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Mechanisms of AMR: Bacteria Won the Battle Against Antibiotics

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Abstract

Antibiotic was discovered in 1926 by Alexander Flaming but only in 1943 after World War-II, penicillin was marketed for all followed by tetracycline and streptomycin. However, high dose uses of antibiotics cause rapid destruction of gut microbiota that help human growth and metabolism by providing vitamins and many other complex biomolecules. It appears that intestinal cells signal to bacteria that are also induced save its soul creating many MDR genes and activating gene transfer mechanisms by combining R-plasmids with F'-plasmids. Thus, large conjugative MDR plasmids have 5-15 *mdr* genes, 6-10 metal resistant genes and two dozen TRA genes for conjugation as well as new protein synthesizing genes like transposases, integrases, topoisomerases, resolvases, restriction endonucleases, DNA ligases and DNA polymerases. Physicians do not know how to cure KPC2 *Klebsiella kneumoniae*, NDM1 *Escherichia coli* or MRSA *Staphylococcus aureus*, MDR *Mycobacterium tuberculosis* and XDR *Acinetobacter baumannii* infections. Sadly, once used ampicillin, oxacillin, streptomycin, cefotaxime, azithromycin, tetracycline, ciprofloxacin and chlormphenicol are useless against those bacteria. Our study with Ganga River water of Kolkata indicated that super drugs like imipenem, colistin, tigecycline, amikacin, ceftizidime, vancomycin, levofloxacin and linezolid resistant bacterial species were generated creating a antibiotic dark age even we had thousand antibiotics in the selves.

Keywords: Anti-microbial resistant; MDR plasmids; Beta-lactamases; Drug efflux genes

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Introduction

Past 75 years are the golden era of drug development and few thousands penicillin drug derivatives are produced targeting bacterial cell wall peptidoglycan synthesis [1,2]. Dr. Selman A. Waksman discovered over twenty antibiotics from bacteria, actinomycetes and fungi including streptomycin and chloramphenicol that eradicated TB and typhoid in 1950s respectively [3]. New era of biology was begun since 1953 with the discovery of structure of DNA, gene structure, regulation of gene expression and advancement of DNA sequencing, chromosomal structure and RDT work [4-7]. Profound impact was seen in bio-molecules separation by ion-exchange and gel filtration chromatography, glycerol or sucrose gradient ultracentrifugation and HPLC followed by chemical structure analysis by Mass, NMR and FTIR [8,9]. However, last two decades doctors found that drug response curve of many diseases have profoundly changed with increase MIC and a dark site of success RDT work using plasmids pBR322 containing amp and *tet* genes were anticipated [10-12]. Now every laboratory use dozens of marker genes (*neo, amp, tet, aac, cat, pac*) derived from drug resistant 5-15 kb R-plasmids. Such plasmids do not transmit *mdr* genes to other bacteria by conjugation and even such genes have identified in 1940 (sequenced in 1965), it took few decades to generate MDR conjugative plasmids that profoundly were making most bacteria drug resistant. Scientist predicted that antibiotic use by patients, antibiotic contamination from industry, antibiotic use in agricultural land and lives stocks growth, all are contributing to *mdr* gene creation and spread [13,14].

Results and Discussion

Pubmed and GenBank search suggested that unresponsiveness of many bacteria to drugs like ampicillin, chloramphenicol, streptomycin and tetracycline as early as 1940s forced drug companies to develop new drug derivatives that effective against

