ANTIBIOTIC RESISTANCE - AN EMERGING HEALTH PROBLEM: CAUSES, WORRIES, CHALLENGES AND SOLUTIONS - A REVIEW

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ARTICLE INFO

Article History:
Received 12th April, 2013
Received in revised form 13th May, 2013
Accepted 16th June, 2013
Published online 18th July, 2013

Key words:
Antibiotic, Microorganism, Resistance, Multidrug resistance, Emergence, Alternative/complementary therapy, Detection, Public health, Antibiotic residues, Prevention, Control.

ABSTRACT

Untreatable bacterial infections become treatable due to the discovery of antibiotics in the previous century but their inappropriate and irrational uses ultimately led to emergence of resistant microbial population. Genes responsible for conferring resistance are transferred horizontally via conjugation; transduction or transformation. Tetracyclines and beta lactams represent 50% each of the total antibiotics used in feeds and global antibiotic consumption respectively. Due to development of antibiotic resistance there may be failure of the resistant bacteria to respond to the prescribed treatment; risk of infectious diseases becoming uncontrollable; financial burden; advanced therapeutic approaches may get jeopardized and ultimately resistant organisms may spread to distant countries and continents. Both intrinsic and acquired resistance mechanisms are involved in bacterial antibiotic resistance. Meticillin-Resistant Staphylococcus aureus (MRSA) and Vancomycin-Resistant Enterococci (VRE) are among the most striking antibiotic resistant microbes in the recent years. Factors driving antibiotic resistance include: inadequate national commitment; improper surveillance; irrational use of drugs; poor disease prevention and insufficient diagnostics and therapeutics etc. Limiting infectious diseases; judicious uses of antibiotics; precise selection and completing the full course of antibiotics; and regular surveillance, monitoring and continuous vigilance are the steps to limit antibiotic resistance. The most common antibiotic-resistant organisms sourced from animals are some strains of E. coli, Salmonella etc., which can also infect humans. Disc diffusion method and Minimum inhibitory concentration (MIC) methods; gas chromatography (GC); High-performance liquid chromatography (HPLC) with UV mass spectrometry (MS) and nano quantity analyte detectors; microfluidic methods and electrochemical methods; European Union four-plate test (EU-4pt); Pioneer Frontier Test (PFT) are used to detect antibiotic residues. Use of penicillin or sulphonamides has raised public health and industrial issues. Prevention and control measures require involvement of various governmental agencies for accurate testing and screening; surveillance and monitoring. Along with this alternative therapeutic approaches viz. bacteriophages; virophage and mycophage; avian egg yolk antibody; cytokines and herbal; panchgavya and vaccine therapy; and diagnostics are the need of hours. The present review discusses all these aspects of antibiotic resistance and their solutions ultimately for social benefit with particular reference to emerging antibiotic resistance in animals and humans, its challenges, detection, antibiotic residues, prevention and control measures along with current and future scenario at international level, which would be helpful for formulating strategies for safeguarding health of animals as well as humans.

INTRODUCTION

The discovery of antibiotics being the wonderful amiable discovery of previous century had turned the untreatable era of bacterial infections into treatable conditions (Waksman, 1973). With the invention of antibiotic drug Penicillin in 1928, Sir Alexander Fleming draw the attention of scientist to look for the microbial products to counteract the pathogenic effects produced by the microorganism until 1940 when reports of penicillinase enzyme appeared. In modern medicine antibiotics were well thought-out as pillars of chemotherapy, however efficacy of these medicinal molecules against many pathogens is vulnerable and threatened owe to developing antibiotic resistance which affects all types/classes of natural, semi-synthetic, and/or completely synthetic antibiotics (Walsh, 2003). Before 1940, antibiotics were considered as the magical drugs for the treatment of various bacterial ailments. But now, increasingly, bacteria are able to resist the curative effects of these medications. These drugs are also being used as growth promoters because as these may also influence the growth rate because of thinning of mucous membrane of the gut, facilitating better absorption and assimilation and producing favorable conditions to beneficial microbes in the gut of animal by destroying harmful bacteria (Brotze-Oesterhel and Brunner, 2008). The very frequent use of antibiotics in animals especially in cases of mastitis, febrile and inflammatory conditions, wounds and bacterial, and viral diseases to check secondary infections have widespread residual effects on products derived from animal origin viz., milk, eggs or meat and their by products. Antimicrobials are also used either directly or indirectly during the production processing, storage and transportation of these food products. Humans are the ultimate consumers of these products with antibiotic residues and thus the presence of these

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residues in routine diet may adversely affect the health. Antibiotics when used inappropriately and irrationally provide favorable conditions for development of resistant group of microbes that can spread very easily. The extraordinary genetic capacities of microbes have utilized the continuous survival pressure as a warning to their life. By expressing various resistance genes and all possible means of horizontal gene transmission in equilibrium with environmental microbiomes to support the rise of antibiotic resistance microorganisms are counteracting antimicrobial approaches, indeed (Sørensen et al., 2005; Walsh, 2006; Davies and Davies, 2010). Countless documents are available describing the genetics, origins, evolution, and mechanisms of antibiotic resistance. In the present review we will have a brief look on the underlying causes, genetic mechanisms including chromosomal or mobile genetic elements responsible for emerging resistance with relevant examples and steps to counteract the developing antibiotic resistance. Termination of the course of antibiotic without following prescribed format is a major cause of development of resistance worldwide. The socioeconomic factors at both individual as well as national level facilitate emergence and spread of antimicrobial resistance. Urban poor people exposed to inclement climate or due to their poor quality of living standard are more prone to develop resistance. The beta-lactam group of antibiotics is responsible for approximately 95% of all milk antibiotic contamination. In the developing countries nowadays multiple antibiotic resistance have been observed among important enteric pathogens viz., Escherichia coli; Salmonella and Shigella; Klebsiella; Vibrio cholerae which have increased the global worry (Calva et al., 1996; Murray and Lopez, 1997; Borroto et al., 2000; Aiello and Larson, 2003; Butaye et al., 2003; Byarugaba, 2004 and 2005; Soulsby, 2005; Aminov and Mackie, 2007; Aminov, 2009).

