CHAPTER

10

Bad Bugs, No Drugs: The Saga of Antibiotic Resistance

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INTRODUCTION

The word "antibiotic" was coined by soil microbiologist Selman Waksman, the nobel prize-winning discoverer of Streptomycin. Antibiotics are among the most important discoveries of medical science. It is not necessary to re-emphasize the important role antibiotics have played in saving millions of lives till date. Anne Sheafe Miller was the first patient to be saved by antibiotics. Medicine had failed Miller. During four weeks of treatment her temperature soared above 106 degrees, and no medications, not even sulfa drugs, had broken the fever. She was dying of streptococcal septicemia. A sample of Penicillin was arranged from Dr. Howard Florey (who expanded on Alexander Fleming's 1928 discovery of penicillin by isolating its active ingredient and demonstrating its therapeutic properties). Miller began receiving her first dose via intravenous drip at 3:30 p.m. on a Saturday. The next morning her temperature, which had hovered between 103 and 106.5 degrees, dropped to normal for the first time in four weeks. By Monday her appetite had returned and she had eaten four full meals. Her bacteria count dropped.[1] Miller owed her last 57 years of life to antibiotics.

Contrary to the popular belief that exposure to antibiotics is a benefaction of the modern era, traces of antibiotics have been found in the skeletal remains of the ancient humans from Africa.[2-4] The presence of antibiotics in the bones can be accounted for only by acknowledging the consumption of these compounds in their diet. Similarly, anecdotes about the antibiotic-like properties of red soils in Jordan that were used historically for treating skin infections, discovery of anti-malarial artemisinin from Artemisia plants (which were used by Chinese since ages as a remedy for many illnesses) and presence of active antimicrobial components in many of the ancient herbal remedies corroborate that antibiotics were in use much before the advent of 'antibiotic era'.

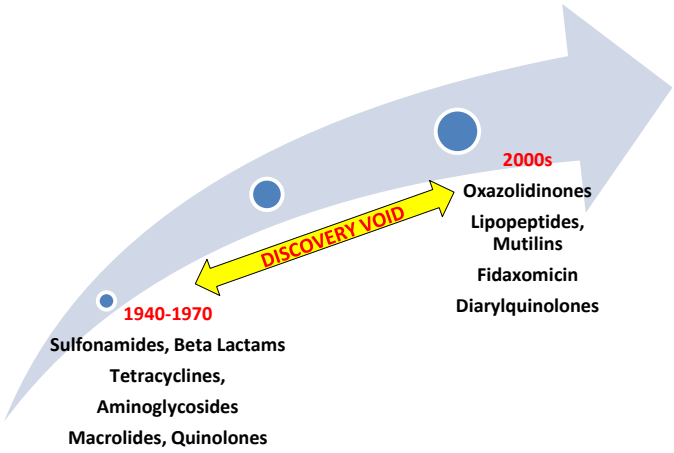

Fig. 1: Timeline showing discovery of new antibiotics

THE ANTIBIOTIC ERA

The foundation of modern antibiotic era is attributed to three exemplary discoveries by Paul Ehrlich, Josef Klarer and Alexander Fleming. Ehrlich is credited with the discovery of Arsphenamine (Salvarsan; first organic antisyphilitic); Klarer synthesized Prontosil (Sulfa drug) and Fleming discovered Penicillin.[5-7] These impeccable milestones in the antibiotic history led to the development of a number of new classes of antibiotics, many of which made their way to the patient's bedside (Figure 1).

As is evident in Figure 1, the period from 1950s to 1970s was the golden age for discovery of new classes of antibiotics, with no new structural classes of antibiotics introduced between 1970s and 2000, representing a serious innovation gap during the genomic era. Therefore, with the decline in the rate of discovery of new drugs, the main approach of combating resistance in the pathogens was modification of the existing classes of antibiotics. As a consequence of this 'discovery void' and 'adaptation' of pathogenic microbes to the available antimicrobials, antibiotic resistance has emerged as the deepest and the gravest crisis staring at the medical fraternity worldwide. A 2011 national survey of infectious disease specialists, conducted by the IDSA Emerging Infections Network, found that more than 60% of participants had seen a pan-resistant, untreatable bacterial infection within the prior year.[8] In 2013, the CDC declared that the human race is now in the "post-antibiotic era," and the World Health Organization, one year later, warned that the antibiotic resistance crisis is becoming dire.