MDR bacteria [2]. Naturally, semi-synthetic drugs were made without choice to overcome the action of multi-drug resistant genes located in bacterial plasmids that inactivate the antibiotics by different mode of actions [10]. 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 1**). However, at the almost same time, resistant bacteria to all these antibiotics were developed and the pharmaceutical companies and investors had lost their money (1-5 billion dollar/drug). What happen to investor if a developed drug is good for few years and then drug resistant microbes were appeared when no one would want to prescribe that antibiotic because uncertainty of cure of such infections? In fact, still now R and D Industry is screening new drug every day and also computer-guided graphics design and stimulation of artificial drug-target interactions are accelerating the new drug development. Screening of new drug from fungi was favorable 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 was good having different phylum but what we did introduced actinomycetes derived drug like neomycin and bacteria derived drug like streptomycin from *Streptomyces griseus*. Then we introduced total synthetic drug against bacteria like ovibactam that was so far good but how long as similar drug sulbactam became useless [15]. What had happened in life of bacteria that all wanted to destroy it by polluting water and consuming antibiotics and as a result bacterium were forced to re-arrange its genes to save its own life (**Figure 2**). In 1960-1980, we produced >1000 tons of antibiotics in industry and 7000 million of global peoples now taken antibiotics almost every month to remove the bacteria from intestine for good health [16]. Does all patients are taking multi-vitamin after each antibiotic dose? Are patients taking probiotics after each antibiotic dose? The answer is simple, in poor nations most doctors do not even prescribe probiotics and multi-vitamins during antibiotic treatment [17]. In Kolkata, I was advised for amoxycillin-cavulinate but sad

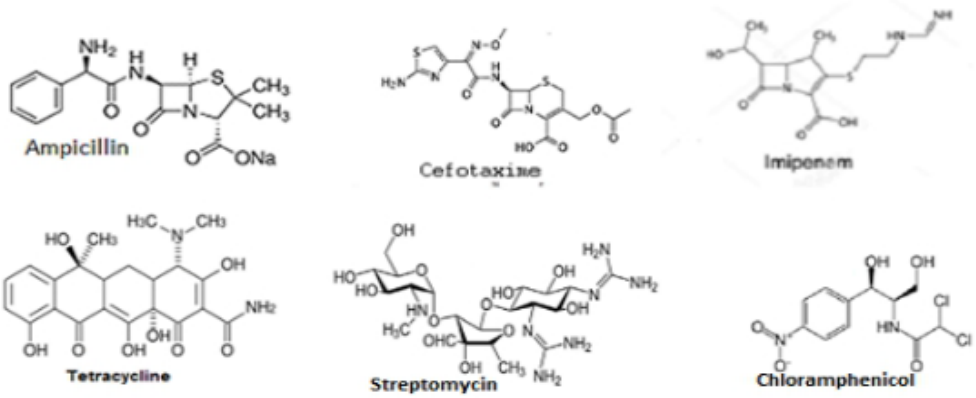

Figure 1 Chemical structures of few early antibiotics that have saved life many fold from TB, sepsis, pneumonia, cholera, skin infection, UTI and fever but now almost all are useless due to AMR.

