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ANTIBIOTIC RESISTANCE: A UNIVERSAL-SCALE COLLAPSE IN DRUG ADMIN

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ABSTRACT

The development of resistance to all kinds of antibiotics in the sensitive bacterial pathogens is a major challenge to infectious disease medicine. The astonishing effects of antibiotics and origin of the genes associated with resistance has been a long mystery. There is growing evidence that the genes that make up this environmental resistome have the potential to be transformed to pathogens and indeed there is some evidence that clinically relevant resistance genes have originated in environmental microbes. Understanding the extent of environmental resistome and its mobilization into pathogenic bacteria is essential for the management and discovery of antibiotics.

KEY WORDS: Antibiotic resistance, Drug administration, Environmental resistome, Host resistance, Efflux.

INTRODUCTION

Antibiotics are organic substances produced by microorganisms, capable of inhibiting the growth or destroying another microorganism at low concentrations.[1] The antibiotics field was initiated when Paul Ehrlich first coined the term ‘magic bullet’, or chemotherapy, to designate the use of antimicrobial compounds to treat microbial infections. In 1910, Ehrlich discovered the first antibiotic drug, Salvarsan, which was used to treat Syphilis. Later Alexander Fleming, discovered Penicillin in 1928. Then, in 1935, Gerhard Domagk discovered the sulfa drugs, that paving the way to the discovery of the anti-TB drug Isoniazid. Then, in 1939, René Dubos became the first scientist to discover an antibiotic after deliberately looking for it in soil microbes. Dubos discovered Gramicidin, which is still used

today to treat skin infections. Finally, in 1943, the first TB drug, Streptomycin, was discovered by Selman Waksman and Albert schatz. Waksman was also the one who coined the term 'antibiotics'. Thus, antibiotics have been used to treat bacterial infections since from 1940s.[2] Thereafter, microbial therapy made remarkable advances during the 20th century, resulting in the excess of optimistic view that infectious diseases would be conquered in the near future.[3] But, the capacity of microorganisms to acquire resistance to antimicrobial agents has surpassed our imagination, and in response to the development of antimicrobial agents, microorganisms have acquired resistance to drugs through a variety of mechanisms and continued to human beings. The ability of microorganisms to become resistant to the major therapies used against them has been recognized in present and the resistances of many of the isolates are increasing at variable rate. For example, the proportion of *Staphylcoccus aureus* resistant to Methicillin increased from the value of merely zero to 70% in Japan within the duration of 15 years. Increasing antimicrobial resistance (AMR) became a major threat to public health because it reduces the effectiveness of antimicrobial treatment, leading to increased morbidity, mortality, and health care expenditure.

Bacteria may be inherently or naturally resistant to an antibiotic, when inherent properties of the bacterium are responsible for preventing antibiotic action. Innate resistance is said to be possessed by bacteria naturally, for e.g. some bacteria are more resistant to certain antibiotics than others. For instance, gram-negative bacteria are inherently resistant to a number of antibiotics such as Vancomycin and fusidic acid, which are highly effective against Gram-positive organisms like S*treptococci* and S*taphylococci*, within the gram-negative group, *Pseudomonas aeruginosa* have notable intractable chemotherapeutic problem as it has an unusual level of intrinsic resistance to many antibiotics. This inherent resistance seems to be associated with the permeability of the complex outer layers of the cell envelope to some drugs, which prevents the attainment of an inhibitory concentration within the cell. The non-specific resistance of gram-negative bacteria is recognized as a limitation in the treatments of infections of these organisms. However, the general pattern of resistance is well known and stable, so that drugs are prescribed of which the infecting organism are not inherently resistant. Bacteria can develop resistance to antibiotics usually but not always after exposure to the antibiotics, this type of resistance results from changes in the bacterial genome. In bacteria, acquired resistance is driven by two genetic processes, which are mutation and selection that are sometimes referred to as vertical evolution and exchange of genes between strains and species which also called as horizontal evolution. In other words, acquired

immunity is a type of immunity acquired by the microorganisms in response to environmental and other changes. For instance, when a new antibiotic is introduced into clinical practice, for treating infections resulting from bacteria not inherently resistant to it, the drug would be quite effective in the beginning but later the treatment may result in failure which is due to newly acquired resistance to the organism due to the new drug was administered. The time before emergence of resistance and the rate of emergence may not be predictable. Resistance of *Staphylococci*, for instance, to Neomycin did not appear until after nine years of its introduction.[4]

