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Mechanisms of AMR: Mdr Genes and Antibiotics Decoys Retard the New Antibiotic Discovery against Superbugs

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Summary

Many microorganisms cause lethal diseases in human causing loss of lives and property worldwide. Many antibiotics are used to cure deadly infections for the past 75 years with no difficulty. Recent outbreaks of multi-drug resistant bacteria have caused millions of death every year and physicians do not know how to cure KPC2 *Klebsiella kneumoniae*, NDM1 *Escherichia coli* or MRSA *Staphylococcus aureus* and XDR *Acinetobacter baumannii* infections. Sadly once used ampicillin, streptomycin, azithromycin, tetracycline, and chlormphenicol are useless against those bacteria. Combination therapy using colistin, imipenem, amakacin, ceftizidime and investigation drug ovibactam sometime are giving good clinical efficacy but not sure. In such a situation, heterogeneous phyto-antibiotics and gene medicines have been welcome by medical authorities but AMR calamity remains as mdr genes (*amp, bla, tet, cat, aac, aad, aph,sul* etc) moved to conjugative plasmids and chromosome of bacteria with target specific alterations in rRNA and porins genes.

Keywords: Mdr genes; Anti-microbial resistant; Gene medicine; Phyto-antibiotics

Introduction

Past 75 years are the golden era of drug development and several types of antibiotics are in centre stage of such discoveries since the discovery of penicillin drug by Alexander Flaming from slime mold Penicillium notatam in 1928 targeting peptidoglycan cell wall biosynthesis of most Gram (+) and Gram (-) bacteria [1]. Since then 1000 derivatives were made alone for penicillins (ampicillin, cefotaxime and imipenem) for better drug usually called penicillinases resistant drug. A professor of biochemistry and microbiology at Rutgers University, Dr. Selman A. Waksman discovered over twenty antibiotics (a word he coined) and introduced procedures of antibiotic production (streptomycin) that led to Nobel Prize in Physiology or Medicine in 1952. However, such dream could not last long as more potent penicillinases called, oxacillinases, cefotaximases, carbapenemases were appeared in bacterial plasmids [2].

New era of biology was begun in 1953 with the discovery of structure of DNA, gene structure, regulation of gene expression and advancement of DNA sequencing, chromosomal structure and RDT work (Figure 1). Profound impact was found by bio-molecules separation by ultra centrifugation and HPLC with chemical structure analysis by Mass, NMR and FTIR. Invitebly we got many life saving drugs with known target site although basic DNA, RNA and protein composition in virus to bacteria to human were same [3].

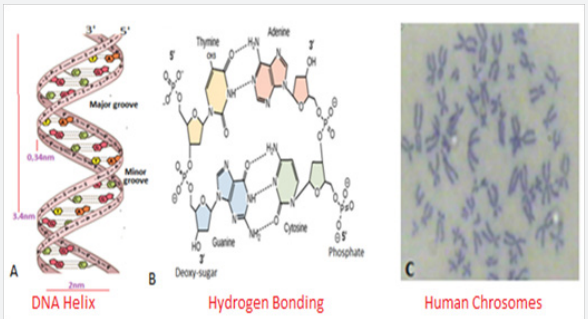

Figure 1: DNA and chromosome structures.

Semi-synthetic drugs and anti-microbial resistance

Naturally, semi-synthetic drugs were made without choice to overcome the action of multi-drug resistant *class* located in bacterial plasmids that inactivate the antibiotics by different

mode of actions. As for example, ampR cell extract was discovered as early as 1940 and amp gene which produces an enzyme, Beta-lactamase was sequenced in 1965. Now one in three bacteria in river and sea water contained amp gene in large conjugative plasmids that also carry 5-10 other mdr genes and 10-15 Tra and Tnp genes [4]. So journey from 1940-1960, described the isolation of tetracycline, streptomycin, sulfa-drug, ampicillin, amoxicillin, cefoxitin, cefotaxime, erythromycin, nalidixic acid, ciprofloxacin, neomycin, polymyxin, enoxacin, norfloxacin (Figure 2). However, at the almost same time, resistant bacteria to all these antibiotics were developed creating pressure to drug industry for more and more new drug development. However, it is not very easy to develop a drug for human use because it needed at least one billion dollar to develop a drug. What happen to investor if a developed drug is good for few years and then drug resistant microbes appeared when no one want to prescribe that antibiotic because uncertainty of cure of such infections and also delay in treatment and also taken of repeated different antibiotics surely toxic to health and time and monetary loss [5].

