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A review on antimicrobial resistance, diagnosis and an alternative approach

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Abstract
Antibiotics are extensively used as therapeutic agents for the treatment of infectious diseases in humans and livestock. Penicillins, tetracyclines, sulphonamides, fluoroquinolones and cephalosporins are major antibiotic classes widely used in animal production. Antibiotic usage was highest in swine, chicken and cattle and extremely low in sheep. The use of antibiotics in extra-label use may pose a strong pressure on human, animal and soil microbiome leads to the emergence of antibiotic resistance and have a serious impact on human and animal health. The antimicrobial alternatives such as phage therapy, prebiotics, probiotics, metals and minerals, organic acids and essential oils may be used to treat the infections and increase immune response. Globally, antimicrobial resistance crisis may be controlled by the implementation of monitoring and surveillance programme for clinically important antimicrobial classes used in animals and human and assessment of antimicrobial resistant genes in animal products and environment, reduced use of antibiotics in animals by enhancing host resistance and decreased exposure to the infectious agent through hand sanitation.

Keywords: Antibiotics, AMR, ARG, antibiotic alternatives, growth promoters, PCR

Introduction
Antibiotics are antimicrobial substance destroy or slow down the growth of the bacteria. These are natural, semi-synthetic or synthetic origin [1], widely used in livestock for the treatment of respiratory, enteric infections, mastitis, metritis and prophylactic mass treatment to control infection and as growth promoters to improve feed efficiency [2]. Several antibiotic classes are extensively administered to food-producing animals *viz.*, tetracyclines, sulfonamides, fluoroquinolones, macrolides, lincosamides, aminoglycosides, beta-lactams, cephalosporins etc. [3]. Nowadays most of antibiotics are lost their curative purpose due to the emergence of antibiotic resistance [4]. Continuous antibiotic usage urges the sensitive bacteria to become resistant for survival, the resistant pathogens can be transferred indirectly to the human through the environment. The emergence of antimicrobial resistant pathogenic bacteria [5] poses a serious impact on public health worldwide [6]. Most of the antibiotics/antibacterial agents were used today are derived from soil bacteria genus Streptomyces [7]. Antibiotics producing soil bacteria contain a variety of self defence against their own antibiotics, and the genetic determinant for self-defence was always co-regulated with antibiotic biosynthetic genes [6, 8]. The increase in antimicrobial resistance has been reported in both commensal and pathogenic bacteria [9], which leads to poor clinical recovery in animals and humans [10]. The understanding of antibiotic usage pattern, the resistant mechanism in bacteria and its impact on human and animal health are essential to trace the alternatives to mitigate antimicrobial resistance.

Antibiotics use in food animals
Antibiotics are widely used in animals to maintain health and productivity as well as to meet the increased demand for animal protein for human consumption. The estimated global antibiotics consumption in food producing animals was 63151 tons in 2010, of which five countries shared more than 50% of consumption by China (23%), United States (13%), Brazil (9%), India (3%) and Germany (3%) [11]. China has become the largest producer and consumer of antibiotics and nearly half of the produced (210,000 tonnes) were utilized for food animals [12], a high volume of sulphonamides, tetracyclines and fluoroquinolones (enrofloxacin, fleroxacin and norfloxacin) were widely used in agricultural sectors in China [13].

The United States utilizing more antibiotics (9702 tonnes) for livestock than the United Kingdom of a European country (404 tonnes) [14]. In livestock, antimicrobial usage was maximum in swine followed by chicken and cattle for the production of meat and very low in sheep [15-16]. The world average annual consumption of antimicrobials for the production of one kilogram of beef, chicken and pork was 45, 148 and 172 mg, respectively [11]. India holding the largest livestock population in the world, accounting for 11.6% and stands fifth place in meat production. Chicken is most commonly consumed meat than others, antibiotics such as tetracycline, doxycycline and ciprofloxacin are commonly used as a growth promoters in poultry production at a sub-therapeutic doses in India [17].

Growth promoters

The antibiotics used as growth promoting agents to accelerate feed efficiency in the poultry and pigs over 60 years. Drugs such as penicillin, oxytetracycline, bacitracin, aureomycin and streptomycin were among the earliest antibiotics reported to yield a growth impact on young birds at sub-optimal dose in feed at 1-20 ppm [18]. It is more frequently used for non-therapeutic than therapeutic applications, developed countries utilizing 50-80% of antibiotics produced [19]. There are 12 classes of antimicrobials and they are arsenicals, polypeptides, glycolipids, tetracyclines, elfamycins, macrolides, lincosamides, polyethers, beta-lactams, quinoxalines, streptogramins and sulphonamides used at different times in the life cycle of poultry, cattle and swine to increase production [20], repeatedly exposing these animals to small doses of antibiotics significantly contributes antimicrobial resistance [21]. Currently, many antibiotics used in food animals are the same as or surrogates of antibiotics used in human medicine [22]. Antibiotics are essential for the treatment of many common human and animal pathogenic bacterial infections and other major surgical procedures [23]. The World Health Organization (WHO) and World Organisation for Animal Health (http://www.oie.int/) developed a comprehensive list as "critically important", "highly important" and "important" based on importance in human and veterinary medicine to balance the animal health and public health [24] (Table 1). The new rules eliminate the use of medically important antibiotics for growth promotion and permit only the use of these drugs for therapeutic or preventive purposes under the supervision of a Veterinarian [25].

Table 1: Classification of veterinary important antimicrobials for food producing animals [24]