The intense use and misuse of antibiotics are undoubtedly the major forces associated with the high numbers of resistant pathogens and commensal bacteria worldwide. Probably antibiotic-resistant bacteria bring out more sensation than its usage of the in animal husbandry and in cattle.[5] Microbial resistance to antibiotics is on the rise, in part because of inappropriate use of antibiotics in human medicine but also because of practice in the agricultural industry.[6] Antibiotic resistance operates through one of four general mechanisms.[7]

- Does not absorb the antibiotic (Reduced permeability)
- Expels it (Enhanced efflux)
- Degrades it (Enzymatic inactivation)
- Has altered the usual molecular target for the antibiotic so that the drug has no effect

Thus Antibiotic-resistant bacteria impose a substantial burden on the human population. In addition to morbidity and mortality caused by infections with resistant pathogens, society as a whole must pay for the development of new antibiotics to keep pace with continually evolving pathogens. It is clear, therefore, that there is a cost associated with antibiotic resistance from the perspective of human society.[8]

Emergence of Antibiotic Resistance in Bacteria

S. aureus is the most resistant bacterium familiar in the clinical setting. This bacterium rapidly acquired resistance to Sulfonamides and Penicillin which are supposed to be more effective initially to this microorganism, but resistant strains that produce penicillinase increased in the 1950s, thus penicillinase-stable methicillin was developed in 1960. However, in the following year, Methicillin-resistant *S. aureus* (MRSA) was isolated in the UK [**9**]. In the latter half of the 1990s, Vancomycin-intermediate *S. aureus* (VISA) was reported in

Japan. It is thought that thickening of the cell wall contributes to decreased sensitivity to this drug. On the other hand, Vancomycin-resistant *S. aureus* (VRSA) reported in the US seemed to acquire the resistance genes horizontally from vancomycin-resistant Enterococci (VRE)[10] *N. gonorrhoeae also* now has shown its ability to also develop high-level resistance to ceftriaxone, which is the last remaining option for empirical first-line treatment of gonorrhea. This STI pathogen seems to be evolving into a potential superbug and, in the near future, it will become untreatable.[11]

Functional roles of antibiotic resistance genes in their native hosts

If antibiotics have functions other than the killing of competitors, then the antibiotic resistant genes may have other functions than providing resistance. Even in antibiotic producers, the presence of an antibiotic resistance gene does not help to resist the action of antibiotic produced by the host. For example, *Streptomyces coelicolor* encodes proteins similar in sequence and indeed in mode of action to those involved in resistance to vancomycin in human pathogens, even though this microorganism does not produce glycopeptide antibiotics. An example of antibiotic resistance determinants with a functional role other than affording resistance is provided by multidrug resistance (MDR) efflux pumps. In bacteria besides offering resistance to antibiotics and other toxic compounds, they contribute to virulence, maintaining homeostasis, detoxification of intracellular metabolites, signaling network by efflux of signal compounds.[12]

Mechanisms of antibiotic resistance

Organisms that are normally sensitive to the action of an antibiotic may sometimes develop resistance or insensitivity to it. This may be due to destroying the antibiotic or by retaining their presence even in the presence of the drug. Antibiotic resistance in the microbes is now a wide spread problem and a serious clinical issue. The microbes develop resistant to antibiotics by any mechanisms like: selection, mutation, phage transduction, and transference while microbial resistance can either be inherent in the organism or acquire through the environment. Factors that have led to the continued occurrence of the bacterial resistance to antimicrobial agents include: over prescription of antibiotics, use of under dose, and use of antibiotic in animal husbandry and agriculture.[13] Antibiotic resistance can occur via three general mechanisms: prevention of interaction of the drug with target, efflux of the antibiotic from the cell, and direct destruction or modification of the compound.[14] *Pseudomonas aeruginosa* is an opportunistic human pathogen characterized by an innate resistance to