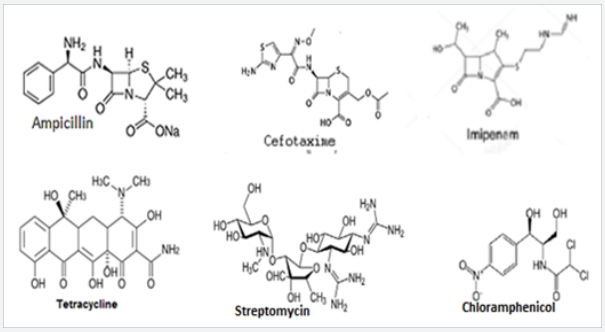

Figure 2: Structure of antibiotics that are mostly useless now.

Drug screening from bacteria against bacteria-a wrong message

In fact, now R & D Industry screening new drugs everyday and also computer-guided graphics design and stimulate artificial drug-target interactions have accelerating the new drug development. Screening of new drug from fungi was favourable in sense that in soil and water there is a battle between bacteria and fungi and so fungi will produce anti-bacterial to kill bacteria. That type of selection is good having different genus but what we did that we introduced the battle between actinomycetes and bacteria like neomycin (1946) and actinomycin (1940). And then we introduced the battle between bacteria against bacteria as for example streptomycin is produced from soil bacteria, *Streptomyces griseus* and also chloramphenicol that eradicate typhoid disease in early decades. What has happened in life of bacteria that all want to destroy it and as a result bacteria are forced to re-arrange its genes to save its life, Hypothesis is not so easy as its own counterpart is enemy and bacteria created many new entity like transposons, integrons, R-plasmids and many DNA rearrangement enzymes like transposes, resolves and integrases and also many topoisomerases and restriction end nucleases [6]. In 1960-1980, we produced 1000 tons of antibiotics in industry and 7000 millions of global peoples now taken antibiotics almost every day or every month to remove the bacteria from intestine and blood to keep healthy. Doctors have forgotten that bacteria needed for human development and intestine should stay (10)12 bacteria for normal synthesis of vitamins which human could not synthesize itself . When such discrepancy was noticed, then probiotic bacteria were used as supplement after each antibiotic therapy. In other word, we used many unnecessary doses of antibiotics as for example, for viral infection, for pain and in food animal growth as well as in agricultural land [7].

Conjugation plasmid-a safe guard of bacteria to transmit genes without failure

However that is too late, as bacteria developed another armour against antibiotics by using its very urgent plasmids used in conjugation (marriage) that means bacteria could form a sex pilus using Tra proteins coded by 62kb plasmid called F'-plasmid which usually did not carry MDR genes. What bacteria did that combined R-plasmid with F'-plasmid and such plasmid is known today as conjugative MDR plasmid which could be large as 100-500kb and such plasmids are hard to purify by plasmid purification method for molecular biological study being contaminated with bacteria chromosome (2000-5000kb) [8]. Never the less CsCl density gradient centrifugation and Pulse Field Gel Electrophoresis have help to isolate such plasmids with purity and also fully sequenced. What we see that such plasmids carry most Tra and Tnp genes including localized mdr genes. What is the advantage of bacteria then? Very advantage for life because such plasmids are very stable in bacteria during cell division and also could donate the non-MDR bacteria of mdr genes to save from deleterious effects of antibiotics and toxic chemicals in water. What is a toxic chemical? Well large industry like mineral Industry, Paint industry, drug industry, paper industry, petroleum industry and excreta from 100 million peoples in many big cities (New York) releases tons of chemicals, antibiotics and heavy metals into sea water that are very harmful to bacterial central dogma enzymes like those involved in replication, transcription and translation. What exactly bacteria did Bacteria simply made 100 different enzymes that destroy antibiotics once it entered into bacteria. But that is not sure as 100 chemicals and detergent in sewage water and bacteria made drug efflux genes (known as tetA, acrAB, mexAB/CD/EF, and ABC genes) that could remove drugs and chemicals from cytoplasm into outside keeping save its cellular enzymes and nucleic acids (Figure 3). That mean whatever the high concentration of pollutants and antibiotics outside water where bacteria live no toxicity because once a chemical enter into bacteria acrAB/C proteins pump it back into environment keeping bacteria safe. What is the consequence? Well bacteria in our body stay alive and divide most to cause sepsis and trauma but condition not likely going to improve by taking prescription

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drugs because no achievable concentration of the drug would be happen in bacterial cytoplasm (to stop protein synthesis) due to bacterial drug efflux pumps (Figure 4).