	Antimicrobial family	Name of the antibiotics	Veterinary Antimicrobials			Specific recommendations
			Clinically important	Highly important	Important	
I.	**Penicillins**					
a	Natural Penicillin	Benethamine penicillin, Benzyl penicillin, Penethamate (hydroiodide), Penicillin procaine, Benzathine penicillin	✓	-	-	Treatment of Septicemia, respiratory and urinary tract infections
b	Amdinopenicillin	Mecillinam				
c	Aminopenicillins	Amoxicillin, Ampicillin, Hetacillin				
d	Aminopenicillin and Beta lactamase inhibitor	Amoxicillin and Clavulanic acid				
e	Carboxy Penicillin	Ticarcillin, Tobicillin,				
f	Ureidopenicillin	Aspoxicillin				
g	Phenoxypenicillins	Phenoxymethylpenicillin, Phenethecillin				
h	Anti-staphylococcal Penicillin	Cloxacillin , Dicloxacillin, Naficillin, Oxacillin				
II.	**Cephlosporins**					
a	First generation Cephalosporins	Cefacetrile, Cefalexin, Cefalotin, Cefapyrin Cefazolin Cefalonium	✓	-	-	Treatment of septicemias, respiratory Infections and mastitis
b	Second generation Cephalosporins	Cefuroxime				
c	Third generation Cephalosporins	Cefoperazone, Ceftiofur Ceftriaxone				
d	Fourth generation Cephalosporins	Cefquinome				
III.	**Aminoglycosides**					
e	Aminocyclitol	Spectinomycin Streptomycin, Dihydrostreptomycin	✓	-	-	Septicaemias, digestive, respiratory and urinary diseases. Gentamicin is indicated for *Pseudomonas aeruginosa* infections. Apramycin and Fortimycin are currently used in animals alone
f	Aminoglycosides + 2 Deoxystreptamine	Kanamycin, Neomycin, Framycetin, Paromomycin, Apramycin, Gentamicin, Tobramycin , Amikacin				
IV.	**Ansamycin-Rifamycins**	Rifampicin, Rifaximin	-	✓	-	Mastitis and Rhodococcus equi

						infections in foals
V.	**Bicyclomycin**	Bicozamycin	-	-	✓	Digestive and respiratory diseases of cattle and septicaemias in fish
VI.	**Phosphonic acid**	Fosfomycin	-	✓	-	This antimicrobial is authorised only in a few countries and critically importance for fish
	VII. Tetracycline					
a	Tetracyclines	Cholrtetracycline, Doxycycline, Oxytetracycline, Tetracycline	✓	-	-	Treatment of many bacterial and chlamydial diseases in animals and birds. There are no alternatives to tetracyclines in the treatment of animals against heartwater (*Ehrlichia ruminantium*) and anaplasmosis (*Anaplasma marginale*)
	VIII. Sulfonamides					
b	Sulfonamides	Sulfachlorpyridazine, Sulfadiazine, Sulfadimerazine, Sulfadimethoxine Sulfadimidine, Sulfadoxine, Sulfafurazole, Sulfaguanidine, Sulfamethazine, Sulfadimethoxazole, Sulfamethoxine, Sulfamonomethoxine, Sulfanilamide, Sulfaquinoxaline		✓	-	Several sulfonamides alone or in combination with diaminopyramidines are very essential to treat bacterial, coccidial and protozoal infections
c	Sulfonamides + Diaminopyrimidines	Sulfamethoxypyridazine, Trimethoprim+Sulfonamide, Ormetoprim+Sulfadimethoxine				
d	Diaminopyrimidines	Baquiloprim, Trimethoprim				
IX.	**Streptogramins**	Virginamycin		-	✓	For prevention of necrotic enteritis (Clostridium perfringens) in poultry
	X. Quinolones					
a	First Generation Quinolones	Flumequin, Miloxacin, Nalidixic acid, Oxolinic acid	✓	-	-	Septicemias, chronic respiratory disease in poultry *(E.coli* colibacillosis in poultry, cattle, swine, fish and other species
b	Second Generation Quinolones (Fluoroquinolones)	Ciprofloxacin, Danofloxacin, Difloxacin, Enrofloxacin, Marbofloxacin, Norfloxacin, Ofloxacin, Orbifloxacin, Sarafloxacin				
XI.	**Quinoxalines**	Carbadox, Olaquindox	-	-	✓	Digestive disease of pigs (e.g. swine dysentery)
XII.	**Amphenicols**	Florphenicol, Thiamphenicol	✓	-	-	It is a useful alternative for respiratory infections in cattle, swine and poultry and some fish diseases. Florfenicol, are used to treat pasteurellosis in cattle and pigs
XIII.	**Plueuromutilins**	Tiamulin, Valnemulin	-	✓	-	Respiratory infections in pigs and poultry, swine dysentery (Brachyspira hyodysenteriae)
XIV.	**Lincosamides**	Pirlimycin, Lincomycin	-	✓	-	Mycoplasmal pneumonia, infectious arthritis and hemorrhagic enteritis of pigs

	XV. Macrolides					
a	Macrolides C14	Erythromycin, Oleandomycin	✓	-	-	Mycoplasma infections in pig and poultry, hemorraghic digestive disease in pigs, respiratory infections and liver abscesses (Fusobacterium necrophorum) in the cattle
b	Macrolides C15	Gamithromycin, Tulathromycin				
c	Macrolides C16	Carbomycin, Josamycin, Kitasamycin, Spiramycin, Tilmicosin, Tylosin, Mirosamycin, Terdecamycin, Tildipirosin, Tylvalosin				
d	Macrolides C17	Sedecamycin				
XVI.	**Orthosomycins**	Avilamycin	-	-	✓	Digestive diseases of poultry and rabbits and necrotic enteritis in chickens
XVII.	**Aminocoumarin**	Novobiocin	-	-	✓	Novobiocin is used in the treatment of mastitis in the form of intramammary creams and sepsis in fish
	XVIII. Polypeptides					
a	Polypeptides	Enramycin, Gramicidin, Bacitracin	-	✓	-	Bacitracin is used against necrotic enteritis in poultry. Polypeptides are indicated in septicaemias, colibacillosis, salmonellosis and urinary infections
b	Polypeptides Cyclic	Colistin, Polymixin				
XIX.	**Fusidic acid**	Fusidic acid	-	-	✓	Ophtalmic diseases in cattle and horses
XX.	**Ionophores**	Lasalocid, Maduramycin, Monensin, Narasin, Salinomycin, Semduramicin	-	-	✓	Intestinal coccidiosis (*Eimeria* spp). This class currently used in animals alone
XXI.	**Arsenical**	Roxarsone, Nitarsone	-	-	✓	Intestinal coccidiosis in avian and swine
XXII.	**Thiostrepton**	Nosiheptide	-	-	✓	Treatment of dermatological conditions in swine and avian

Source: OIE, 2018 [24]

Prophylactic and metaphylactic use

Antibiotics are prophylactically used when dairy herds or flocks are exposed to infectious pathogens considered to be at risk. In poultry and livestock, mass administration of antibiotics is often practiced when transporting or moving young animals to prevent respiratory and intestinal illness, when animals have been subjected to stressful or unfavourable environmental conditions. In United States, 16% of lactating dairy cows were treated for clinical mastitis [26]. Penicillin, cephalosporins or other beta-lactam drugs are prophylactically used as intra-mammary infusions in all dairy cows following each lactation to prevent and control future mastitis [27]. Metaphylactically healthy beef calves are administered with a therapeutic dose of antibiotics to mitigate anticipated outbreaks of respiratory disease and for prevention of liver abscess in beef calves with tylosin[26, 28,29].