In these days, however, due to the advancement in the field of molecular biology and biotechnology to combat the antibiotic resistance arising due to the ever increasing threat of zoonotic diseases including food-borne zoonosis several new therapies have come into existence (Deb et al., 2013a; Dhama et al., 2013a,b,c; Tiwari et al., 2012; Mahima et al., 2012).

- The term “antibiotic resistance” is defined as “a property of bacteria that confers the capacity to inactivate or exclude antibiotics, or a mechanism that blocks the inhibitory or killing effects of antibiotics to which it was previously sensitive, leading to survival despite exposure to antimicrobials” (Whyte et al., 2002).
- This is a consequence of the use, particularly misuse and indiscriminate use (Low or high doses, inappropriate use, casual use without prescription), of antimicrobial medicines and develop when a microorganism mutates or acquires a resistance (R) gene (Zhang et al., 2006).
- The antibiotic resistance may disseminate in the nature either by clonal spread of resistant clonal lineages or by horizontal gene transfer methods (Witte, 2004).
- Journey of antibiotic has seen various phases comprised of dark ages, preantibiotic era; primordial phase including advent of chemotherapy via the sulphonamides; golden years, the halcyon ages, pre golden ages, pre golden phase.
- A wide array of biochemical and physiological mechanisms are responsible for development of resistance (Wright, 2007).
- Genes responsible for conferring resistance can be transferred horizontally between bacterial cells by conjugation, by involvement of bacteriophage through transduction or from environmental factors by transformation (Anderson, 1968; Hotchkiss and Gabor, 1970; Ciz et al., 2005; Mathew et al., 2007; Canton, 2009).
- Bacteria may acquire genes which encode for enzymes capable of destroying antibiotics hence nullifying the action of drug by being resistant (Abraham and Chain, 1940; Martinez et al., 2007).
- When a bacterium harbors many a resistance genes together, then it is called multidrug resistant (MDR), extremely drug-resistant (XDR) strains and/or totally drug resistant (TDR) strains as documented in cases of tuberculosis or informally, a superbug or super bacterium. Certain MDR pathogens may show resistance to second and even third-line antibiotics. They acquire them sequentially and such phenomenon is illustrated by Staphylococcus aureus when they pose a threat of nosocomial infection. Pseudomonas aeruginosa additionally is responsible for a greater degree of intrinsic resistance (Thomas et al., 1998; Girou et al., 2006; Wright, 2010).

- The resistant bacteria in animals can be transmitted to humans via three pathways:
  - Consumption of improper/undercooked contaminated meat, milk or eggs.
  - Close or direct contact with animals.
  - Through the environment (Hawkey and Jones, 2009).
- Unchecked/Uncontrolled and widespread application of antibiotics in both human and veterinary medicines is equally responsible for increase in prevalence of antibiotic-resistant bacterial infections, and emergence of new antimicrobial resistant pathogenic strains of various infectious agents (Hidron et al., 2008).
- A major source of antibiotic overseuse is the extensive use of these drugs in livestock sector for promoting the growth and production of animals including poultry for getting more profitability to compensate unsanitary conditions present in livestock industry and agriculture (Toole et al., 1997; Richet et al., 2001).
- Staphylococcus aureus is amongst important and major resistant bacterial pathogens to the general population. Methicillin-resistant Staphylococcus aureus (MRSA) is nowadays frequent in hospital settings. MRSA has become a major community-acquired (CA) pathogen CA-MRSA, with enhanced virulence and transmission characteristics. In United States, resistance to penicillin; Methicillin; tetracycline and erythromycin groups of drugs have become more common. The emergence of glycopeptide intermediate Staphylococcus aureus (GISA) or vancomycin-intermediate Staphylococcus aureus (VISA) have made the situation more critical (Skurray and Firth, 1997; Chan et al., 2011; Xie et al., 2011).
- Tetracyclines being used since many a years in livestock and poultry sector represent almost 50% of the total antibiotics used in feeds.
- B-lactams group of antibiotic used for treating the infectious diseases in food animals, represents 50% of the global antibiotic consumption (Hirakata et al., 2005; Okeke et al., 2005).

**Antibiotic resistance – An issue of global worry**

- Antimicrobial resistance is an alarming public health threat worldwide which demands scrupulous investigations (Levy and Marshall, 2004; Zhang et al., 2006).
- Infections caused by resistant bacteria often fail to respond to the standard/prescribed treatment, which results into prolonged illness and greater mortality risk or death. Many pathogens have developed resistance over the years in different regions of the world including developing nations (Byarugaba, 2005; Islam, 2007).
- Antibiotic resistance reduces the effectiveness of treatment because animals/patients remain chronically ill, thus potentially spreading resistant microorganisms to others (Mitema et al., 2001 and 2004).
- Many infectious diseases from becoming uncontrollable could overwhelm the progress made towards reaching the targets of the health-related Goals of United Nations Millennium Development (MDGs) set for 2015 (Primhomed et al., 2007).
- This gradual increase in antibiotic resistance may add to financial burden to the general public because of the inefficacy of first line medicines (Larsson et al., 2000).
- In absence of effective antimicrobials against infections, the success of treatments such as organ transplantation, cancer
chemotherapy and major surgery would be jeopardized (Radyowijati and Haak, 2002).
• With the continued emergence of multidrug resistant strains, the future may again be in hopeless conditions of pre-antibiotic era of untreatable infections (Boucher, et al., 2009; Rossolini and Thaller, 2010).
• In present scenario of globalization, resistant microorganisms may spread rapidly to distant countries and continents. Ever changing characteristics of microbes; selective pressures in the use of antibiotics along with social and technological changes all have acted as contributory factors (Aarestrup et al., 2001; Nugent et al., 2008).