Figure 3: Ultra structure of 1-1.5 µm length MDR bacteria (8000X).

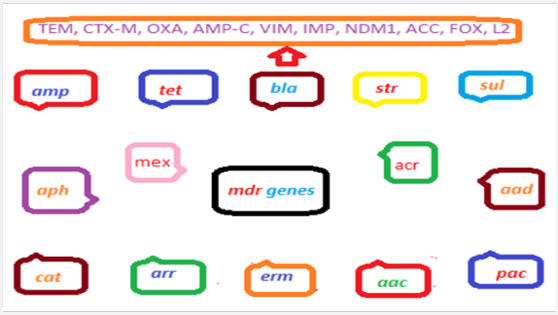

Figure 4: Different mdr genes where β-lactamase gene (bla) is most diversified

Bacteria moved mdr genes into chromosome to increase gene dose further

Did any other genetic changes happen that we should be worry? Yes, bacteria also made safe guard by combing mdr genes into their chromosome and few bacteria like *Staphylococus aureus* and *Acinetobacter baumannii* and also household bacteria like *Escherichia coli* genome-MDR-islands were sequenced confirming the calamity (Figure 4). That is not the end, porin membrane proteins are also mutated in such a way that antibiotics receptors altered and no drug could enter into bacteria at low drug concentration giving MDR. Further, ribosomal ribonucleic acids (23S, 16S rRNAs) gathered few mutations (usually very conserved) and many ribosomal proteins and drugs interactions did altered causing MDR. On one word, bacteria have achieved many shrouds against antibiotics and drug companies did not know where to start [9].

As for example, we discovered at least twenty types' bacterial beta-lactamases (mdr genes) that were sequenced. Again in each type beta-lactamase gene, hundreds of mutations were discovered that sometime gave high drug resistance increasing drug MIC or totally resistant. Gen Bank analysis clearly showed that each conjugative plasmid in *Pseudomonas aeruginosa, Klebsiella kneumoniae, Escherichia coli* and *Salmonella typhi* have many mdr genes giving resistant to 5-10 antibiotics from different groups with different mode of ations (Table 1). Interestingly such plasmids carry mdr genes in one locus with activation by transposon promoter-enhancers and Tra genes are located in clusters (Figure 5). It is very easy to isolate MDR bacteria in water by adding antibiotics in media at 50µg/ml and then isolate plasmid DNA by alkaline lyses method and then do PCR reactions in presence of mar gene specific primers as shown (Figure 6) where *Escherichia coli* KT-1_mdr bacterial plasmids were amplified with mcr, tet, bla VIM and acrAB mdr genes specific primers. Such PCR product could be confirmed by di-deoxy DNA sequencing as shown in (Figure 7) where blaTEM gene was found in every ampicillin resistant bacteria we have isolated from Ganga River water of Kolkata [3,10].

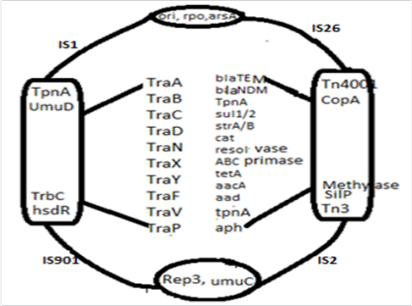

Figure 5: A structure of MDR conjugative plasmid which donates mdr genes into many bacteria [4].