SUPERBUGS WITH SUPER-RESISTANCE

'Superbugs' are the microbes associated with significant morbidity and mortality subsequent to multiple mutations conferring high levels of resistance to the antibiotic classes specifically recommended for their treatment. The microbes which acquire this 'super-resistance' have increased virulence and transmissibility and limited therapeutic options. A superbug infection leads to prolonged duration of hospitalisation as well as increased cost of treatment. These include infections with Acinetobacter baumannii, Burkholderia cepacia, Campylobacter jejuni, Citrobacter freundii, Clostridium difficile, Enterobacter spp., Enterococcus faecium,

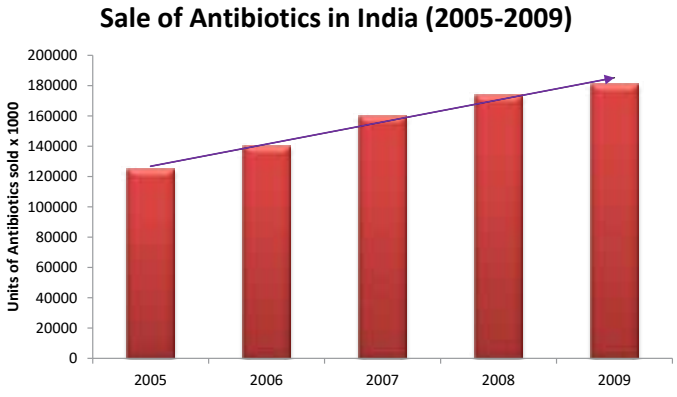

Fig. 2: Antibiotic sales in India by type[12]

Enterococcus faecalis, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella spp., Serratia spp., Staphylococcus aureus, Staphylococcus epidermidis, Stenotrophomonas maltophilia, and Streptococcus pneumoniae.

Among gram-positive pathogens, resistant S. aureus and Enterococcus species currently are the biggest threats. Methicillin Resistant Staphylococcus aureus (MRSA) is amongst the most frequently occurring of all antibiotic resistant threats. (10) Resistant S. pneumoniae infections are another leading cause of death among adults 50 years of age or older. Nearly one-third of severe S. pneumoniae cases are fully resistant to one or more clinically relevant antibiotics.(9) M. tuberculosis can also be resistant to one or more of the first-line drugs. Treatment of drug-resistant TB is complex. Extensively drug-resistant TB (XDR-TB) has fewer treatment options available for patients and that too at the expense of efficacy.(9) An enzyme called New Delhi metallo-beta-lactamase (NDM-1) is present in some gram negative Enterobacteriaceae including E.coli and Klebsiella that makes them resistant to virtually all beta-lactams, including carbapenems. Many of the bacterial pathogens associated with epidemics of human disease have evolved into multidrug-resistant (MDR) forms subsequent to antibiotic use. Acinetobacter baumannii is a more recent nosocomial Gram-negative pathogen, which derives its infectious properties from its robust survival and biodegradation capabilities in the environment and high rates of natural transformation. Recent genome sequence studies have identified at least 28 genomic islands encoding antibiotic resistance determinants. (11) Some strains of MDR P. aeruginosa have been found to be resistant to nearly all antibiotics, including aminoglycosides, cephalosporins, fluoroquinolones, and carbapenems. However, in terms of the number of infections and consequences, Vibrio cholerae should be at the head of the superbug list.