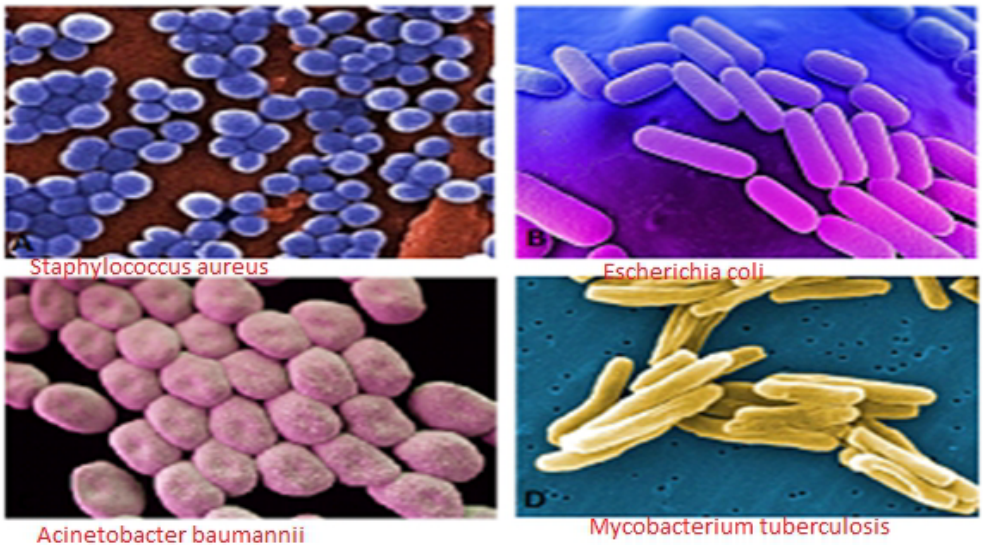

Figure 2 EM structures of few MDR bacteria (1-2 micro meter length) that are highly contaminated and transmitted in human causing acute problem of society (8000X) [10].

thing was not advised to take probiotic capsule or multivitamin capsule! So, in my opinion, in most poor and even developing countries we are creating serious health hazards by prescribing antibiotics to all peoples whose metabolism are surely reduced due to lack of coenzymes like FAD, NAD+, COA-SH, THFA, Biotin and vitamin-B12 [18]. The scenario is fatal as we boil most foods destroying vitamins and we do not take raw vegetables and fruits that are great source of vitamins. G-20 leaders and scientists are gathered in Germany recently and have issued Action Plan to reduce superbug horror, but the calamity remains as new therapeutics are not going to develop very soon. Surely, phage therapy clinical trials are ongoing, but phage resistant factors are slowing the FDA approval [17]. Nanotechnology applications are underway to deliver toxic drugs but are very costly. Phyto-antibiotics will be considered again but we have cut all plants for crops and a new agricultural land development is necessary but not possible as South Asian countries are highly populated and needs more and more foods which are getting costly day by day. We are educated in science but still we are ill minded as we want to develop poison, hydrogen bomb and nuclear power for mass destruction, not for generating good civilization! How we define human is best creature in this Earth which is still fabricated with many small countries having no scientific development but fight each other like primitive age peoples! We need one nation research and development plans, so all discoveries reach common man at reduced cost. If bacteria can save itself from drugs, then we have to develop new technology to control pathogens. We have new brands of computer, aeroplane, mobile phone, paint, cosmetics, detergent, company with share market, car but we cannot say a name of new antibiotic that available this year against MDR bacteria [19].

It is noteworthy to state the mechanisms that bacteria developed. The first antimicrobial machinery bacteria were created was small drug resistant plasmid or R-plasmids. During the discovery of restriction enzymes and DNA ligase as well as bacterial transformation principle, we developed plasmid pBR322 from un-characterized three resistant plasmids isolated from ampicillin and tetracycline resistant bacteria (1950s). Very soon we made probe to locate *mdr* genes in many bacteria known as Southern hybridization (1970s) and also able to locate such genes in unknown plasmids by PCR reaction (1980s). Finally, di-deoxy Sanger DNA sequencing principle helped to understand genetic structure of *mdr* genes and their associated promoter-enhancer-repressor elements that were induced by antibiotics [6,20]. However, bacteria continued its mission to live in presence of antibiotics and created MDR conjugative plasmid combining R-plasmid with F'-plasmid generating a platform for more space for *mdr* genes in plasmid and more stability and also more active in participation of battle against antibiotics to transfer *mdr* genes to all resident intestinal bacteria to save symbiosis (**Figure 3**) (**Table 1**). So MDR conjugative plasmid now-a-day 50-500 kb and its sequence is known but the functions of 1/4 genes are still unknown [21].