Pigs in intensive, indoor systems often receive antibiotic treatment at each stage of their lives until slaughter, usually at less than 6 months old. Suckling and nursery pigs are commonly affected with a gastrointestinal infection such as post weaning diarrhoea due to *Escherichia coli*, salmonellosis and respiratory diseases [30]. In Thailand, the largest proportion of medicated feed was applied to suckling and nursery pigs (39.7%) and fatteners (37.3%), the drugs like lincomycin, tiamulin and tylosin are commonly used in fatteners to prevent diseases [31]. In Belgium, 93% of pigs were treated prophylactically and 7% metaprophylactic. The most frequently used oral antimicrobials in pig herds are colistin (30.7%), amoxicillin (30.0%), trimethoprim-sulfonamides (13.1%), doxycycline (9.9%) and tylosin (8.1%). Injectable antimicrobials such as tulathromycin (45.0%), long acting ceftiofur (40.1%) and amoxicillin (8.4%) [32].

Evolution of antimicrobial resistance

Ever since there has been the discovery of antibiotics and alongside the development of resistance to new antibiotics are takes place. In fact, germs will always look for ways to survive and resist new drugs [33] (Table 2).

Table 2: Development of antimicrobial resistance to various antibiotics

Antibiotics	Year of introduction for therapy	Antimicrobial resistance gene identified bacteria	Antimicrobial resistance identified year
Penicillin	1941	*Staphylococcus aureus*	1942
		Streptococcus pneumonia	1967
		Pencillinase producing *Neisseria gonorrhoea*	1976
Vancomycin	1958	Plasmid mediated vancomycin-resistant *Enterococcus faecium*	1988
		Vancomycin-resistant Staphylococcus aureus	2002
Methicillin	1960	Methicillin resistant Staphylococcus aureus	1960
Extended spectrum cephlosporins (Cefotaxime)	1980	Extended spectrum β-lactamase producing *Escherichia coli*	1983
Azithromycin	1980	*Neisseria gonorrhoea*	1983
Imipenem	1985	Carbapenemase producing *Klebsiella pneumonia* (KPC)	1996
Ciprofloxacin	1987	*Neisseria gonorrhoea*	2007
Daptomycin	2003	*Staphylococcus aureus*	2004
Ceftazidime-Avibactam	2015	Carbapenemase producing *Klebsiella pneumonia* (KPC)	2015

Source: cdc.gov/drugresistance/about.html) [33]

Antimicrobial resistance mechanism

Bacteria may be intrinsically resistant to more than one class of antibacterial agents or may acquire resistance by a spontaneous mutation in housekeeping structural or regulatory genes of the bacterial chromosome or via the acquisition of resistance genes from other organisms [34]. Acquisition of new genetic material by antimicrobial susceptible bacteria from resistant strains of bacteria may occur through horizontal gene transfer (HGT) mechanisms includes transformation, conjugation and transduction with transposons-mobile genetic elements found in plasmids, which helps to facilitate the incorporation of multiple resistance genes into the host genome or plasmids [35]. The majority of commensal bacteria's are harmless but, it acquires antibiotic resistance gene by HGT and transfer resistance genes to pathogenic bacteria [34], acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to express efflux systems, alteration of target site that prevents the drug binding to exert antimicrobial action or to produce an alternative metabolic pathway that bypasses the action of the drug [36] (Table 3).

Table 3: Mode of action and resistant mechanism for commonly used antibiotics

Antibiotic class	Examples	Mode of action	Mode of development of resistance
B-Lactams	Pencillin, Cephalosporin, Penems (Meropenem), Monobactams (Aztreonam)	Inhibit cell wall synthesis	Hydrolysis, efflux, altered target site
Sulfonamides	Sulfamethoxazole, Sulphaquinoxaline	Inhibit folic acid metabolism	efflux, altered target
Aminoglycosides	Gentamicin, Kanamycin, Amikacin, Streptomycin Spectinomycin	Inhibit Protein synthesis	Phosphorylation , acetylation, nucleotidylation, efflux, altered target
Tetracycline	Oxytetracycline, Minocycline, Doxycyline	Inhibit protein synthesis	Monooxygenation, efflux, altered target
Macrolides	Erythromycin Azithromycin	Inhibit protein synthesis	Hydrolysis, glycosylation, phosphorylation, efflux, altered target site
Lincosomides	Clindamycin	Inhibit protein synthesis	Nucleotidylation, efflux, altered target
Oxazolidinones	Linezolid	Inhibit protein synthesis	Efflux, altered target
Glycopeptides	Vancomycin, Teichoplanin	Inhibit cell wall synthesis	Reprogramming peptidoglycan biosynthesis
Quinolones	Ciprofloxacin, Enrofloxacin Pefloxacin	Inhibit DNA replication	Acetylation, efflux, altered target
Streptogramins	Synercid	Inhibit protein synthesis	C-O lyase (type B streptogramins), acetylation (type A streptogramins), efflux, altered target
Lipopeptides	Daptomycin	Act on cell membrane	Altered target
Rifamycins	Rifampin	Inhibit transcription	ADP-Ribosylation, efflux, altered target
Phenicols	Chloamphenicol	Inhibit protein synthesis	efflux, altered target
Pyrimidines	Trimethoprim	Interfere with folate metabolism	efflux, altered target
Cationic peptides	Colistin	Act on Cell membrane	efflux, altered target

Source: Davis and Davis, 2010 [36]