multiple antimicrobial agents. A major contribution to this intrinsic multidrug resistance is provided by a number of broadly-specific multidrug efflux systems, including MexAB-OprM and MexXY-OprM. In addition to these, two additional tripartite efflux systems, namely MexCD-OprJ and MexEF-OprN, promote acquired multidrug resistance as a result of mutational hyper expression of the efflux genes. In addition to antibiotics, these pumps promote the export of numerous dyes, detergents, inhibitors, disinfectants, organic solvents and homoserine lactones which involved in quorum sensing. The efflux pump proteins are highly homologous and consist of a cytoplasmic membrane associated with drug-proton antiporter of the Resistance-Nodulation-Division (RND) family, an outer membrane channel-forming protein [sometimes called outer membrane factor (OMF)] and a periplasmic membrane fusion protein (MFP). Homologues of these systems have been described in *Stenotrophomonas maltophilia, Burkholderia cepacia, Burkholderia pseudomallei* and the non-pathogen *Pseudomonas putida*, where they play a role in export of resistance to multiple antimicrobial agents and/or organic solvents. Although the natural function of these multidrug efflux systems is largely unknown, their contribution to antibiotic resistance and their conservation in a number of important human pathogens makes them logical targets for therapeutic intervention.[15]

Fluoroquinolones have some of the properties of an 'ideal' antimicrobial agent. Because of their potent broad spectrum activity and absence of transferable mechanism of resistance or inactivating enzymes, it was hope that clinical resistance to this group of drugs would not occur. However, over the years, due to intense selective pressure and relative lack of potency of the available quinolones against some strains, bacteria have evolved at least two mechanisms of resistance: (I) alteration of molecular targets, and (II) reduction of drug accumulation. DNA gyrase and topoisomerase IV are the two molecular targets of f1uoroquinolones. Mutations in specified regions (quinolone resistance determining region) genes coding for the gyrase and/or topoisomerase leads to an efflux pump effective in pumping out hydrophilic quinolones. New f1uoroquinolones recognizes both molecular targets and have improved pharmacokinetic properties offer hope of higher potency, thereby reducing the probability of resistance development. *Helicobacter pylori* a Gram-negative rod which colonizes in the stomach of approximately half the world's population. First identified in 1983 as a pathogen, it has now been accepted as the causative agent of several gastric disorders ranging from chronic active gastritis and peptic ulcer disease to gastric cancer. The recognition of *H. pylori* as a pathogen has had a significant impact on gastro enterologic

practice for diagnosis and treatment. The frequent indication of eradication therapy and the limited choice of antibiotics have resulted in the development of antibiotic resistance in *H. pylori*, which significantly impairs the treatment of *H. pylori*-associated disorders. The prevalence of antibiotic resistance of *H. pylori* shows regional variation per antibiotic, but can be as high as 95%. The molecular mechanisms of antibiotic resistance characterized thus far are all based on point mutations located on chromosome. This is in contrast with antibiotic resistance mechanisms observed in other bacteria, where they are frequently located on plasmids, transposons or integrons.

In *H. pylori*, the majority of antibiotic resistances probably arise through *de novo*. The *recA* gene encodes a protein that plays an essential role in DNA recombination and DNA repair. In *H. pylori*, a *recA* homologue has been identified and functionally characterized. *H. pylori* mutants with an inactivated *recA* gene are severely impaired in their ability to survive UV light treatment and exhibit enhanced susceptibility to metronidazole. Although transfer of the *recA* gene of several metronidazole-resistant *H. pylori* strains to metronidazole-susceptible *H. pylori* strains resulted in an increased MIC of metronidazole. Rifabutin and several other derivatives of rifampin are bactericidal antibiotics that bind to the β-subunit of DNA-dependent RNA polymerase, resulting in the inhibition of transcription. The β-subunit of this complex is encoded by the *rpoB* gene. Until a few years back resistance against rifamycins and rifabutin *in vivo* was very rare, however, the incidence of rifamycin and rifabutin resistance is increasing now. In *H. pylori* resistance to these antibiotics is linked to various point mutations in the *rpoB* gene.[16]

Raising antibiotic use

Antibiotic use has been increasing steadily in recent years; from 2005 to 2009 the units of antibiotics sold have increased about 40 per cent. Increased sales of cephalosporins were striking up to 60 per cent over that five-year period, but some increase was seen in most antibiotic classes. In comparison, a pilot survey conducted at private retail pharmacies in 2004[17] and a survey in the same areas in 2008 found increased use of cephalosporins, but decreased use of macrolides. The fact that antibiotic use increasing is not, itself the indicative of a problem, but evidence from studies of prescribing patterns suggests that antibiotics are often used in inappropriate ways.[18]