Classical examples of antibacterial resistance

The development and distribution of antibiotic-resistant microbes in the biosphere is one of the good examples of the Darwinian theory of selection and survival of fittest. Reason behind this is anthropogenic due to misuse or overuse of such drugs superimposing selection pressure on the nature. The indiscriminate use of antimycotic agents and antibiotics to promote growth of farm animals and to prevent their infection rather than to cure infections most of times lead to drug resistance, emergence of antibiotic-resistant pathogenic microbes and development of “superbugs” and super-resistant strains, microbes harboring enhanced morbidity and mortality potentials due to multiple mutations endowing high levels of resistance against antibiotic drugs and thereby reduces the efficacy of the antimicrobials in the battle with various infections. (Meynell and Datta, 1967; Bryskier, 2005; Liu and Pop, 2009). Literature reveals more than 20,000 potential resistance genes (r genes) are existing in nature which are of nearly 400 different types among various species, fortunately they all have not been expressed yet (Depardieu et al., 2007; Dantas et al., 2008).

Some of the common examples of MDR organisms are Methicillin-Resistant Staphylococcus aureus (MRSA), Vancomycin-Resistant Enterococci (VRE) among Gram positive bacteria, Klebsiella pneumoniae carbapenemase (KPC) producing Gram-negatives, Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria, Multidrug resistant S. enteritica serovar Typhimurium DT 104 (ACSSuT phenotype), Imipenem-resistant or MDR Organisms Acinetobacter baumannii, Acinetobacter lwoffii, Pseudomonas aeruginosa, Bacteroides spp., Clindamycin-resistant Clostridium difficile, Streptomycin-resistant Thermus thermophilus, E. coli resistant to multiple fluoroquinolone, resistance of Mycobacterium tuberculosis to isoniazid, rifampin, and multiple antifungal resistant Scenedosporium prolificans infections (Gregory et al., 2001; Shoemaker et al., 2001; Traksaomoon et al., 2001; Enright et al., 2002; Goddard et al., 2003; Inoue et al., 2004; Nandi et al., 2004; Gomez and Neyfakh, 2006; World Health Organization, 2006; Perez et al., 2007; Reinert et al., 2007; Boucher and Corey, 2008; Maragakis and Perl, 2008; DeLeo and Chambers, 2009; Li et al., 2013). E. coli has developed low-level of resistance against fluoroquinolones and ciprofloxacin due to activity of qnrA1 and qnrS1 genes and because of mutation in gyrA gene respectively (Andersson, 2006; Allou et al., 2009). Similarly qnrB is quinolone-resistance determinant (Da Re et al., 2009). Rifamycin, broad-spectrum antibiotics target bacterial transcription by inhibition of RNA polymerase but mutational alteration of the drug target (ribosome) by ADP-ribosylation is the predominant mechanism initiating resistance and as an additive for the formation of rifampin resistome (Llano-Sotoelo et al., 2002; Recht and Puglisi, 2001; Baysarowich et al., 2008). Actinomyces group of bacteria produces vast range of bioactive secondary metabolites/antibiotics which are strain specific rather than species-specific. To be specific they possess specific genotypes encoding for strain specific antibiotics out of which few have Aminoglycoside antibiotic (AG) inactivation enzymes (β-lactamases) while few strains of actinomycetes are AG producers showing multiple patterns of resistance (Benveniste and Davies, 1973; Ogawara et al., 1999). Sulfonamide and trimethoprim resistance in Streptococcus agalactiae has been observed due to presence of amplification of a naturally occurring gene (Brochet et al., 2008). Multilocus sequence typing has confirmed the antimicrobial resistance in Streptococcus pneumoniae isolates (Doern et al., 2001). Whole-genome sequencing is another approach for tracking the in vivo evolution of multidrug resistance in various bacteria, Staphylococcus aureus being a significant example (Mwangi et al., 2007).

Major mechanism of antifungal drug resistance is any change in the cell wall or plasma membrane, which leads to impaired drug uptake by the fungal cells and over expression of efflux pumps of the ABC (ATP binding cassette) transporter and MFS (major facilitator superfamily), drug transporters which belong to two different superfamilies (Mendez and Salas, 2001; Poole, 2005). These are multifunctional proteins, which mediate important physiological functions and are the most prominent contributors to phenomenon of Multiple Drug Resistance (MDR) in microorganisms. Hence, an alarming increase in bacterial resistance and increased proportion of MDR strains of bacteria has been documented. The macrolides and many related antibiotics act by binding with 50S ribosome subunit. Resistance can be acquired by modification of the RNA or protein components of the ribosome. A specific rRNA modification mechanism producing resistance to all antibiotics is spreading rapidly specifically against quinolones (Zengel et al., 1977; Yassin and Mankin, 2007; Roberts, 2008).