We should not blame only prescription drugs for MDR bacteria generation. Well, large industry like mineral Industry, paint

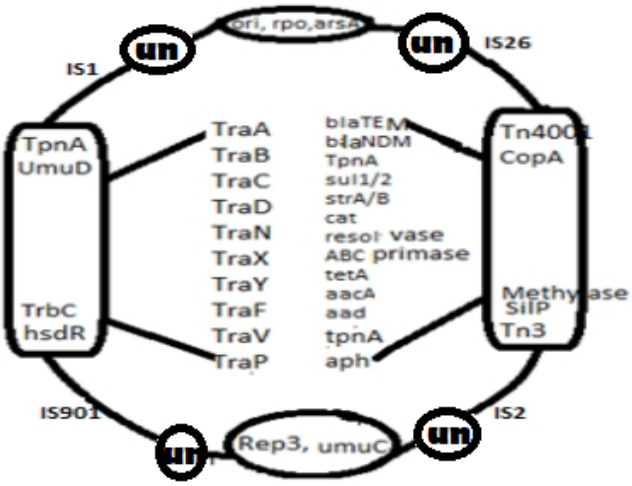

Figure 3 Prototype structure of MDR conjugative plasmid that contribute AMR and highly donate *mdr* genes to normal intestinal and river bacteria [10]. *Tra* genes codes proteins for sex pili and un represent unknown genes that likely are involved in AMR (plasmids pKPX-1/2, pNDM-HK, pCTXM-637 and also accession numbers, AP012056, KC543497, JN420336, CP011634, HG530658 and LN555650).

industry, drug industry, paper industry, petroleum industry and excreta from 100 million peoples in many big cities (New York, Mumbai, Kolkata) release tons of chemicals, antibiotics, heavy metals and organic nutrients into river water that are mostly harmful to bacterial central dogma enzymes, but organic nutrients do foster bacterial growth facilitating a huge platform of mutant bacteria creation other than human intestine [22]. So apart from MDR genes (*bla, cat, neo, aac, aph, amp*); bacteria also created many drug efflux genes by modification of >200 ABC transporter genes which are ATP-driven network of 12-14 channels membrane proteins involved in nutrient transport across the membrane into cytoplasm [23] (**Figure 4** for many *mdr* genes). As toxic chemicals and heavy metals are increased in water, it is necessary to develop more gateways to remove toxic chemicals from bacterial cytoplasm. Now few thousand drug efflux genes were sequenced, but their roles in removing antibiotics remains a field of active research. Never-the-less, RND, MFS, MATE types drug efflux genes and proteins have well studied with their capacity to remove penicillins, aminoglycosides and fluroquinolones antibiotics [24]. Perhaps most important frightening fact is such genes are also getting accumulated in MDR conjugative plasmids and chromosome. A 150 kb IncA/C plasmid pMRV150 in *Vibrio cholerae* 0139 strain was found resistant to common antibiotics, ampicillin, tetracycline, gentamycin and chloramphenicol. A IncC hybrid 165 kb plasmid in *Proteus mirabilis* was discovered in 2017 with 15 *mdr* genes including most deadly blaNDM-1 and blaCTX-M-65 and indicated that how severe recombination was facilitated in the human intestine during antibiotics exposure [25]. We detected many *mdr* genes in Kolkata superbugs (accession numbers: KU898253, KY769876-

Table 1 Multidrug-resistant enzymes with their NCBI GenBank ID and functions contributing AMR [10]. Accession numbers are useful to retrieve primary amino acid sequence and crystal structure of MDR proteins (www.ncbi.nlm.nih.gov/protein).