Enteric pathogens *E. coli* and *Klebsiella pneumonia* have evolved resistance to the most recent generation β-lactam antibiotics by acquiring plasmids carrying extended-spectrum β-lactamases and carbapenemases [34]. Enterococci are intrinsically resistant to cephalosporins and penicillin due to the expression of low-affinity penicillin binding proteins (PBPs) that bind weakly to beta-lactam antibiotics [37]. *Saphylococcus aureus* is resistant to several antibiotics starting with Penicillin to the most recent antibiotics linezolid and daptomycin. Resistance mechanisms involve the production of inactivating enzyme penicillinase and aminoglycoside-modification enzymes (Penicillin and aminoglycosides), alteration of target binding site, trapping of antibiotics and upregulating of efflux pumps, which extrude drug from the cell (Fluoroquinolones and tetracycline) and for linezolid and dapomycin through spontaneous mutation [38]. Strains of *Pseudomonas aeruginosa* are known to utilize their high levels of intrinsic and acquired resistance mechanisms to most of the antibiotics. In addition, adaptive antibiotic resistance of *P. aeruginosa* is a recently characterized mechanism, which includes biofilm mediated resistance and formation of multidrug-tolerant persister cells, and is responsible for recalcitrance and relapse of infections [39]. A biofilm is an aggregate of microorganisms that adhere to each other on a living or non-living surface, and are embedded within a self produced matrix of extracellular polymeric substances (EPSs), including exopolysaccharides, proteins, metabolites and extracellular DNA (eDNA) [40-41]. The microbial cells grown in biofilms are less sensitive to antimicrobial agents and host immune response than the cells grown in free aqueous suspension [42]. This bacterial tolerance/persistence is responsible for the chronicity of disease [43]. Moreover, biofilm formation can also be harmful to host tissues since they can promote the phagocyte release of lysosomal enzymes, reactive oxygen and nitrogen species [44]. Administration of the third generation cephalosporin ceftiofur in dairy cows increased β-lactam and multidrug resistance genes in faeces [45-46]. *Proteus mirabilis* is a common cause of urinary tract infections (UTI). Emergence and spread of multidrug resistant *P. mirabilis* isolate, including those producing ESBLs, AmpC cephalosporinases and carbapenemases are more frequently reported and showed resistance to β-Lactams, aminoglycosides, fluoroquinolones, ceftazidime, cefotaxime, aztreonam and nitrofurans [47].

Antimicrobial resistant (AMR) bacteria in animal products

Any antimicrobial use, whether in humans, animals, plants or food processing technology, could lead to bacterial resistance [48]. The spread of AMR bacteria (AMRB) between animals and humans via the food chain and the exchange of AMR genes requires holistic approaches for risk mitigation [49]. The emergence of livestock-associated (LA) methicillin-resistant *Staphylococcus aureus* (MRSA) among and within livestock species is a relevant issue from both human and animal health perspectives [50]. The presence of MRSA in bovine milk and dairy environments poses a potential risk to farmworkers and veterinarians [51-52]. Limited data are available about the prevalence and genetic spread of MRSA in dairy environments in the United States. The low occurrence of MRSA (0-0.6%) among *S. aureus* isolates from bovine milk were reported. The herd prevalence of MRSA was 4% in Minnesota dairy farms and displayed resistance to β-lactams, cephalosporins, and lincosamides [53].

Mahami *et al.* [54] reported that the *E. coli* was the most common isolate in pasteurized and unpasteurized milk, *Staphylococcus epidermidis* in powdered milk, *E. coli* and *Enterococcus faecalis* in imported pasteurized skimmed milk, *Klebsiella* sp in soya milk and all isolates showed 100% multi-drug resistant (MDR) to ampicillin, tetracycline, chloramphenicol, gentamycin, cotrimoxazole, cefuroxime and cefotaxime in Accra, Ghana. The MDR *E. coli* (52.5%), *Proteus* spp (77.7%) and *S. aureus* (40%) were isolated in both buffalo and chicken raw meats in Nepal. Chicken meat isolates had higher antimicrobial resistance rates in comparison to buffalo meat isolates, particularly amoxicillin, tetracycline, cotrimoxazole and nalidixic acid. [55]. The ESBL producing of *E. coli* isolates were more commonly detected in pork (76.7%) than broilers (40%) [56]. Yang *et al.* [57] reported that the *Salmonella derby*, *S.typhimurium, S. london, S. rissen, S. weltevreden,* and *S.enteritidis in* different meat samples of China and showed resistance to tetracycline (65.6%), ampicillin (45.4%), trimethoprim-sulfamethoxazole (40.8%), streptomycin (40.4%) and nalidixic acid (35.8%) The pork had a higher prevalence of (37.3%) *Salmonella* contamination followed by beef (16.1%), mutton (10.9%), dumplings (6.6%) and smoked pork (3.6%) [57].

Antimicrobial resistant bacteria in the environment

Antibiotics are extensively used as therapeutic agents for the treatment of infectious diseases in human and livestock [58]. The use of antibiotics in large amount may pose a strong pressure on humans, animal and soil microbiome results in the emergence of antibiotic resistance [59-60]. In animals, most of the consumed antibiotics, 30 to 90% are excreted into the manure and urine [61]. Antibiotics can enter the environment through many ways water, sewage, effluent from pharmaceutical industries, hospital effluents, irrigation with wastewater, runoff from an agricultural field containing livestock manure [62-63]. Daily intake of antibiotic residues from the environment may largely enter the human gastrointestinal tract (harbouring diverse group of bacterial species 95% beneficial and 55% harmful and opportunistic pathogens)[59, 64] alter the intestinal microbiome composition due to the broad-spectrum influence of antibiotic on the host-associated microbial community rather than target bacteria [65]. Antibiotic therapy could lead to composition changes of intestinal microbiota with an increase in gram positive bacteria, concurrent reduction of beneficial bacteria [66], and also induce the emergence of antibiotic-resistant bacteria, which could persist in human intestines for years and excreted through faeces [63]. Cattle faeces carry more number of antibiotics than swine, administration of the third generation cephalosporin ceftiofur in dairy cows increased the excretion of β-lactam and multidrug resistance genes in faeces [46, 67]. High prevalence of cefotaxime resistant bacteria *E.coli* has been reported in faecal samples of grazing animals in farms raised without antibiotic supplementation in North and Central Florida in the United States. Cattle faeces had higher cefotaxime resistant bacteria (CRB) than others. Environmental samples had a higher prevalence of CRB in water (88.6%), soil (98.7%) and forage samples (95.7%). Soil microbiota of CRB from farms is clustered closer together with that of Phylum Proteobacteria [68]. The *E.coli, K.pneumonia* and *Staphylococcus lentus* isolated from poultry house environment showed multi-drug resistant to penicillins, fluoroquinolones, third and fourth generation cephalosporins and carbapenems [69]. The *E. coli* isolated from faeces of all animals in the northern region of Ghana, Africa showed resistance to tetracycline (54.5%), doxycycline (27.9%), ciprofloxacin (11.2%), gentamicin (7.4%) and ceftazidime (5.6%), which was higher in poultry followed by pigs, sheep, cattle and the least was in goats [70].