Mechanism of development of antibiotic resistance

Now sufficient documentary evidences are available specifying that antibiotic resistance is an ancient phenomenon and environmental microorganisms, including antibiotic producers, are source of such resistant determinants found in pathogenic microorganisms (Silver and Falkow, 1970; Kopecko and Punch, 1971; Bartoloni et al., 2009). Resistance to antibiotics may be either intrinsic (or natural) and acquired. Microbes lacking the target site are not affected by drugs that form the basis of the concept of intrinsic resistance (Fajardo et al., 2008). The differences in the chemical nature of the drugs and the membrane structure of the microbes are responsible for low permeability of drug under natural circumstances due to presence of inactivating microbial enzymes or alternative enzyme for the enzyme that may get inhibited by the antibiotic like the beta lactamases responsible for resistance against Beta lactam antibiotics and third generation cephalosporins (von Baam and Marre, 2005; Rossolini and Thaller, 2010). It may also be due to mutation in the target; post-transcriptional as well as post-translational modification of the drug target; reduced uptake and active efflux of the drug; chromosomally encoded multidrug resistance; R-factor; typical expression or suppression of genes in vivo that may be entirely different from the condition in vitro (Watanabe, 1971; Hall, 1997; Fluit et al., 2001; McKeegan et al., 2002; Poole, 2005; Piddock, 2006b; Torok et al., 2012). Gene deletion is also considered as a mechanism of development of antibiotic resistance as demonstrated in the case of Burkholderia pseudomallei, the bacterium has developed resistance against cefazidime due to deletion of Penicillin binding protein gene encoding (PBP 3) but this mechanism is extremely uncommon (Torok et al., 2012). The role of human intestinal microbial flora in the transfer of antibiotic resistant genes to pathogenic bacteria is also screened (Salyers et al., 2004; Sommer et al., 2009). The hypothesis that the organism could acquire gene of resistance from soil/nature or aquatic environments is also investigated recently, as most of the antibiotics were obtained from soil borne organism and the soil is also suspected to be the source of resistance genes (Riesenfeld et al., 2004; D’Costa et al., 2006; D’Costa et al., 2007; Baquero, et al., 2008; Martinez, 2009; Szczepanowski et al., 2009; Allen et al., 2010; Walsh, 2013). The stress to which bacteria are exposed both within the host and also in the environment, results in development of adaptive mechanism like development of antibiotic resistance and formation of biofilms for their survival (Poole, 2012). Recently, development of rifampicin resistance among E. coli growing in an
antibiotic free environment at higher temperatures resulting from mutations of the rpoB gene was reported (Pallecchi et al., 2007; Pallecchi et al., 2008; Rodriguez-Verdugo et al., 2013).

Non-chromosomal antibiotic resistance

Mobile genetic elements such as transposons, prophages, integrons, resistance islands (R factor), self replicating plasmids all carry resistance genes into sensitive microorganisms (Normark and Normark, 2002; Guerin et al., 2010). Plasmid-mediated quinolone resistance is emerging globally as a multifaceted threat (Rownd, 1969; Robisch et al., 2006; Stráhilevitz et al., 2009). Spread of plasmid is very rapid in between heterogeneous bacterial communities (Dionisio et al., 2002). Integrons are genetic elements associated with the resistance (r) genes related with transferable plasmid-mediated resistance (Gillings et al., 2008). OXA beta-lactamase genes responsible for resistance against Beta lactam antibiotics have been observed to be present on plasmids for several millions years (Davies, 1995; Barlow and Hall, 2002). Microarray technology involving gene signatures of microorganisms should be explored to elucidate and understand mechanisms of antibiotic action and developing resistance in more detail (Brazas and Hancock, 2005). Methyleneomycin antibiotic is produced by two strains of Streptomyces where the genes for production and resistance both are located on the transferrable plasmids i.e. pSV1 and SCP1, mrr being the methyleneomycin resistance gene (Chater and Bruton, 1985). Development of multiple resistance in microorganisms indicates molecular basis of new defense strategy of tiny super bugs (Aleksun and Levy, 2007). R-plasmid pTP10 of the Corynebacterium xerosis carries resistance genes for erythromycin chloramphenicol, kanamycin, and tetracycline antibiotics (Tauch et al., 1995). Genetic transformation of Escherichia coli and Proteus mirabilis explains the involvement of molecular nature of circular R-factors/plasmid DNA (Cohen and Miller, 1969; Cohen and Miller, 1970; Davies et al., 1971; Cohen et al., 1972). Conjugal plasmids are part of enterobacteria such as Plasmid F of Escherichia coli K-12, R-Factor NR1 in Proteus mirabilis and other conjugative plasmids encoding antibiotic resistances in Salmonella, Shigella, Klebsiella, Proteus, and Escherichia (Watanabe et al., 1964; Rownd and Mickel, 1971; Silver and Cohen, 1972). They are considered as 'pre-antibiotic' plasmids means they were existing before the development of modern resistance encoded by modern R plasmids and it has been confirmed by incompatibility tests or Inc groups (Datta and Hughes, 1983; Nordmann and Poirel, 2005).

Microbial genetics explains existence of chromosomal and non-chromosomal r genes

A large number of bacteria and fungi acts as source of wonderful drugs. Antibiotics were produced as a means of self-defense by various bacteria and fungi as microbes are exposed to various organic, inorganic and industrial influents in the nature. To specify, antibiotic producing microorganisms harbor r-genes and R-factors in plasmids to confer their own protection from inhibitory or lethal actions of these secondary metabolites naturally. Currently due to excessive survival pressure microorganisms are either expressing these r-genes and R-factors or following various alternate approaches by episome mediated gene transfer or modifications in targets to overcome bacterial activity of antibiotics for their prolonged survival in the nature (White et al., 2005; Hopwood, 2007).

Reasons / Factors That Drive Antibiotic Resistance

- Inadequate national commitment to a comprehensive and coordinated response, ill-defined accountability and insufficient engagement of communities.
- Improper surveillance, tracking and monitoring systems.
- Lack of basic knowledge on the complexity of the processes that contribute to emergence and dissemination of resistance is one of the primary reasons of no significant accomplishment.