The CDC assessed antibiotic-resistant bacterial infections according to seven factors: clinical impact, economic impact, incidence, 10-year projection of incidence, transmissibility, availability of effective antibiotics, and barriers to prevention. Based on these, the threat level of each bacterium has been classified as "urgent," "serious," or "concerning". The 'urgent threats' include Clostridium difficile, Carbapenem resistant Enterobacteriaceae, Drug resistant Neisseria gonnorrhoeae. Multidrug resistant Acinetobacter, Pseudomonas, Salmonella, Shigella; drug resistant Streptococcus, tuberculosis, Campylobacter, Candida; extended spectrum beta lactamase producing Enterobacteriaceae (ESBLs) complete the list of 'serious' threats. Vancomycin resistant Staphylococcus aureus, erythromycin resistant Group A Streptococcus and clindamycin resistant Group B Streptococcus are the 'concerning' threats.[9]

CAUSES OF ANTIBIOTIC RESISTANCE

Antibiotic resistance is a complex problem requiring a collaborative effort of microbiologist, ecologist, health care specialist, educationist, policy makers, legislative bodies, agricultural and pharmaceutical industry and the public to deal with. There are no examples of antibacterial agents against which bacteria have not been able to develop resistance.l[9] In the last 15 years, significant deficiencies have occurred in the development and availability of new antibiotic (discovery void). Therefore, the implementation of strategies to preserve the activity of existing antimicrobial agents has become an urgent public health priority. By knowing how resistance evolves and spreads in a population, steps can be introduced to prevent or at least delay the spread.

The various mechanisms of development of antibiotic resistance can be 'bullet' or'target' related.The 'bullet' (drug) related mechanisms are: modification (so the efficiency is lost, as in the case of acetylation of aminoglycosides), destruction (as the beta-lactam antibiotics by the action of beta-lactamases), and expulsion (pumped out from the cell as in efflux pump mechanisms of resistance). Target (microbe) related means can be: protection by modification (mutations in RNA polymerase conferring resistance to rifampicin; modification by an enzyme (methylation of an adenine residue in 23S rRNA making it insensitive to macrolides); replacement (ribosomal protection proteins conferring resistance to tetracyclines); and protection at cellular or population levels (formation of a protective barrier by secretion).

Overuse of Antibiotics

In 2011, the World Health Organisation (WHO) warned: "Combat Drug Resistance – No Action Today, No Cure Tomorrow." The slogan was coined, urging governments to ensure responsible use of antibiotics in order to prevent drug-resistant viruses and bacteria, or 'super bugs'. Brazil, Russia, India, China, and South Africa account for 76 percent of the increase in antibiotic use around the world. A combination of increasing income and affordability, over the counter availability of antibiotics, willingness of physicians to prescribe antibiotics freely, patient expectations and a high background of infections that should ideally be contained by better sanitation and vaccination have contributed to the overuse. (Figure 2)

Epidemiological studies have demonstrated a direct relationship between antibiotic consumption and the

emergence and dissemination of resistant bacteria strains. While antibiotic resistance has predominantly been a clinical problem in hospital settings, recent data show resistant organisms have also been detected in patients in primary care. Most of the antibiotics are unregulated and available over the counter without a prescription. Antibiotics remove drug-sensitive competitors, leaving resistant bacteria behind to reproduce as a result of natural selection. This lack of regulation results in prescription of antibiotics that are easily accessible, plentiful, and cheap, which promotes overuse. Apart from increasing the resistance, antibiotic overuse is associated with increased incidence of more severe diseases, increased duration of the disease, increased risk of complications, increased mortality rate, increased healthcare costs, increased risk of adverse effects and increased medicalization of self-limiting infectious conditions. A reduction in antibiotic consumption leads to a reduction of resistance. Bergman et al. in their study focusing on macrolide resistant Streptococcus pyogenes showed that a reduction in macrolide use is associated with reduction in antibiotic resistance (9.2% in 1997 to 7.4% in 2000).[13]

Inappropriate Prescribing

Antibiotic prescribing is influenced by several factors, including cultural aspects, socio-economic factors, cultural beliefs of the patient and the prescriber and patient demand. According to the CDC vital signs report, about one-third prescriptions to treat urinary tract infections and prescriptions for the critical and common drug vancomycin included a potential error i.e. many patients are given drugs without proper testing or evaluation, or were given drugs for too long. Clinicians in some hospitals prescribed three times as many antibiotics than actually needed. Inappropriately prescribed antibiotics contribute to the promotion of antibiotic resistant bacteria by supporting genetic alterations, such as changes in gene expression, horizontal gene transfer and mutagenesis; which can increase the virulence as well as transmission of the infectious agent. Literature review reveals that indications of antibiotic use, choice of the agent, dose and the duration of antibiotic therapy is incorrect in 30% to 50% of cases. 30% to 60% of the antibiotics prescribed in intensive care units (ICUs) have been found to be unnecessary, inappropriate, or suboptimal.[14] These incorrectly prescribed antibiotics have a questionable therapeutic benefit and expose the patients to potential complications of antibiotic therapy including strain diversification in organisms and induction of broad proteomic alterations.[15]

