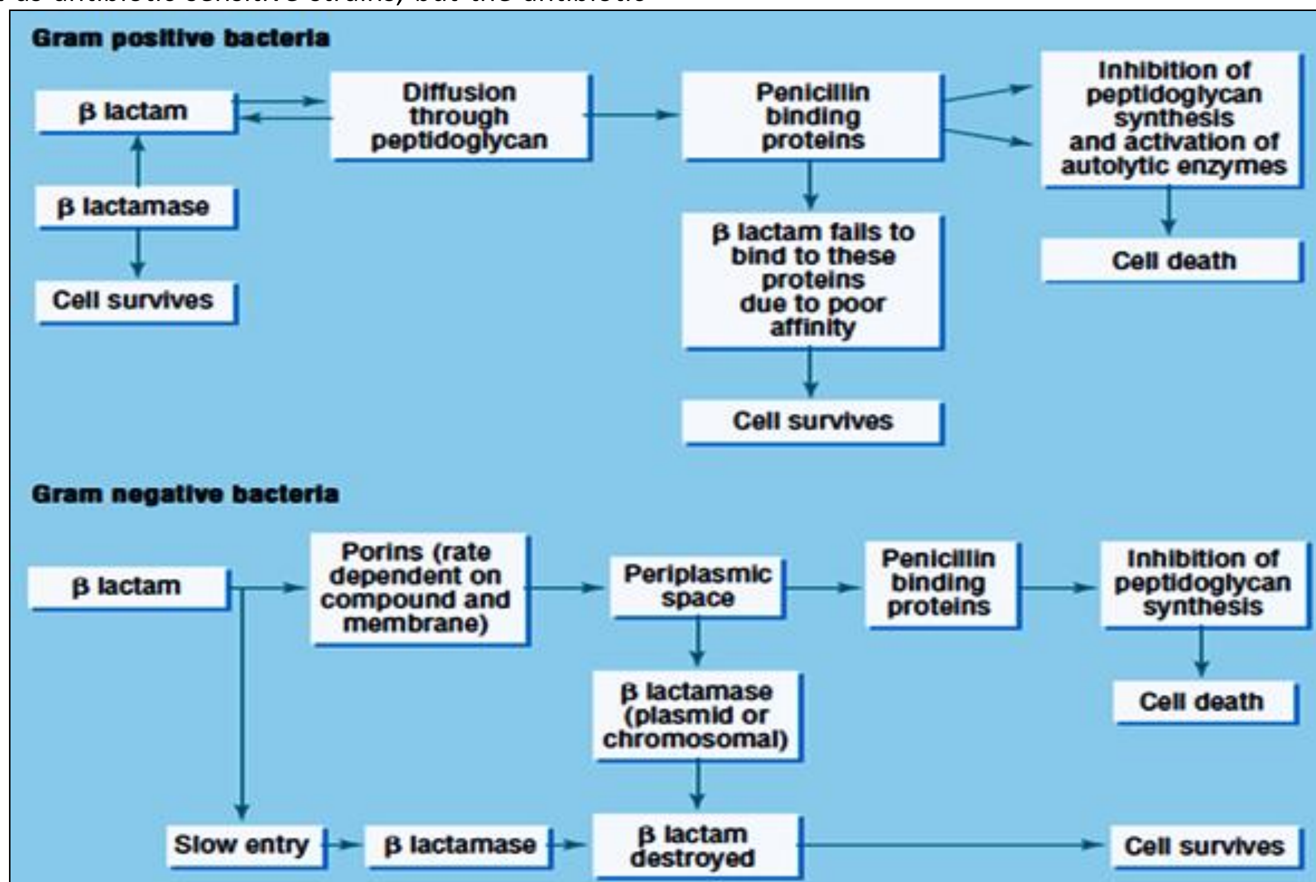

FIG. 2: INTERPLAY OF β-LACTAM ANTIBIOTICS WITH GRAM =VE AND GRAM –VE BACTERIA

In the case of imipenem resistant *Pseudomonas aeruginosa*, lack of the specific D2 porin confers resistance, as imipenem cannot penetrate the cell. This mechanism is also seen with low level resistance to fluoroquinolones and aminoglycosides. Increased efflux via an energy- requiring transport pump is a well recognized mechanism for resistance to tetracyclines and is encoded by a wide range of related genes, such as tet(A), that have become distributed in the Enterobacteriaceae [4].

Alterations in the primary site of action may mean that the antibiotic penetrates the cell and reaches the target site but is unable to inhibit the activity of the tar- get because of structural changes in the molecule. Enterococci are regarded as being inherently resistant to cephalosporins because the enzymes responsible for cell wall synthesis (production of the polymer peptidoglycan)—known as penicillin binding proteins —have a low affinity for them and therefore are not inhibited [5, 6].