- Though steps have been taken at international, national, and local levels against this serious problem by proposing resolutions and a variety of recommendations have also been propounded but still the development of antibiotic resistance is relentless.
- Inappropriate and irrational use/dosing of drugs/medicines by humans and given even to their companion animals.
- Paucity of knowledge- Informations regarding natural biological functions of all the antibiotics is not completely known and understood.
- Poor disease prevention and control practices.
- Insufficient diagnostics, medicines, nosocomial transmission and vaccines (Finland, 1979).
- Consistent lack of skilled staff, deficit in training of R & D staffs persons and limited resources in order to enforce infection control practices to be followed.
- Inadequacy or less availability of proper guidelines for rational usage of antibiotics.
- Increased international trade and travel act as force for inter-continental spread.
- Reduction in the speed of development of new antimicrobials as laboratory and clinical trials take much time than the emergence of resistant genes (Baltz, 2006; Douthwaite, 2010; Rossolini and Thaller, 2010).

Steps to limit antibiotic resistance

- Limiting infectious diseases forms the fundamental step in reducing antimicrobial resistance. It is thus needed to give special attention to health transition from acute diseases in neonates towards chronic diseases in adults in order to limit the risk of indiscriminate spread of disease in various age groups.
- Importance to build and sustain a greater effort to reduce antibiotic resistance is provided by international commitment towards specific diseases including tuberculosis for which resistance is a major concern (Shah et al., 2007; Thami et al., 2007; Velayati et al., 2009).
- Use antibiotics only in case of bacterial infections. In this regard, the introduction of fluoroquinolone group of antibiotics in the south-eastern Asian countries requires a special mention as it has illustrated a negative impact due to overuse. This proves that judicious use of antibiotics is the need of hour (Linton, 1977).
- Antibiotic therapy should be prescribed only after performing the antimicrobial sensitivity testing in the laboratories.
- Selection of such antibiotic should be promoted, which targets the infection causing microbe instead of broad-spectrum antibiotics. This will cause reduction in the development of early signs of antibiotic resistance provided there is feasibility of feedback intervention.
- Completion of full course of antibiotics should be followed. Any negligence in this regard ultimately encourages the pathogen to persist in the host.
- Virulence inhibitors should be used instead of antibiotics as this will check the occurrence and expression of disease.
- Stimulation of immune system of host, can also involve promoting gut microbiomes and commensals to enhance competitive colonization such as use of prebiotics and probiotics (Marshall et al., 2009; Dhama et al., 2011a).
- The burden imposed upon human health by antibiotic resistance especially in rural setting is huge but cannot be quantified with precision. For this reason regular surveillance, monitoring and continuous vigilance are of utmost importance.
- Strict hygienic practices and monitoring of antibiotic use in hospital settings to prevent spread of nosocomial spread of antibiotic resistance (Bergstrom et al., 2004).

(Duse, 1998; Newton et al., 2001; World Health Organization, 2001; Siriravin and Dowell, 2002; von Baum and Marre, 2005; Falagas and Karveli, 2006; Zhang et al., 2006; Chetley et al., 2007; Iseman, 2007).
The antibiotic resistance in animals

Sometimes the bacterium causing disease to both humans and domestic animals may be common. The animals act as reservoir for these resistant strains because of indiscriminate use of antimicrobials (Wegener et al., 1999). It is not surprising that antibiotics are widely used in agricultural and animal husbandry settings to treat the infections and to promote the growth and production of animals (Payne et al., 2007). More than half of the total amount of antimicrobials consumed worldwide is reported to be as growth promoter (Wegener et al., 1999). The most common antibiotic-resistant organisms sourced from animals are some strains of *Salmonella*, *Campylobacter*, *Enterococci*, and *E. coli*, which could also infect humans as well. Although it is difficult to establish an obvious link, when animal pathogens/bacteria are exposed to an antibiotic used in animals which is related to an antibiotic used in humans, they may develop common resistances; the antibiotic resistant organisms in animals or their antibiotic resistant genes could spread to humans. As per World Health Organization (WHO), excessive and unnecessary use of antibiotics, particularly as growth promoters in livestock animals and poultry destined for human consumption, may lead to a growing risk to human health. In 1998, the European Union followed WHO recommendations and banned the use of antimicrobials in animals prescribed for the treatment of human infections as well as all antibiotic use for growth promotion in animals. An increase in international trade of animals and animal products is also a concern in the spread of antibiotic resistant clonal lineages. Use of fluoroquinolone drugs in veterinary medicine was reported to be associated with the development of resistance among S. enteritica DT 104 (Witte, 2004; Kahn and Line, 2005; Musgrove et al., 2006; Kikavu et al., 2007; Luangtongkum et al., 2009; WHO, 2013).

Many countries have banned some specific drugs/antibiotics. DDT, diclofenac, paracetamol, aspirin, analgin, furazolidone, piperazine, nitrofurazone, penicillin as skin or eye ointment, tetracycline as liquid concentrate of antibiotics. FDA prohibits the extra label use of DDT and related food products is also a concern in the spread of antibiotic resistant clonal lineages. Use of fluoroquinolone drugs in veterinary medicine was reported to be associated with the development of resistance among S. enteritica DT 104 (Witte, 2004; Kahn and Line, 2005; Musgrove et al., 2006; Kikavu et al., 2007; Luangtongkum et al., 2009; WHO, 2013).