Resistance to antibiotics

Antibiotic resistance has a low-priority area in most developing and developed countries, when compared with HIV/AIDS, tuberculosis, malaria, pneumonia, and many other infectious diseases, the loss of antibiotics in future does not capture the same attention. Resistance against certain antibiotics is already at high levels in certain places in India (and around the world), but the problem has remained largely unknown because relatively few studies were published and nationwide surveillance was not being carried out. But the issue came to the fore in India when New Delhi metallo-ß-lactamase-1 (NDM-1), first reported in 2009, made front-page news in 2010. NDM-1 is an enzyme produced by the gene $bla_{\text{NDM-1}}$, named as New Delhi because Swedish patient in whom it was first identified had undergone surgery in a New Delhi hospital.[19] The gene was carried on plasmids and could be transferred between different bacterial species, between *Klebsiella pneumoniae* and *Escherichia coli* most importantly, conferred broad resistance to most antibiotics, including carbapenems. Later studies reported NDM-1 in a tertiary-care centre in Mumbai.[20] The controversy heated up when a paper reported the gene in multidrug-resistant *Enterobacteriaceae* in hospitals in Chennai and Haryana and in isolates from patients represented in the UK's National Reference Library (a high percentage of whom had travelled to the subcontinent).[21] A further study in which NDM-1 was detected in drinking water and sewage water in New Delhi[22] added to the concern and the focus on India.

Antibiotic resistance surveillance has been limited to small-scale efforts by the Indian Council of Medical Research (ICMR) and some private agencies on a pilot basis. The Invasive Bacterial Infection Surveillance (IBIS) project produced valuable information on pneumonia in India, though it was unable to meet its goal of establishing a permanent surveillance system for antibiotic resistance.[23]

Nosocomial infections by antibiotic resistant pathogens

The Center for Diseases control and Prevention (CDC), the Food and Drug Administration (FDA) and the National Institute of Allergy and Infectious Diseases (NIAID) states that antibiotic resistance is one of the world's most critical health problems.[24] Diseases such as tuberculosis, gonorrhea, bacterial pneumonia, and *Enterococcal*, *staphylococcal* infections are now more difficult to treat than their infection in decades ago.[25] Antibiotic resistance is an increasingly difficult problem in hospitals. About 25 to 35% of hospitals patients are under antibiotic treatment for active infections or to prevent potential infections. The large

volume of antibiotic use exerts enormous selective pressure for the emergence and spread of antibiotic-resistant bacteria. Therefore, untreatable bacteria, such as some strains of (VRE) Vancomycin-resistant *Enterococcus*, and hard-to-treat bacteria are much more common in hospitals than in the community. Between November 1990 and October 1992, 55 hospital patients infected with ceftazidime-resistant *E. coli, K pneumonia,* or both were identified. Of 35 admitted from 8 nursing homes, 31 harbored the resistant strain on admission. All the strains were resistant to Ceftazimide, Gentamicin, and Tobramycin, 96% were resistant to trimethoprim-sulfamethoxazole and 41% to ciprofloxacin hydrochloride. Plasmid studies on isolates from 20 hospital and nursing home patients revealed that 17 had a common 54-kilobase plasmid, which conferred ceftazidime resistance via ESBL TEM-10, and mediated resistance to trimethoprim-sulfamethoxazole, Gentamicin, and Tobramycin; all 20 isolates harbored this EBRL. Molecular fingerprinting showed 7 different strain types of resistant *E. coli,* and *K. pneumonia,* distributed among the nursing homes.[26]

Sources of resistance in environments

Although resistant organisms are found naturally in the environment, most resistance is associated with man-made impacts, either agricultural or direct human impact. Antibiotic use in humans can lead to resistance in the environment via discharge of domestic sewage, hospital wastewater, and industrial pollution. In addition to use in humans, antibiotics are added to animal feed to treat infections, as prophylactics, and in sub therapeutic doses as growth promoters. Although no definitive numbers are available, almost half of the antimicrobial agents used in the United States were used in animal feed. There are a variety of positive effects from using antibiotics in animal feed, namely, inhibition of harmful gut flora which leads to increased growth rates and decreased mortality which allowed more usage of antibiotics in the livestock management. However, this practice has resulted in selection of antibiotic resistant organisms in the guts of food animals, from which they enter human. In many countries, producers add antibiotics to the feed of terrestrial food animals in sub therapeutic concentrations (doses lower than those needed to treat infections) to improve growth of their livestock, thus acting as antibiotic growth promoters (AGPs). The mechanisms by which AGPs affect feed efficiency and weight gain are not fully known.