Most strains of *Streptococcus pneumoniae* are highly susceptible to both penicillins and cephalosporins but can acquire DNA from other bacteria, which changes the enzyme so that they develop a low affinity for penicillins and hence become resistant to inhibition by penicillins. The altered enzyme still synthesizes peptidoglycan but it now has a different structure. Mutants of *Streptococcus pyogenes* that are resistant to penicillin and express altered penicillin binding proteins can be selected in the laboratory, but they have not been seen in patients, possibly because the cell wall can no longer bind the anti-phagocytic M protein.

The final mechanism by which bacteria may protect themselves from antibiotics is the production of an alternative target (usually an enzyme) that is resistant to inhibition by the antibiotic while continuing to produce the original sensitive target. This allows bacteria to survive in the face of selection: the alternative enzyme "bypasses" the effect of the antibiotic (**Fig. 3**). The best known example of this mechanism is probably the alternative penicillin binding protein (PBP2a), which is produced in addition to the "normal" penicillin binding proteins by methicillin resistant *Staphylococcus aureus* (MRSA) [10, 11].

The protein is encoded by the mecA gene, and because PBP2a is not inhibited by antibiotics such as flucloxacillin the cell continues to synthesize peptidoglycan and hence has a structurally sound cell wall. If an enterococcus acquires the vanA gene cluster, however, it can now make an alternative cell wall precursor ending in d-alanine-d-lactate, to which vancomycin does not bind [7].

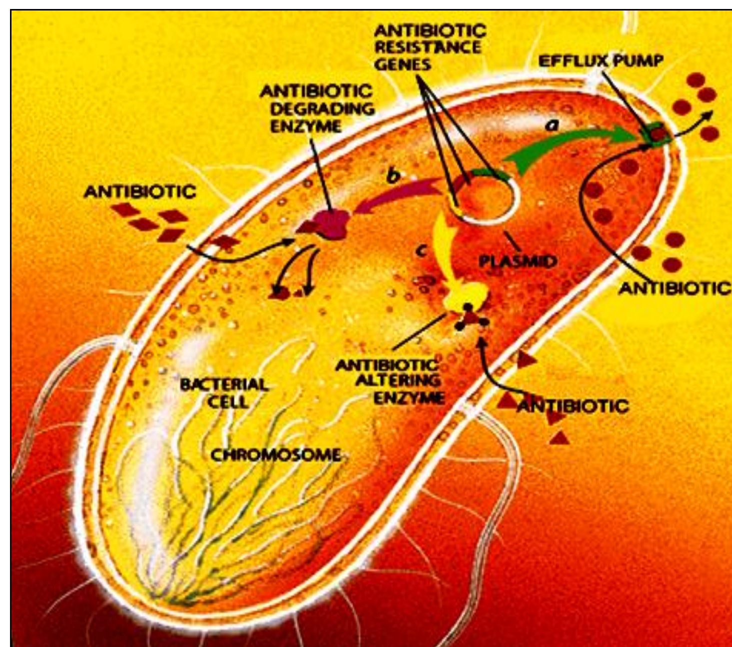

FIG. 3: MAJOR MECHANISM OF ANTIBIOTIC RESISTANCE

The goal in reducing antibiotic resistance in bacteria-

1. To overcome resistance in bacterial species.
2. To minimize the problem of resistance.
3. To give more effect of antibiotics to pathogens.
4. Make use of the older antibiotics by creating sensitivity towards it by the pathogens.

Methodologies that can be used to overcome Antibiotic Resistance: The methodologies to overcome the reduction of antibiotic resistance in bacteria, following are the procedures could be followed up [14, 15];

1. **Collection of Bacterial Isolates:** Bacterial isolates can be collected from different specimens like clinical specimens like throat swab, urine, sputum, etc and other from food industries specimens, agricultural specimens, and pharmaceutical specimens.

2. **Selection of Bacterial Isolates:** Bacterial isolates obtained from different specimens then either the Gram positive or the Gram negative strains will be taken for the further experiments.

3. **Antibiotic Susceptibility Testing:** Different isolates can be obtained in pure form and their antibiotic susceptibility testing is to be done on Muller-Hinton (MH) agar by Kirby Bauer methods on different group of antibiotics. Their sensitivity and resistance to antibiotic will be noted.

4. **Bacterial Growth Rate Evaluation:** Bacterial growth rate can be evaluated by inoculating the desired isolate in Muller Hilton Broth. Growth rate can be measured by turbidetric method at 0, 6, 12 hours intervals. Optical density of turbidity can be taken by spectrophotometer at 600nm.