The manure microbiome can influence the soil microbiome through direct competition and transfer antibiotic resistance genes (ARGs) [71], the high levels of antibiotic resistant bacteria in manure and manure application has been shown to significantly increase the abundance of ARGs in soil[72-73] dispersed into surrounding waterways via runoff and drainage [74-75]. The *E.coli* isolated from the hospital effluents showed 95% resistant to third generation cephalosporin followed by domestic waste with hospital effluents in India. More than 22 tetracycline or oxytetracycline resistant genes have been found in bacterial isolates of water environments [76], tetracycline resistance genes *tetM*, *tetO*, *tetQ*, *tetW*, *tetC*, *tetH*, and *tetZ* have been reported in lagoons and groundwater adjacent to swine production[77-78]. Most of the environmental tet genes code for transport proteins, which pump the antibiotics out of the bacterial cell and keep the intercellular concentrations low to make ribosomes function normally [76, 79]. Several macrolide resistant genes (erm) have been detected in *Enterococcus* spp. isolated from poultry raising wastewaters [80]. The ARGs may be transferred to pathogenic bacteria in the environment through horizontal gene transfer; agricultural dissemination of ARGs into the environment may perpetuate the antibiotic resistance [81].

The resistome of the soils was assessed by screening the total soil DNA for clinically relevant and soil-derived antibiotic resistance genes. The predominant resistant microbiotas isolated from soil were *Pseudomonas*, *Stenotrophomonas*, *Sphingobacterium* and *Chryseobacterium* genera are largely dependent on the efflux mechanisms. Resistant mechanism of soil *Pseudomonas* spp mostly relying on Resistance-nodulation division (RND) to confers the multi-drug resistance. Soil *Stenotrophomonas* spp and *Chryseobacterium* spp develop resistance through RND and ATP- binding cassette (ABC) mediated drug efflux system [82]. The *Stenotrophomonas maltophilia* usually found in soil, sewage, water, plants, animals and humans and showed resistance to tetracycline, quinolones and chloramphenicol [83-84]. *Chryseobacterium meningosepticum* is typically found in the environment that causes neonatal meningitis and showed widespread resistance to vancomycin, erythromycin and clindamycin [85].

Detection of antimicrobial resistant genes in bacteria and environment

Traditionally, monitoring of antimicrobial resistance in bacteria rely on culture and antimicrobial susceptibility testing. It is non-expensive, time- consuming and non-conclusive and may miss most of the bacteria uncultivable [86]. Currently, molecular methods are used to detect antibiotic resistant genes includes polymerase chain reaction (PCR), quantitative real time PCR (RT-PCR), multiplex PCR, whole genome sequencing, DNA microarray, metagenomics[87] and Matrix- assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF-MS) is a new method and could become more common in future AMR characterization [86].

Conventional PCR and Multiplex PCR

PCR assays have been mostly used in both pure cultures and mixed environmental samples for detection of specific antibiotic resistant genes (ARGs) encoding resistances to β-lactams [88-89], aminoglycosides [76, 82, 90], sulphonamide [91-92], tetracycline [76], macrolides [76], chloramphenicol [76, 82], fluoroquinolones [93-94], vancomycin [88] and rifampin [82] (Table 4). It involves the extraction of DNA from target samples, amplification using a specific primer (16S rRNA gene) and visualization of amplified PCR gene products in agarose gels stained with ethidium bromide or other fluorescent DNA chelating dyes.

Multiplex PCR is often used for simultaneous detection of environmental ARGs with various primer pairs at the same time [76]. PCR is considered a rapid and convenient method for the detection of multiple ARGs in isolated bacteria or environmental DNA [95-96]. However, conventional PCR is less suitable for the detection of point mutations within the target genes. DNA sequencing is commonly used to detect the ARGs in PCR products [86, 97]. The RT-PCR can detect single point mutations in a given gene if sequence-specific DNA probes are used for targeting the mutation area.

Table 4: Detection of AMR genes in bacteria and environment

Antibiotic class	Name of the antibiotics	Antimicrobial resistant genes	Function	Resistant bacteria	Reference
B-lactams	Penicillin (Ampicillin)	bla_{SHV}, bla_{TEM}, bla_{IPM}, ^{bla}GES	β-lactamase encoding penicillin resistance, Metallo-β-lactamase	*Aeromonas* sp, *Enterobacter* sp, *Salmonella* sp, *Staphylococci* sp, *Vibrio* sp, Acinetobacter sp	[82]
		AmpC, OXA-8, VIM, KPC, OXA-48	Carbapenemase	Enterobacteriaceae family	[82, 88]
	Methicillin	mec A	-	Methicillin resistant *Staphylococcus aureus*	[88]
	Cephalosporin	BlaCMY, CTX-M, OXA-1, CMY-2	β-lactamase encoding cephalosporin resistance	*E.coli*	[82]
Aminoglycosides	Streptomycin	aadA1, ant	Adenylyl transferases	*Aeromonas* sp *Citrobacter* sp, *Shigella* sp	[76]
	Gentamicin	aac (aac(3)-IV)	Aminoglycoside acetyltransferases	*Enterococci* sp *Streptococci* sp	[76, 82, 90]
	Neomycin	aph, neptII	Phosphotransferase	Microbial communities	[76]
Sulfonamide		Sul I, Sul II, Sul III, dfr	Dihydropteroate synthase	*Aeromonas sp, E.coli, Listeria* sp, *Actinobacter* sp. *Salmonella* sp, *Vibrio* sp	[91-92]
Tetracycline		tet A, B,C,D and E	Efflux pump resistance	*Aeromonas sp, E.coli, Listeria sp, Pseudomonas sp, Salmonella* sp, *Vibrio* sp	[76]
		tet M,O,S, Q and W	Ribosomal protection proteins	Aeromonas, *Bacillus* sp, *E.coli Lactococcus* sp, *Pseudoalteromonas* sp, Vibrio *sp*	[76]
Macrolides		erm A, B,C,F,T and	Ribosomal RNA	*Enterococcus* sp, *Streptococcus* sp	[76]