AGPs were introduced globally for routine use in feed, regardless of the animals' health status or the risk of bacterial infection which led to an explosive increase in the overall usage of antibiotics in many countries. For example, in the United States, the use of antibiotics as

AGPs increased fiftyfold between 1951 to 1978 (from 110 tonnes to 5580 tonnes), while there was only a tenfold increase in the use of antibiotics to treat infections in people and animals, during which many bacterial strains from both human and animal sources that were previously susceptible to antibiotics became resistant. Similar results were reported for several other countries, in England (United Kingdom), the prevalence of tetracycline-resistant *Escherichia coli* in poultry increased from 3.5% to 63.2% within four years (1957–1960) of the antibiotic's use in poultry.[27]

Poultry production

The poultry industry has become one of the largest livestock industries throughout the world. In 1991, 600 billion eggs and 40 million tons of poultry meat were produced globally. Antibiotics are highly used throughout the poultry production process. It is reported that 80% of the poultry raised in U.S are fed with antibiotics. Although many classes of antibiotics are used, fluoroquinolones, avoparcin, and virginiamycin are of precise concern, since resistance to these drugs is associated with resistance the human therapeutic drugs ciprofloxacin, vancomycin, and quinupristin/dalfopristin, respectively.[28]

The use of antibiotics in poultry leads to resistant organisms within the chickens and all over the production environment. Resistant strains of many organisms, including *Staphylococcus*, *Streptococcus*, *Clostridium*, *Pseudomonas*, and *Aeromonas*, have been isolated from these sources. Resistant *E. coli* and strains with MDR resistances to tetracycline, streptomycin, sulfonamides, gentamicin, fluoroquinolones, and virtually all other antibiotics, have been isolated. From live chickens oxytetracycline was identified and compared to a control group within 12 weeks, 70% of the *E. coli* isolates in the experimental group were resistant to more than two antibiotics, including Streptomycin and Ampicillin.[29]

Swine production

Pigs are grown in confinement like poultry, and both pneumonia and diarrhea are common problems. Most pigs are given antibiotics through their feed for growth promotion or disease prevention. In such instances, Tylosin, Sulfonamides, and Tetracyclines are the most commonly used antibiotics, but other drugs are also employed. Most reports on antibiotic resistance in isolates from swine involve *Enterococcus* spp. In 1998 and 1999 in Belgium, 82% of swine samples yielded *E. faecium* isolates resistant to tylosin and 97% resistant to Oxytetracycline.[30]

Cattle production

Antibiotics are used in cattle production in much the same way as in poultry and swine production. Cattle production includes beef, veal, and dairy cows with beef cows generally shipped to feedlots and kept in large groups. Antibiotics are administered individually or to groups of animals via food and water to achieve growth promotion or to prevent diseases, i.e., pneumonia and diarrhea.[31] In 1999, 83% of feedlots dispensed at least one antibiotic in food or water; these included virginiamycin, tetracycline, tylosin, and neomycin.[31] In 1999, 90% of veal calves and 60% of beef cows were estimated to have been fed antibiotics (Dairy calves are also housed in groups and are often administered antibiotics, including penicillin, cephalosporin, erythromycin, and tetracycline. The pathogens isolated from the cattle are *E. coli*, *Salmonella*, *Enterococcus*, and *Campylobacter* spp. were 20% of *Enterococcus faecium* isolates were resistant to ampicillin and 80% to tetracycline.[19]

Patterns of antimicrobial use and resistance

A community based survey, stool samples were obtained from 296 healthy children selected by modified cluster sampling in Camiri and all 25 eligible children in Javillo. In Camiri over 90% and in Javillo over 70% of children carry *E.coli* resistant to ampicillin, trimethoprim-sulphamethoxazole or tetracycline. Over, 63% of children carried MDR to ampicillin, TMP/SMX, tetracycline and chloramphenicol. In the simulated patients study, antimicrobials were dispensed inappropriately for 92% od adults and 40% of the children with watery diarrhea, and were under-prescribed for males with urethral discharge (67%) or females with fever and dysuria (58%). Thus existence of a large reservoir of resistance genes in healthy individuals in developing countries represents a threat to the success of antimicrobial therapy throughout the world.[1]

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