Extensive Use in Farm Animals

Approximately 80 percent of the antibiotics sold in the United States are used in meat and poultry production. These are said to improve the overall health of the animals and hence are widely used as growth supplements in livestock to produce larger yield and a higher-quality product. The antibiotics used in livestock are ingested by humans when they consume food. Antibiotic use in food-producing animals kills or suppresses susceptible bacteria, allowing antibiotic-resistant bacteria to thrive which are then transmitted to humans and may lead to adverse health consequences. Both resistant bacteria, as well as significant volumes of antibiotics consumed, are then excreted by animals releasing resistant bacteria into the environment as well as causing the environment to be contaminated with antibiotics, providing further opportunities for exposure to bacteria and creating additional selective pressure that leads to the development of drug resistance.

Colistin is the last resort antibiotic against multi-resistant bacteria, especially those resistant to carbapenems. Liu and colleagues examined areas in China where colistin is routinely given to pigs and they found colistin-resistant E. coli in more than 20 percent of animals and in 15 percent of raw meat samples. Among these bacteria, all had colistin resistance that could easily be transferred between different bacteria. They also found that about one percent of hospital patients sampled were infected by E. coli or Klebsiella bacteria that had the same piece of DNA, making them resistant to colistin too, thereby highlighting the need for a more cautious, preventive approach.[16]

Antibiotics (tetracyclines and streptomycin) are also sprayed on fruit trees as pesticides in the west, resulting in a considerable geographical spread and exposure of microorganisms in the environment to growth-inhibiting agents. The precise impact of agricultural antibiotic use on resistance levels in the general population is not known anywhere, but the evidence points to a link.

Lack of New Classes of Antibiotics

The vast majority of antimicrobial classes in use today have been isolated in the golden era of antibiotic discovery from a small number of ecological niches and taxonomic groups. So the treatment options for already existing multidrug-resistant bacterial infections are limited, resulting in high morbidity and mortality. The development of newer antibiotics is driven by two factors: economy and regulations. Investing into the development of newer antibiotics is considered a poor economic decision for a pharmaceutical as antibiotics are not as profitable as drugs that treat chronic conditions. The net present value (NPV) of a new antibiotic is only about $50 million, compared to approximately $1 billion for a drug used to treat a neuromuscular disease.[17] In addition, difficulties in pursuing regulatory approval has essentially stalled the development of new antibiotics, leaving fewer options to treat resistant bacteria.

Genetics of resistance

The genetics of resistance is a poorly understood subject despite having been studied extensively. Putative antibiotic r genes are omnipresent in natural environments. Also there are a large number of low-molecular-weight natural products identified to have antibiotic activity in the laboratory. The science is still ignorant of the roles of millions of low-molecular-weight organic compounds that are produced by bacteria, other

microbes, and plants. However the existing knowledge on the molecular mechanisms of resistance to antibiotics in the animal kingdom suggests that these can be disseminated by one or more gene transfer mechanisms. Multidrug resistance in bacteria is a result of accumulation of multiple genes, each coding for resistance to a single drug, on R plasmids. The different phenomena associated with resistance development, which have been the focus of interest include: gene pickup, heterologous expression, horizontal gene transfer and mutations. (eg. a random mutation of genes encoding β-lactamase enzymes has lead to the emergence of an increasingly extended spectrum of resistance, a specific rRNA modification that engenders resistance to all antibiotics acting at 50sRNA has been described).[18] Integrons are especially powerful in producing multidrug resistance because they assemble several resistance genes in a correct orientation on the R plasmid and provide a strong promoter for their expression. Another mechanism of multidrug resistance is the active pumping out of drugs by multidrug efflux pumps (for example, the RND superfamily pumps in gram-negative bacteria, the MFS, ABC, SMR and MATE superfamilies in both gram negative and gram positive bacteria). These are especially important because they are usually coded by chromosomal genes and can be overexpressed easily. In addition these can pump out most of the antibiotics currently in use.