### Antibiotic resistance detection methods

Rising frequency of fungal infection as well as increased reports of resistance to antibacterial and antifungal agents indicates the importance of *in vitro* antibiotic and antifungal susceptibility testing. It reflects the necessity to assess drug sensitivity patterns before recommendation of any therapeutic agent. Different methods are available to assess the sensitivity of microbial agent against different concentrations of antibiotic and antifungal agents. For this, diffusion method and of Minimum inhibitory concentration (MIC) methods are used by observing the zone of inhibition and reduction of turbidity of broth culture, respectively. In disc diffusion methods, small discs containing antibiotics are placed over a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, develops around the antibiotic disc. Sensitive drug produces large clear zone over the bacterial lawn as per the concentration of drug incorporated in the antibiotic/antifungal discs, referred as zone of inhibition. Measurement of MIC of any drug is more sensitive technique comparatively, is used in R&D labs of different pharmaceuticals companies to set an appropriate dose/concentration of drug after the assessment of sensitivity testing and before launching any medicine in the market. According to prescribed dosage medicines should be used to avoid the development of antibiotic resistance. Antibiotic susceptibility tests are used to determine the inhibitory activity of any antibacterial agent against bacteria, thereby suggesting for appropriate therapy. With the current increase in incidence of antibiotic resistant nosocomially acquired infection, it is also assuming greater clinical significance. Additionally, the sensitivity pattern of an organism may be important epidemiologically when used to identify certain strains within a given species (Oka, 1995; Joint FAO/ WHO Food Standards Programme, 2001; Danko et al., 2005).

### Detection Methods for Antibiotic Residues

A number of methods have been defined for detection of antibiotic residues these include microbiological approaches, immunochemical techniques, gas chromatography (GC) methods, high-performance liquid chromatography (HPLC) with UV, mass spectrometry (MS) and nano quantity analyte detectors, microfluidic methods and electrochemical methods. The microbiological methods are preferred because of their convenience, low cost and broad-spectrum characteristics. Sarcina lutea kidney test is one of the first officially recognized microbiological method followed by Bacillus subtilis BGA test. The European Union four-plate test (EU4pt) comprising of three plates of agar medium inoculated with *Bacillus subtilis* at varying pH has found its application in detecting sulphonamide residues in meat. Recently Premi®Test has been certified by French Association for Normalization (AFNOR) validating the analytical effectiveness for a particular field of application commercially. To indicate whether antibiotic residues are present or not screening tests are valuable and are rapid and easy to use and at the same time reliable. They may be broad (detects beta-lactams and cephalosporins; aminoglycosides and tetracyclines; sulphonamides as well as macrolides) or narrow spectrum (covers only beta-lactams). Instead of the presence of commercial screening test methods non-commercial antibiotic residue screening test like *AS 1766.3.11* method is gaining much attention these days. Mass spectrometry (MS), a technique of chromatographic detection is indispensable for contaminant confirmation. At present, more than 80% of the analytical techniques for the determination of veterinary drugs are Liquid chromatography–mass spectrometry (LC-MS, or alternatively HPLC-MS) based, especially when detection is performed by multi-stage MS. Single-stage MS is still used for screening purposes and for quantification of maximum residue limits (MRLs) of substances; in fact, LC tandem MS is recommended for the detection of unauthorized and banned substances, whose detection capability (CCb) should be as low as reasonably achievable. Enzyme linked immunosorbent assay (ELISA) and fluorescence immunoassays (FIA) are excellent survey tools because of their high-throughput, user friendliness, and field portability. These assays are optimized to provide the greatest sensitivity at or near the regulatory safe/tolerance levels for the appropriate antibiotics. Frontier Post Test (FPT) can be performed to detect the day-to-day variation in the concentration of antibiotic residues in milk by using *Bacillus subtilis* starter culture (Andrews et al., 2000; CH 6.2. 2004; CH 12.16. 2004; Stead et al., 2004; Koreňková et al., 2007; Gautin et al., 2008; Pikkenaart et al., 2008; Pikkenaart, 2009; Plozza et al., 2011; www.dairysafevic.gov.au).

### Residue Limits

Internationally recognized organizations such as World Health Organization (WHO), Food and Agriculture Organization (FAO), Veterinary Medicines Directorate (VMD) of the European Union as well as Food and Drug Administration (FDA) of the US, have set tolerance or maximum residue limits (MRLs), acceptable daily intakes for humans and withholding times for pharmacologically active substances including antimicrobial agents prior to marketing. Certain individuals develop tolerance to drugs and antibiotic-tolerant status of any individual may depend on physiological adaptations without direct connections to antibiotic target activity or to medicinal/drug uptake, efflux, or inactivation of drug inside the body of patient (Piddock, 2006a). FDA prohibits the extra label use of chloramphenicol, furazolidone, nitrofurazone, sulphonamide drugs, and fluoroquinolones in lactating animals. The maximum residual
limit of antibiotic varies from country to country and with the antibiotics (Chee-Sanford et al., 2009). For example the MRL of different groups of antibiotics in milk are as betalactam antibiotics (4-100), aminoglycosides (100-1500) and macrolides (50-200) (Althaus et al., 2001; Ghidini et al., 2002; Pikkermaat, 2009; Sierra et al., 2009; Turnipseed et al., 2009).