		X	methylation		
Chloramphenicol	Chloramphenicol	catA1	Chloramphenicol Acetyltransferases	*Pseudomonas* sp	[76]
		CmlA	Transporter resistance	*E.coli*	[76]
		bcr/cfl	Efflux pump		[82]
Quinolones	Fluoroquinolones	*Qnr A* , B & *S*	Efflux pump	*E.coli*, *K.pneumonia*, *P.mirabillis*	[93], [94]
Glycopeptides	Vancomycin	Van A and B	-	*Enterococcus faecium*	[88]
Rifampin		arr- like-1 arr- like-2	rifampin ADP-ribosyltransferases	Soil	[82]

DNA Microarray

DNA microarrays are genomic tools that have been used to detect drug resistant genes, and are commonly used for gene profiling and expression studies. Identibac microarrays have been used to determine the presence of AMR genes in both aerobic and anaerobic gram-negative bacteria isolated from human faeces [86]. DNA microarray was constructed of 70mer oligonucelotide probes designed to detect the genes encoding resistance to aminoglycosides, β-lactams, chloramphenicols, glycopeptides, heavy metals, lincosamides, macrolides, metronidazoles, polyketides, quaternary ammonium compounds, streptogramins, sulfonamides, tetracyclines, and trimethoprims as well as resistance transfer genes for some strains of *Salmonella enterica* [98].

Metagenomic analysis

Metagenomics is a molecular tool used to analyze the DNA of microbial community acquired from environmental samples without culture [99]. It has helped to elucidate a strong correlation between AMR and microbiome through the discovery of complex microbial communities and their functional components involved in AMR in bacteria in clinical and environmental samples [100]. Metagenomics employs two distinct approaches for analysis: sequence-driven and function driven [101]. Both are based on next-generation sequencing techniques that have been developed by commercial organizations. In the sequence-driven method, multiple sequence reads are generated and analyzed using sequence-analysis software [102]. Functional metagenomics has helped in the discovery of new antimicrobial resistant determinants and mobilome and novel mechanisms of antibiotic resistance [103], identification of new ARGs in natural environments overlooks the compulsion of having previous knowledge of these genes [104-105].

Matrix- assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF-MS is a powerful analytical tool, which has been recently introduced in many clinical laboratories for the identification of bacterial species in clinical samples [86]. It is used to analyze biomolecules such as DNA, carbohydrates, proteins and peptides by their ability to become ionized and enter the gas phase and then measuring their time of flight. Here, the mass to charge (m/z) ratio of the resulting molecular fragments is analyzed to produce a molecular signature and will detect protein between the mass range of 2 and 20 kDa [106-107]. Analysis can be made directly on biological samples of blood and urine and each spectrum can be compared to commercial databases containing species-specific spectral information of microorganism for species identification[106], specific proteins or enzymes as well as smaller biomolecules such as antimicrobial agents and their degradation products [106, 108]. A large proportion of AMR determinants are proteins, so it is in principle possible to detect these or proteolytic fragments of AMR, directly in the molecular signature from the MALDI-TOF-MS, thus providing an on-the-fly resistance profile [86,109]. MALDI-TOF-MS is commonly used for rapid identification of microorganisms and to select the target antibiotics for treatment to improve the clinical outcome in hospital settings [110-111].

An alternate approach to antimicrobial resistance

Investigation of an alternative approach to tackle the antimicrobial resistance problem globally most important[112]. The antibiotic alternatives could regulate both commensal and pathogenic bacteria populations. The antimicrobial alternatives such as phage therapy, prebiotics, probiotics, metals and minerals, organic acids and essential oils are commonly used to enhance host resistance and also to reduce the disease incidence in the population [113].

Bacteriophage

Bacteriophages are viruses that can infect and kill the bacteria without any negative effect on human or animal cells used to overcome the problem of microbial resistance [114]. However, Phage therapy is not widely used currently and is approved in few countries [115], but it has the potential to control the colonization of *E.coli*, *Salmonella* spp and *Campylobacter* spp in chicken [113].

Metals and minerals

The metals such as copper (Cu^{2+})' zinc (Zn^{2+}), and silver (Ag^{+}) have antibacterial activity, it can disrupt bacterial protein functions, generate reactive oxygen species and causes damage to bacterial DNA [116-117]. The metallic copper has intrinsically antimicrobial properties and increasing attention in the face of growing antibiotic resistant bacteria [118]. Copper-containing compounds such as $CuSO_4$ and $Cu(OH)_2$ are used as the traditional inorganic antibacterial agents and antifungal activity [119]. Copper ions have demonstrated antimicrobial activity against a wide range of microorganisms, such as *Staphylococcus aureus*, *Salmonella enteritids*, *Campylobacter jejuni*, *Escherichia coli*, and *Listeria monocytogenes* [120]. Additionally, copper has been determined to be an effective antimicrobial for udder washes and prove active against a panel of bacteria and yeasts associated with bovine mastitis [121]. Copper and Zinc salts are commonly added to animal feeds in concentrations above dietary requirements to improve growth. Similarly, Zinc oxide added to pig diets has been effective in reducing post-weaning diarrhoea [122]. Metal nanoparticles such as silver, copper oxide, and zinc oxide are of particular interest for their antimicrobial properties. Copper oxide nanoparticles had excellent antimicrobial activity against various bacteria [123]. Zinc oxide nanoparticles have been demonstrated as effective bactericidal agents against antibiotic resistant *S. aureus* and *S. epidermidis* [124]. Silver nanoparticles have also been shown to be effective against bacterial and fungal species, including some important pathogens [113, 125].

Prebiotics and probiotics
Prebiotics are potential alternatives to antibiotics used for growth promotion. They are indigestible carbohydrates source fructans, oligofructose, inulin, fructooligosaccharides, galactan, galactooligosaccharides, starch, pectin, fiber components and milk oligosaccharides, alter the colon microflora in favor of a healthier gastrointestinal environment[126]. Probiotics are natural feed additives used to improve health and growth performance. Bacteria like bifidobacteria, lactobacilli, yeast and fungai *Saccharomyces cerevisiae* and Kluyveromyces are used as probiotics [127-128]. Administration of *Lactobacillus sporogenes* (100 mg/kg feed) increased body weight and improved feed conversion ratio in broilers and increased egg production in laying hens [126,129].