5. **Preparation of yeast cell wall preparation:** The yeast cell wall preparation may be included in the composition in an amount of from about 0.01% (W/V) to about 1.0% (W/V). Typically the yeast cell wall preparation is derived from a species selected from the group consisting of *Saccharomyces, Candida, Kluyveromyres, Torulaspora*, and mixture thereof.

6. **Curing:** Plasmids curing can be done by aliquots (final inoculum concentration approximately $1X10^5$ CFU/ml) of different isolates will be added to tubes containing 1 ml aliquots of increasing concentration of P-YCWP or YCWP (0, 0.01, 0.1, 0.3, 0.5, 1.0, 1.0, 3.0% w/v). A control culture will established by adding bacterial isolates as described above to aliquots of increasing concentration of mannose. Tubes were incubated at 35°C for 24 hours [16, 17].

7. **Checking the minimum curing agent:** After the incubation 1 ml aliquots will be placed on Maconkey agar and the growth was checked.

8. **Re-checking sensitivity of the isolates:** The isolates can again be done for the antibiotic susceptibility of different antibiotics by Kirby Bauer method on MHB to check the strain sensitivity toward the resistant antibiotics.

9. **Plasmid evaluation:** Plasmid are evaluated by microbial lysis and extraction of DNA (Mini-prep system,) followed by electrophoretic sepration on 1% agarose gel. Gel was stained with ethidium bromide (EB) against a DNA molecular weight standard. The gels were photographed using a VersaDoc-Imaging system under short wave UV light, filter.

10. **Antibiotic Absorption:** Ability of P-YCWP to adsorb antibiotic can be evaluated by growing the isolates in MHB containing antibiotic an increasing concentration of P-YCWP. Growth rate can be determined by turbidimetric method as described earlier.

11. **Curing Over Time:** Percent cure rate (reduction in resistance to antibiotic) over time can be evaluated by growing duplicate aliquots of the isolates in MHB containing 0, 0.3, or 0.5% P-YCWP, with sampling at 0,2,4,6 and 8 hour. Cure rate is determined.

12. **Agglutination:** The isolates should be evaluated for ability to agglutinate P-YCWP and YCWP by growing the isolates into TIF slants (10g peptone, 5g NaCl, 5g yeast extract and 15g bacto agar per litre) for 24 hr. The grown isolates should be suspended in PBS and mixed with low concentration of mannose and to see in low magnifying microscope to see the ability to agglutinate as sugar play role in the agglutination.

13. **Conjugation:** Conjugation can be studied in broth can be performed by growing the culture for overnight in LB medium containing the appropriate antibiotic. Donor and recipient isolates were diluted into LB medium without antibiotics. The number of donor and recipient cell were estimated before mating.

14. **Conjugation in Solid Media:** Overnight cultures of donor containing a broad host range plasmid and recipient strains were grown in LB medium containing the proper antibiotic as described. Then aliquots were placed on the agar media for the growth and the presence of transconjugants was determined.

15. **Conjugative Transfer Rate:** Rate of conjugative transfer can be measured by a number of

transconjugants per donor per minute over 10 minute periods in mating broth [2].

16. **Conjugation in Fecal Sample:** For mating experiments, 100g of fecal sample was weighed into a sterile Stomacher bag. Donor and recipient isolates are to be re-suspended in LB broth and added to the fecal samples, followed by addition of yeast cell wall treatment or control. Samples were stomached for 60seconds and incubated. At intervals (10, 30, 60, 120, 180, 720 min), 10g of feces were diluted in 90 ml PBS in a sterile stomacher bag, stomached, and aliquots (100 µl) plated on LB agar plus the choice antibiotics to determine the number of transconjugants. The donor bacteria and recipient gene can be determined for the resistant and sensitivity toward the antibiotics.

The benefits of reducing Antibiotic Resistance:

1. All the antibiotics will become active towards the pathogens.
2. Decrease in the multidrug resistance.

CONCLUSIONS: The problem of drug resistance was not very serious in the control of animal diseases nearly a decade or more ago. It is clear that bacteria will continue to develop resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information, that is, putting old resistance genes into new hosts [10, 11]. In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming the norm, complicating treatment and increasing both human morbidity and financial costs.

Prudent use of antibacterial drugs using the appropriate drug at the appropriate dosage and for the appropriate duration is one important means of reducing the selective pressure that helps resistant organisms emerge. The other vital aspect of controlling the spread of multidrug-resistant organisms is providing sufficient personnel and resources for infection control in all healthcare facilities. New antibacterial agents with different mechanisms of action are also needed.

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