Public health and Industrial aspects

The non-restrictive use of antibiotics in animal rearing may lead to problems due to presence of harmful residues in foods including meat, milk, eggs and raw materials of animal origin. The antibiotic resistant bacteria found among farm animals can enter the food chain and may affect human health. The presence of antibiotic residues in milk gives rise to health penalties. Human health problems importantly may result from intake of sub-chronic exposure like allergic reactions in sensitive persons, toxicity, carcinogenic effects, even though the legitimacy of some of these reactions is sometimes debated or under question (Doyle, 2006). Penicillins especially, as well as other β-lactam antibiotics such as cephalosporins and carbapenems could cause allergies if high levels of residues persist in milk is consumed by penicillin-allergic persons. Tetracyclines residues have side effects of teeth staining of young children. Development and spread of antibiotic resistance represents a serious threat with potential public health implications and negative impacts. This is more likely in instances where the drug is fed continuously over a long period of time, when used at an extra label dose, beyond recommendations/prescriptions. Veterinary drug residues in food and food products of animal’s sources are considered notorious public health hazards. Penicillin in chicken was reported to have caused severe anaphylactic reaction in a consumer. Skin allergies in persons hypersensitive to sulfonamides though generate resistant population of bacteria but the presence of residues in food products have got little effect on the selection of resistant population of bacteria in human. One interesting aspect is that essential role is played by lactic acid bacteria for acidification of milk as they allow protein precipitation together with the dosage administered, and the withdrawal periods for milk, meat and egg collection. Rapid testing and screening methodologies for the analysis of antibiotic residues and instant grading and prohibition of food containing antibiotics more than MRL.

Prevention and control strategies for antibiotic resistance

- Introducing the awareness programmes to make individuals and organizations aware of the problem through education by veterinary personnel, organizations, medical professionals, NGOs and governmental agencies.
- Record all treatment dates, medicines/drugs used, batch numbers together with the dosage administered, and the withdrawal periods for milk, meat and egg collection.
- Adequate processing of milk will help in inactivation of antibiotics.
- Use of UV irradiation also helps in antibiotic inactivation.
- All milk from treated animals must be discarded.
- Irrational use of antibiotics in field veterinary practices should always be discouraged.
- Development of simple and economical field tests to identify drug residue in edible animal products.
- Fruitful life of antimicrobial drugs could be increased by adaptation of appropriate usage policies that discourage overuse and misuse, and encourage judicious usage practices.
- Improve diagnostic testing practices
- Transmission of infectious pathogens should be checked and intensive strategies for prevention and control of infection should be practiced.
- Regular vaccination programmes should be followed to avoid establishment and development of disease.
- There should be holistic approach and sound strategy to prevent and control emerging antibiotic resistance problems in human and veterinary medicine as well as agriculture.
- Development, improvement and execution of ideology for appropriate and judicious antimicrobial drug use in the production of food animals and plants for food-safety concerns and ultimate health benefits for both medico and veterinary perspectives i.e. safeguarding health issues of animals and humans both.
- Improved animal husbandry and food production practices to reduce the spread of infection and infectious pathogens.
- Regulatory bodies and frameworks need to address for appropriate antimicrobial drug use in agriculture and veterinary medicine while ensuring that such use does not pose a risk to human health.
- Several action items to strengthen; expand and coordinate prevailing national as well as international surveillance systems for resistant groups of microbes must form an integral part of the action plan. To address barriers to timely disseminate and update surveillance data and in order to gather information on the use of antibiotics additional action items are required.
- Surveillance system viz. National Healthcare Safety Network (NHSN) finds its appropriateness in preventing and restricting antibiotic resistance. It has shown its expansion in order to improve the capacity for collection and analysis of multidrug-resistant organisms (MDROs) and use of antibiotics. NHSN in collaboration with National Antimicrobial Resistance Monitoring System (NARMS); National Tuberculosis Surveillance System (NTSS) and United States Department of Agriculture (USDA) have undertaken initiatives in war footage to take care of such sensitive issues in developed nations of the world. (Food and Drug Administration, 2009; Interagency Task Force on Antimicrobial Resistance, 2012; Union of concerned scientists, 2012; www.usda.gov).

WHO has selected combating antimicrobial resistance as the theme for World Health Day, 2011 - wherein WHO issues an international/global call for concerted action to halt the spread of antimicrobial resistance and recommends a six-point policy package for governments and regulatory bodies (WHO, 2013) as mentioned below:

- develop and implement a comprehensive, financed national plan
- strengthen surveillance and laboratory capacity
- ensure uninterrupted access to essential medicines of assured quality
- regulate and promote rational use of medicines
- enhance prevention and control of infection
- foster innovation of research and development of new tools.

Alternative and complementary therapies

To overcome with hurdles of microbial resistance various alternative emerging novel therapies are coming into picture such as herbal medication, ethno-veterinary medicines, bacteriophage therapy, cytokine therapy, mycophage therapy, panchgavya therapies etc. which are opening new avenues to fight against these superbugs (Deb et al., 2013b; Dhamma et al., 2013a,b,d,e).

Bacteriophage therapy

Bacteriophages are super-bugs which are considered as bacteria eaters, bacteriophages thrive over bacteria specifically, as they are viruses of bacteria. They enter their host bacterium through specific receptors and are unable to infect eukaryotic cells due to the absence
of receptors in them. Due to their lytic mode of life-cycle, endolysins and holin enzyme system phages can kill Gram-ve, Gram-ve, Acid-fast and many other bacteria as well. Oral administration or topical application of phages have been attempted for a wide range of bacterial infections, caused by wounds or surgical intervention like skin grafting (Mathur etal., 2003; Tiwari etal., 2011; Tiwari etal., 2012; Tiwari etal., 2013a; Ghannam and Mohammadi, 2012).

Virophages and Mycophages

These are viruses which act specifically against viruses and fungi, respectively. Virophage and Mycophage therapies are new emerging concepts, as antiviral drugs and antifungal drugs require long-term medications and may have many side effects. The mycophages and virophages can be modified and used in therapeutic preparations for the treatment of diseases against many pathogenic fungi and certain viruses and can thus reduce antifungal and anti-viral resistance to a certain extent (Ghabrial, 1980; Koonin, 2012; Tiwari etal., 2013b).