Major Characteristics of Different MDR Genes in bacteria and Sequence information				
Genes	**Subclass**	**AA**	**Protein Id number**	**Function of the MDR proteins**
bla	blaTEM	286 aa	AAB59737, ABI20744	Degrade ampicillin, amoxycillin
	blaCTX-M	291 aa	ABN09669, CAA63262	Degrade cefotaxime as well
	blaKPC	293 aa	AAG13410, ACB71165	Degrade cephalosporin and imipenem
	blaNDM1	270 aa	AGC54622, AFQ31613	Degrade carbapenems as well
	blaOXA1	276 aa	AFG30109, CAC82805	Degrade oxacillin better than ampicillin
	blaAMP-C	382 aa	AAD28044, AHN62490	Degrade early penicillins but imipenem
tet	tetA	399 aa	CAA53389, AHC55487	Kicks out tetracycline
	tetB	401 aa	AKJ20239	Tetracycline efflux isomer
	tetC	396 aa	AGL61405	Remove tetracycline better
str	strA	267 aa	AAA26443, ALF35537	Phosphorylate streptomycin
	strB	278 aa	CED95339, AFU91634	Phosphorylate streptomycin
cat	catB3	210 aa	ABP52023, ABC69169	Acetylate chloramphenicol
aac	aacA1	185 aa	BAB72153, BAO48019	Acetylate aminoglycosides
	aacA4	152 aa	AEZ05102, AFV31448	Acetylate ciprofloxacin
	aac(3')	286 aa	AAA21890	Acetylate tobramycin
aad	aadA1	263 aa	AAK13440, ABG23480	Adenylate drugs like streptomycin
	aadA4	263 aa	AAV34365	Adenylate many drugs
aph	aph(6')	266 aa	CAA25854, CAA68516	Phosphorylate aminoglycosides
	aph(4')	341 aa	CAA24743, CAA27276	4'-Phosphorylating isomers
	aph(3')	264 aa	AAA85506, CAA23892	3'-phosphorylating enzyme
RND	acrAB/EF	1027aa	WP_001132469	Acriflavin type efflux proteins
	mexAB	1045aa	WP_023101049	Doxorubicin efflux isomer
MFS	norA	388 aa	BAA14147	Norfloxacin efflux enzyme
	mdtE	385 aa	ABG71588	Fluoroquinolone efflux isomer
ABC	macB	664 aa	EMU20415	Macrolides efflux proteins
	DrrA	316 aa	CCK28451	Drug efflux 12 channels membrane protein
SMR	Mmr	107 aa	KPU48951	Drug efflux 4 channels protein
	emrE	110 aa	CAA77936	Drug efflux small isomer
EMR	emrA/B	~243aa	CAA26964, CAA68299	Drug efflux protein
MCR	mcr-1	541aa	WP_049589868	LipidA-phosphoethanolamine transferase
VanR	vanA	287aa	AKE81063	Vancomycin resistant proteins
DHPS	sul1/2	~279aa	AKG90139, BAN19562	Sulfamethaxozole resistant protein isomers

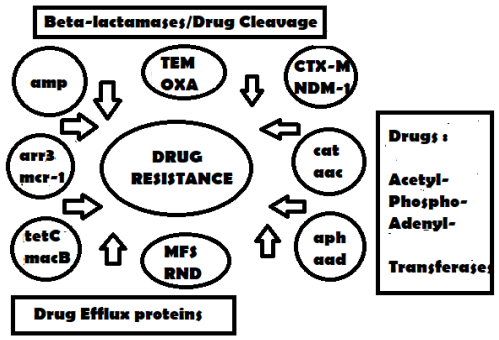

Figure 4 Demonstration of some *mdr* genes that all contribute to AMR. Mostly 20 types Beta-lactamases with few thousand mutant isomers are important, then drug modifying enzymes (acetyl-, Phospho-, and adenyl-transferases) are highly contaminated, as well as many drug efflux genes including *tet* and *mex* isomers. Further *sul1/2, mecA, penA, gyrAB, strAB, dhfr mdr* genes are also highly contaminated (not shown here).

KY769883) and confirmed by PCR and DNA sequencing (accession number: KY769875 and in preparation) (**Figure 5**).