Organic acids
Organic acids are composed of individual or blends of several acids that have antimicrobial activities similar to those of antibiotics [130]. It has been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids are effective by altering the pH of the gastrointestinal tract [131-132], several other acids like lactic, citric, fumaric and sorbic acids and their salts calcium formate and calcium propionate are used as feed additives [133-134]. The addition of organic acids in the diet improves the proliferation of *Lactobacillus* sp and reduce the harmful bacteria [135-136] and protects the young chicks by competitive exclusion of most common enteric pathogens *Salmonella* sp *Campylobacter* sp and *Escherichia coli* [137-138]. It also enhances nutrient utilization, growth, immune response and feed conversion ratio in poultry [134].

Essential oils
Essential oils are complex mixtures of volatile compounds isolated from the whole plant or plant parts by pressing and distillation [139-140]. Most constituents of essential oils are terpenoids and phenylpropanoids. Terpenoids are more abundant than phenylpropanoids [141], posses antibacterial and antioxidant activities. The plant families with medicinal properties include garlic (Alliaceae), anise (Apiaceae), oregano (Lamiaceae), thyme (Myrtaceae) cinnamon (Lauraceae), black pepper (Piperaceae) and turmeric (Zingiberaceae) [142], lemongrass and rosewood have high bactericidal activity rather than bacteriostatic effects [143]. Thymol is a derivative of p-Cymene extracted from *Thymus vulgaris*, eugenol is oil of clove and carvacrol oil extracted from *Origanum vulgare* has high antimicrobial activity against *E.coli* and *S. typhimurium* [144]. The carvacrol, cinnamaldehyde, and capsicum oleoresin increased the gut lactobacilli and the ratio of lactobacilli to Enterobacteria in the jejunum and cecum of early-weaned piglets [145-146].

Conclusion
The global antimicrobial resistance is controlled by the countrywide implementation of monitoring and surveillance programme (antimicrobial usage, antimicrobial resistance genes in animals, animal products and environment). Knowledge on a new mechanism of antibiotic resistance in bacteria and its rapid identification, implementation of one health approach, judicious use of antibiotics, regulation of antibiotic sale, research and development for innovation of new antibiotics class, restricted use of newer antibiotics and reducing the incidence of infection through hand sanitation.

References

1. Chafer-Pericas C, Maquieira A, Puchades R. Fast screening methods to detect antibiotic residues in food samples. Trends in Analytical Chemistry 2010;29(9):1038-1049.
2. Layada S, Benouareth DM, Coucke W, Andjelkovic M. Assessment of antibiotic residues in commercial and farm milk collected in the region of Guelma (Algeria). International Journal of Food Contamination 2016, 1-16.
3. Jank L, Martins MT, Arsand JB, Motta TMC, Feijo TC, Castilhos TDS *et al*. Liquid chromatography–tandem mass spectrometry multiclass method for 46 antibiotics residues in milk and meat: Development and validation. Food Analytical Methods 2017;10:2152-2164.
4. Schjorring S, Krogfelt KA. Assessment of bacterial antibiotic resistance transfer in the gut. International Journal of Microbiology 2011, 312956.
5. Lefferts LY. A consumer perspective: to what extent does antimicrobial use in animal husbandry contribute to resistance associated human health problems?. In: *OIE* International Standards on Antimicrobial Resistance, 2003, 90-95.
6. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Frontiers in Microbiology 2018;9:2928.
7. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Meier-Kolthoff JP, *et al*. Taxonomy, physiology, and natural products of actinobacteria. Microbiology and Molecular Biology Reviews 2016;80:1-43.
8. Mak S, Xu Y, Nodwell JR. The expression of antibiotic resistance genes in antibiotic-producing bacteria. Molecular Microbiology 2014;93:391-402.
9. Marshall BM, Ochieng DJ, Levy SB. Commensals: Underappreciated reservoir of antibiotic resistance. Microbe 2009;4(5):231-238.
10. Gandra S, Josh J, Trett A, Lamkang AS, Laxminarayan R. Scoping Report on Antimicrobial Resistance in India. Washington, DC: Center for Disease Dynamics, Economics & Policy 2017, 1-130.
11. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA *et al*. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. The Lancet Infectious Diseases 2014;14(8):742-50.
12. Collignon P, Voss A. China, what antibiotics and what volumes are used in food production animals?. Antimicrobial Resistance and Infection Control 2015;4:16.
13. Xu J, Sangthong R, Virasakdi C. Antibiotic use in chickenfarms in Northbwestern China. Antimicrobial Resistance & Infection Control 2020;9:10.
14. Veterinary Medicine Directorate. Veterinary antimicrobial resistance and sales and surveillance (www.gov.uk/government/publications/veterinary-