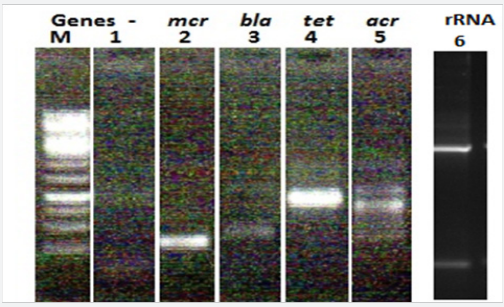

Figure 6: PCR amplification of E. coli KT-1_mdr plasmids using acrA,tetC,mcr and blaVIN genes specific primers [8, 16].

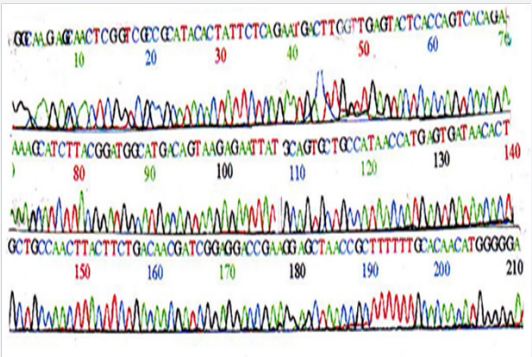

Figure 7: Di-deoxy sequencing of blaTEM gene fragment from P. aeruginosa DB-1_mdr (Accession number KY769875) plasmid which present in most MDR bacteria in Kolkata.

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Table 1: Localization of multiple mdr genes in single MDR conjugative plasmids from different superbugs [10,16].

MDR Genes in Large Plasmids Involving ESBL and XRD Pathogens				
Accession Number	**Size (Kb)**	**MDR Genes Profiles with Bla Genes, Drug Modifying and Drug Efflux Genes**	**Gen Bank Year**	**Pathogenic Bacterial Name**
AP012056	141	tetA,aac3'/6,cat,sul2,blaOXA1/CTXM15/TEMI,strBA	2013	*K. pneumoniae*
KM877269	249	aad,hph,aac6',aac3',blaOXA1,catB,arr3,sul1	2015	*S. enterica*
LN555650	299	terF,sul1,strA,catB,blaACC1,aacA4,blaVIM1	2015	*S. enterica*
JN420336	267	blaNDM1,blaOXA1,aac6',qnrB1,catB,blaCTXM,	2012	*K. pneumoniae*
KC543497	501	Ter2,blaOXA10,MFS, blaTEM-8,aac	2014	*P. aerogenosa*
CP0011634	95	aph,blaTEM,aac3',MFS, dhfr,aad,aar2,bla NDM1	2014	*K. pneumoniae*
AP012055	250	bla NDM1,ccdA,ccdB,aadA2,catA1,qacA1	2013	*K. pneumoniae*
NC_022078	317	ABC,merB,cat,aph*,aac3',cmr,tetA,blaKPC	2015	*K.pneumoniae*
CP011634	227	blaOXA and bla ACCI= blaACC1; bla VIM= blaVIM	2015	*k. oxytoca*
HG530658	223	bla ACC1,strA,aadA2,aaC3'	2015	*E. coli*
NC_019375	180	blaOXA and bla blaACC1; blaVIM	2014	*P. stuartii*
NC_022522	168	blaOXA and bla blaACC1; blaVIM	2014	*S. enterica*
LC055503	160	blaSHV12,aac6', blaOXA10, aadA1, sul1, blaDHA1	2015	*K. pneumoniae*
HG941719	135	blaTEM/CTXM/OXA,aad,mphA,aac6',sull,tetA	2014	*E. coli*

Conclusion

It is very evident that superbugs were highly contaminated in water resources of India similar to other Asian and American countries [11,12]. WHO warned that if alternative to antibiotics were not discovered, very fatal human loss might be occurring in the future? Likely herbal antibiotics research has given priority in India as there is enough medicinal plants and spices available as described in Sanskrit books Charaka Samhita and Veda [8]. However, gene medicines (ribozymes, miRNA, antisencse RNA, and DNA nanotechnology have benn welcome to stop the horror of MDR bacterial pathogenesis. MDR phenomenon is ancient and also universally have detected in viral pathogenesis, cancer cells and parasitic diseases [13,14]. More sadly, bacteria have acquired promoter induction system by antibiotics and many transcription factor repressors (tetR, acrR) have been accumulated in conjugative plasmid. What it mean that if you take imipenem then it will activate MDR genes causing more AMR and simply patient will die on antibiotic treatment [15].

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