HOW TO CONTROL OR REDUCE ANTIBIOTIC RESISTANCE DEVELOPMENT

Antibiotic resistance is a universal and inevitable phenomenon. Over the years a large number of solutions have been proposed by knowledgeable experts and all the major international health groups. The various proposals for action are discussed.

Development of national action plan and guidelines on antibiotic use: Such action plans serve as a road map to achieve the goal of slowing the emergence of resistant bacteria and prevent the spread of resistant infections. In June 2016, National Centre for Disease Control in collaboration with Ministry of Health and Family Welfare, Govt. Of India has issued National Treatment Guidelines for Antimicrobial Use in Infectious Diseases.[19]

International collaboration: At UN, in September 2016, world leaders, for the first time committed to taking a broad, coordinated approach to address the root causes of antibiotic resistance across multiple sectors, especially human health, animal health and agriculture. This international co-ordination for antibiotic-resistance prevention, surveillance, control, and antibiotic research and development is expected to impact positively on the currently grim scenario.

Establishing a database for antibiotic use and resistance: There is an urgent need to establish methods for surveillance on antibiotic consumption and resistance profiles by microbial species, drug, and date; which can serve as a guide on the future use of antimicrobials.

Avoid inappropriate antibiotic use: Approximately 60% of the adults who seek medical care for viral infections are prescribed antibiotics due to various reasons discussed previously. A 2005 Cochrane review concluded that the only intervention sufficient to impact bacterial resistance was "delayed prescription," meaning that antibiotic prescriptions are to be filled a few days later if symptoms do not improve.[20] The important part is also to comply with the drug use regimen. The contributing factor to the dissemination of antibiotic resistance, even in the case of absolute compliance, may be the practice of empirical prescription of antibiotics.

Addressing antibiotic abuse in farm animals: There is good evidence that use of antimicrobials in farm animals has serious consequences to human health and no clear benefit to farmers, although quantification of this effect is a tough task. Bans on antibiotic abuse in farm animals in the European Union have been accompanied by significant declines in antibiotic resistance in humans and animals.[21]

Prevention of nosocomial infections: Implementation of the scientific advances to practice to reduce rates of multiple common nosocomial infections including Clostridium difficile–associated diarrhea, ventilator-associated pneumonia, catheter-associated urinary tract infects, selected surgical site infections, and MRSA bacteremia is the need of hour. Inpatient Healthcare Providers need to

- Know what types of drug-resistant infections are present in their facility and patients
- Request immediate alerts when the lab identifies drug-resistant infections
- Prescribe antibiotics wisely, de-escalate the antibiotics based on culture sensitivity analysis
- Remove temporary medical devices such as catheters and ventilators as soon as they are no longer needed
- Develop an antibiotic stewardship programme and institutional antibiotic policy to preserve and properly use existing antibiotics.

Promote development of newer antibiotics: Strict regulatory barriers in the past two decades hampered the research into development of newer antibiotics. However more recently, the regulatory authorities have relaxed the norms which are expected to encourage substantially smaller, less-expensive, and faster clinical trials. Newer approaches for antimicrobial development include:

- Manipulating bacterial signaling and communication: eg. probiotics
- Use of antibiotics in combination: eg. efflux pump inhibitors (EPIs) combined with antibiotics
- Increased sampling in diverse environments and increased application of the techniques of metagenomics

- To identify bioactive compounds produced by currently unknown and uncultured microorganisms

- Development of small-molecule libraries customized for bacterial targets.

Antibiotic recycling: The use of previously approved drugs and outmoded antibiotics show promise as an alternative combinatorial drug strategy for treating infections caused by drug-resistant bacteria.