Cytokine therapy

These are intercellular regulatory proteins, which play a pivotal role in initiating, maintaining, and regulating immunologic homeostatic and inflammatory processes. Due to their multiple function, they are promising candidates for therapeutic interference in infectious and autoimmune diseases, in immunocompromised patients with AIDS. The immunoglobulin Fc fragment based cytokines provides superior therapeutic approach. Nevertheless, the development of new vaccines necessitates the development of new types of adjuvants to ensure an appropriate immune response (Antachopoulos and Roolides, 2005; Jazayeri and Carroll, 2008; Nicholls etal., 2010; Dhamae etal., 2013d).

Avian egg antibodies therapy

Chicken are capable of producing antigen specific antibodies (IgY), which have function similar to IgG in response to antigen. It can be used to treat microbes, which do not respond to antibiotics. Treatment with these antibodies is safer, more efficient and less expensive in comparison to antibiotics. Specific IgY antibodies have been developed against different bacterial or viral pathogens viz., rotavirus, bovine respiratory syncytial virus, coronavirus, infectious bursal disease virus, E. coli, Salmonella, Edwardsiella, Yersinia, Staphylococcus, Streptococcus and Pseudomonas (Yegani and Korver, 2007; Shaban etal., 2007; Michael etal., 2010; Wilmar and Tamburgi, 2010; Dhamae etal., 2011b; Ferella etal., 2012; Deb etal., 2013a).

Herbal therapy

Various herbs and their extract have been proved to have antimicrobial, antiviral or antifungal activities. For example neem, giloy, onion, garlic, mustard, red chilli, turmeric, clove, cinnamon, saffron, curry leaf, fenugreek, ginger etc. Also these do not possess development of resistance like that of antibiotics, and are also comparatively safer and cost-effective. Globally many researches are going on exploring the role of plants and their extracts in enhancing the immunity of man and animals and thereby encouraging avoidance of antibiotics. Herbal therapy is also gaining much attention these days in the treatment of subclinical mastitis and uses of Terminalia chebula and Terminalia belerica in this regard are found to be significant (Hawari, and Fawzi A. 2008; Hashemi and Davoodi, 2012; Mahima etal., 2012; Vashney etal., 2012; Deb etal., 2013c; Mahima etal., 2013).

Panchgavya therapy

Nowadays, Panchgavya therapy is gaining much importance because cow urine (an important component of Panchgavya) is able to kill a number of bacteria that show antibiotic resistance. The antibiotic resistance germs of tuberculosis can be killed by cow dung and urine, particularly cow urine acts as a bioenhancer for anti-tubercular drugs, for which it is gaining much importance in the international market as an anti-tubercular agent (Dhamae etal., 2005a,b; Jain and Mishra, 2011; Randhawa and Kullar, 2011; Dhamae etal., 2013e).

Diagnostics

An interesting fact is that improvement in the field of diagnosis has also helped in the early detection of antibiotic resistance apart from alternative and complementary therapies. In this regard development of molecular beacon based polymerase chain reaction and DNA chips for detection of methicillin resistant Staph. aureus (MRSA); liquid culture systems and molecular line probe assays in the detection of drug-resistant tuberculosis needs a special mention (Fluit etal., 2001; Woodford and Sundsfjord, 2005; O’Grady etal., 2011; Wilson, 2011; Deb etal., 2013c).

Vaccines

Molecular biology has created an enormous impact on vaccine development, leading to development of DNA vaccines; Subunit vaccine, anti-idiotypic and virosome vaccine, virus-based nanoparticles and virus like particles, biotechnologically engineered vaccine. These do not have the problem of resistance because they enhance the body’s natural defense system, while an antibiotic works differently. However, due to evolution of new strains that escape from immunity induced by particular vaccine may develop for example in case of influenza/flu viruses, so an update regarding vaccine strain is necessary annually. Plant based oral vaccine importantly have gained popularity in human medicine and has been found to be protective against diseases like Pneumonia, Cholera and Tetanus. Development of anti-staphylococcal and other more effective vaccines is under way (Mengeling etal., 1997; Mercenier etal., 2001; Dhamae etal., 2008; Daniell etal., 2009; Dhamae etal., 2013f).

Conclusion and Future Perspectives

Antibiotics have revolutionized medicine in many aspects; their discovery was a turning point in human history. With the growing development as well as emergence of antibiotic resistance microbes, it is vital that we no longer take the availability of effective antibiotics for granted. We must respond to this burning issue and growing problem, and our response needs to be holistic and thorough for an ultimate fruitful cause to have check on this precarious problem. There is a need of cost-effective, sustainable control program in animal husbandry practices viz., dairy, piggery, poultry industry etc. through the use of simple approaches. Implementation of an affordable prevention and control program at farm level may result in a reduction of antibiotic residues in food products of animal origin (milk, meat, and eggs), which would result in checking the residual and toxic effects to our forks (avoiding this menace from farm to forks). The regulatory policies are required to be put in place by the governmental authorities to address the problems of farmers. These alone cannot solve the inherent residue problems. Control strategies that focus on implementing on-farm measures to reduce the risk for contamination of animal products should be more sustainable. Identification of the factors and strategies promoting appropriate antimicrobial use or discouraging inappropriate use will facilitate the implementation strategies. Identify factors and strategies that promote appropriate antimicrobial use (i.e., best practices) or discourage inappropriate use in all types of healthcare settings, including in-patient and out-patient facilities, clinics and offices facilitate the implementation of these strategies. Advancement in identification of new sources of natural antibiotic products and their diversity, creative approaches to discover novel antibiotics and their controlled introduction to therapy, inhibitors of drug resistance, microbial virulence inhibitors, and alternative therapies like phage therapy, cytokine therapy, mycophage therapy, panchgavya therapy etc are some of the ways that can help us to tackle the challenges of antibiotic resistance in the 21st century. By building on our current efforts and thinking for future in right perspectives, we can extend the
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