The adaptation of environment is natural, and bacteria also continued its mission to defect antibiotics by mobilizing *mdr* genes and drug efflux genes (*acrAB, mexCD, norA, macAB*) into bacterial chromosome and such genetic islands are well characterized in *Escherichia coli, Salmonella typhi*, *Pseudomonas aeruginosa, Mycobacterium tuberculosis, Helicobacter pylori* (cag/T4SS islands) and *Acinetobacter baumannii* [26-28]. That is not the end, porin membrane proteins are also mutated in such a way that antibiotics receptors are altered, and no drug could enter into bacteria at low drug concentration giving MDR. Further, ribosomal ribonucleic acids (23S, 16S rRNA genes) are gathered few mutations (usually very conserved) as well as ribosomal proteins with alteration of drugs interactions causing MDR. In one word, bacteria have achieved many shrouds against antibiotics and drug companies did not know where to start [29].

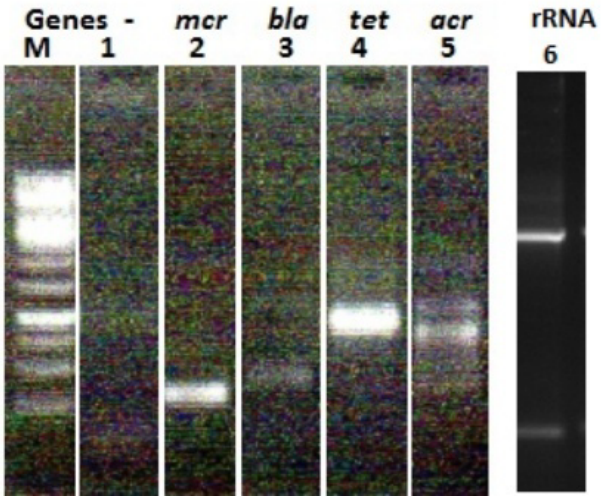

Figure 5 Detection of *mdr* genes by PCR using sequence specific primers, dXTPS, $MgCl_2$, Taq DNA polymerase and plasmid from a MDR *Escherichia coli*. The reactions were run on a 1% agarose gel at 2V/cm in 1x TAE buffer, stained with 0.5 µg/ml ethidium bromide and was taken photograph under UV illumination. The result on one MDR bacteria from Kolkata superbug is presented here indicating plasmid has *tet, mcr, bla,* and *acr* genes [32].

Conclusion

WHO warned that if alternative to antibiotics would not discovered soon, 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 [30]. However, gene medicines (ribozymes, miRNA, antisense RNA) and DNA nanotechnology have been welcome to stop the horror of MDR bacterial pathogenesis. More sadly, bacteria have acquired promoter induction system by antibiotics and many transcription factor repressors (tetR, mtrR, acrR) have been found in conjugative plasmids [31]. Those TFs induced efflux pumps (mexAB, mtrCDF) and Beta-lactamases (blaNDM1, blaOXA23) could destroy antibiotics and such mechanisms remain elusive. In such a situation, heterogeneous phyto-antibiotics and gene medicines (antisense RNA, ribozyme, Caspase-Cas, miRNA) with different efficient drug delivery modes like DNA nanotechnology are important R and D for new hope. Phage therapy research has forwarded strongly and is waiting for FDA approval [20]. WHO suggested that all countries should follow AMR Action Plan by increasing R and D for new therapeutic intervention as well as by reducing antibiotic use in human, food animal and agricultural land. In other words, we need strong country, one nation platform to keep this Earth as good habitat for human and we have to culture science at every step for our growth. As rate of MDR generation against common antibiotics was high (5% to 10% per year) with 20% to 40% bacteria in water and >95% human pathogens are ampicillin resistant now, it could be assumed that bacteria won the battle against antibiotics and we had entered in an antibiotic dark age as before 1926 [32]. Most importantly, we have houses, schools and shops but no play grounds causing obese, hypertensive and diabetic children with weak immune system that may also contribute a wild spread of tuberculosis (TB) in India. Indian Government has lunched Ganga River Mission but no way we can remove the MDR bacteria and fungi from its water which was consumed raw in religious occasion. In South Asian countries phyto-antibiotic research has given priority [33,34]. But we also need gut microbiota protection awareness and reduced antibiotic use [18].

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