CONCLUSION

Rapidly emerging resistant bacteria threaten the remarkable health benefits that have been achieved with antibiotics. Resistance mechanisms are pandemic and create an enormous clinical and financial burden on health care systems. We need to learn to be more precise in targeting the pathogens and limit the indiscriminate use of antimicrobials. There is no perfect antibiotic, and once the most appropriate use of any new compound is identified, it is essential that prescription of the antibiotic be restricted to that use only. Synchronized efforts to implement new policies, renew research efforts for development of new agents to treat bacterial infections and pursue the preventive strategies to manage the crisis, as discussed, are greatly needed.

REFERENCES

1. http://yalemedicine.yale.edu/autumn1999/features/capsule/55396/ (Accessed on 9th September, 2016)
2. Bassett EJ, Keith MS, Armelagos GJ, Martin DL and Villanueva AR. Tetracycline-labeled human bone from ancient Sudanese Nubia (A.D. 350). *Science* 1980; 209:1532–1534.
3. Cook M, Molto E and Anderson C. Fluorochrome labelling in Roman period skeletons from Dakhleh Oasis, Egypt. *Am J Phys Anthropol* 1989; 80:137–143.
4. Nelson ML, Dinardo A, Hochberg J and Armelagos GJ. Brief communication: mass spectroscopic characterization of tetracycline in the skeletal remains of an ancient population from Sudanese Nubia 350–550 CE. *Am J Phys Anthropol* 2010; 143:151–154.
5. Ehrlich P, and Hata S. Die Experimentelle Chemotherapie der Spirilosen. 1910. Berlin: Julius Springer.
6. Domagk G. Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *Dtsch Med Wochensch* 1935; 61:250.
7. Fleming, A. On antibacterial action of culture of Penicillium, with special reference to their use in isolation of B. influenzae. *Br J Exp Pathol* 1929; 10:226–236.
8. Spellberg B, Gilbert DN. The fuure of antibioyics and resistance: a tribute to a career of leadership by John Bartlett. *Clin Infect Dis* 2014; 59 suppl 2: S71-S75.
9. Centers for Disease Control and Prevention, Office of Infectious Disease. Antibiotic resistance threats in the United States, 2013. April 2013. Available at: http://www.cdc.gov/drugresistance/ threat-report-2013.
10. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. *Clin Opin Pharmacol* 2014; 18:56-60.
11. Gomez MJ and Neyfakh AA. Genes involved in intrinsic antibiotic resistance of Acinetobacter baylyi. Antimicrob. *Agents Chemother* 2006; 50:3562–3567.
12. Ganguly NK, Arora N K, Chandy SJ, Fairoze MN, Gill JS, Gupta U, et al. Rationalizing antibiotic use to limit antibiotic resistance in India +. *Indian J Med Res* 2011; 134:281-94.
13. Bergman M., Huikko S., Pihlajamäki M., Laippala P., Palva E., Huovinen P., et al. Effect of macrolide consumption on erythromycin resistance in Streptococcus pyogenes in Finland in 1997–2001. *Clin Infect Dis* 2004; 38:1251–1256.
14. Luyt CE, Brechot N, Trouillet JL, Chastre J. Antibiotic stewardship in the intensive care unit. *Crit Care* 2014; 18:480.
15. Viswanathan VK. Off-label abuse of antibiotics by bacteria. *Gut Microbes* 2014; 5:3–4.
16. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016; 16:161-8.
17. Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. *Clin Infect Dis* 2013; 56:1445–1450.
18. Long, K. S., J. Poehlsgaard, C. Kehrenberg, S. Schwartz, and B. Vester. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob. *Agents Chemother* 2006; 50:2500–2505.
19. www.ncdc.gov.in/writereaddata/linkimages/AMR_guideline7001495889.pdf. (Accessed on 4th September 2016)
20. Arnold SR, Strauss SE. Interventions to improve antibiotic prescribing practices in ambulatory care. Cochrane Database Syst Rev 2005;19:CD003539.
21. Aarestrup FM, Jensen VF, Emborg HD, Jacobsen E, Wegener HC. Changes in the use of antimicrobials and the effects on productivity of swine farms in Denmark. *Am J Vet Res* 2010; 71:726-33.