antimicrobial reistant-sales-surveillance-2016). Accessed on 16.12.2020
15. Cuong NV, Padungtod P, Thwaites G, Carrique-Mas JJ. Antimicrobial usage in animal production: A review of the literature with a focus on low-middle income countries. Antibiotics 2018;7(3):75.
16. United States Department Agriculture, Overview of U.S. Livestock, poultry and aquaculture production in 2015 (www.aphis.usda.gov/nahms). 2016. Accessed on 16.08.2020.
17. Sivaraman. Antibiotic use in food animals: India overview. ReAct Asia-Pacific Christian Medical College, Vellore, Tamil Nadu, India.(www.reactgroup.org) 2018.
18. Jukes TH, Williams WL. Nutritional effects of antibiotics. Pharmacological Reviews 1953;5(4):381-420.
19. Cully M. Public health: The politics of antibiotics. Nature 2014;509:S16-17.
20. Sarmah AK, Meyer MT, Boxall AB. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 2006;65:725-59.
21. Van TTH, Yidana Z, Smooker PM, Coloe PJ. Antibiotic use in food animals Worldwide, with focus on Africa: Pluses and minuses. Journal of Global Antimicrobial Resistance 2020;20170-177.
22. Elliott KA, Kenny C, Madan JA. Policy paper. A global treaty to reduce antimicrobial use in livestock. Washington, DC: Center for Global Development 2017 https://www.cgdev.org/sites/ default/files/global-treaty-reduce-antimicrobial-use-livestock.pdf. Accessed on 15.11.2020.
23. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N *et al*. Antibiotic resistance-the need for global solutions. The Lancet Infectious Diseases 2013; 13:1057-1098.
24. OIE,2018.https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/A_OIE_List_antimicrobials_May2018.pdf. Accessed on 15.12.2020.
25. Food and Drug administration, FDA Announces final decision about veterinary Medicine 2005. http://www.fda.gov/oc/antimicrobials/baytril.html. Accessed on 17.12.2020
26. Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: Perspective, policy, and potential. Public Health Reports 2012;127:4-22.
27. Department of Agriculture (US). Dairy part III: reference of dairy cattle health and management practices in the United States, 2007. Fort Collins (CO): USDA, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System 2007, September 2008. Aavailable at URL: http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_dr_PartIII_ rev.pdf. Accessed on 14.11.2020.
28. Aarestrup FM. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic and Clinical Pharmacology and Toxicology 2005;96:271-281.
29. Department of Agriculture (US). Feedlot part III: health management and biosecurity in U.S. feedlots, 1999. Fort Collins (CO): USDA, Animal and Plant Health Inspection Service, Veterinary Services, National Animal Health Monitoring System 1999 December 2000. available at URL: http://www.aphis.sda.gov/animal_health/nahms/ feedlot/downloads/ feedlot99/Feedlot99_dr_PartIII.pdf. Accessed on 14.11.2020
30. Xiao-Shen L, Liu B-Guang, Dong P, Fu-lin L, Yuan L, Hu GZ. The prevalence of mcr -1 and resistance characteristics of Escherichia coli isolates from diseased and healthy pigs. Diagnostic Microbiology and Infectious Disease 2018;91:63-65.
31. Lekagul A, Tangcharoensathien V, Mills A, Rushton J, Yeung S. How antibiotics are used in pig farming: a mixed-methods study of pig farmers, feed mills and veterinarians in Thailand. BMJ Global Health 2020;5(2):e001918.
32. Callensa B, Persoonsa D, Maesa D, Laanena M, Postmaa M, Boyenb F *et al*. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig Herds. Preventive Veterinary Medicine 2012;106(1):53-62.
33. Centers for disease control and prevention, National centre for emerging and zoonotic infectious diseases (NCEZID), Division of healthcare quality promotion (DHQP), Available at cdc.gov/drugresistance/about.html, Accessed on 09.04.2020.
34. MacLean RC, Millan AS. The evolution of antibiotic resistance. Science 2019;365(6458):1082-1083.
35. Tenover FC. Mechanisms of Antimicrobial Resistance in Bacteria. The American Journal of Medicine 2006;119(6A):S3-S10.
36. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews 2010;74(3):417-433.
37. Torres C, Alonso CA, Ruiz-Ripa L, Leon-Sampedro R, Campo RD, Coque TM. Antimicrobial resistance in *Enterococcus* spp. of animal origin. Microbiology Spectrum 2018;6(4):ARBA-0032.
38. Pantosi A, Sanchini A, Monaco M. Mechanisms of antibiotic reistance in *Staphylococcus aureus*. Future Microbiology 2007;2(3):323-324.
39. Panga Z, Raudonisb R, Glickc BR, Lina TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology Advances 2019;37:177-192.
40. Donlan, RM. Biofilms: microbial life on surfaces. Emerging Infectious Disease 2002;8:881-890.
41. Das T, Sehar S, Manefield M. The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. Environmental Microbiology Reports 2013;5(6):778-786.
42. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358:135-138.
43. Burki S, Frey J, Pilo P. Virulence, persistence and dissemination of *Mycoplasma bovis*. Veterinary Microbiology 2015;179(1-2):15-22.
44. Hermeyer K, Jacobson B, Spergser J, Rosengarten R, Hewicker-Traut Wein M. Detection of *Mycoplasma bovis* by in-situ hybridization and expression of inducible nitric oxide synthase, nitrotyrosine and manganese superoxide dismutase in the lungs of experimentally-infected calves. Journal of Comparative Pathology 2011;145:240-250.
45. Chambers HF. The changing epidemiology of *Staphylococcus aureus*?. Emerging Infectious Diseases 2001;7:178-182.
46. Argudín MA, Deplano A, Meghraoui A, Dodemont M, Heinrichs A, Denis O *et al*. Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics (Basel) 2017;6(2):12.

47. Girlich D, Bonnin RA, Dortet L, Naas T. Genetics of acquired antibiotic resistance genes in Proteus spp. Frontiers in Microbiology 2020;11:256.
48. OIE Standards on antimicrobial resistances 2003 http://www.oie.int. Accessed on 08.10.2020.
49. Jans C, Sarno E, Collineau L, Meile L, Katharina DC, Stark KDC, Stephan R. Consumer exposure to antimicrobial resistant bacteria from food at Swiss retail level. Frontiers in Microbiology. 2018; 9:362.
50. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant Staphylococcus aureus in Swine Farming. Emerging Infectious Diseases. 2005; 11:1965-1966.
51. Lee JH. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Applied and Environmental Microbiology. 2003; 69:6489-6494.
52. Juhasz-Kaszanyitzky E, Janosi S, Somogyi P, Dan A. MRSA transmission between cows and humans. Emerging Infectious Diseases. 2007; 13:630-632.
53. Haran KP, Godden SM, Boxrud D, Jawahir S, Bender JB, Sreevatsan S. Prevalence and characterization of *Staphylococcus aureus*, including methicillin resistant *Staphylococcus aureus*, isolated from bulk tank milk from Minnesota dairy farms. Journal of Clinical Microbiology. 2011; 50(3): 688-695.
54. Mahami T, Odonkor S, Yaro M, Adu-Gyamfi A. Prevalence of antibiotic resistant bacteria in milk sold in Accra. International Research Journal of Microbiology. 2011; 2(4):126-132.
55. Saud B, Paudel G, Khichaju S, Bajracharya D, Dhungana G, Awasthi MS, Shrestha V. Multidrug-resistant bacteria from raw meat of buffalo and chicken, Nepal. Veterinary Medicine International. 2019;06:1-7.
56. Boonyasiri A, Tangkoskul T, Seenama C, Saiyarin J, Tiengrim S, Thamlikitkul V. Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. Pathogens and Global Health. 2014; 108(5): 235-245.
57. Yang X, Wu Q, Zhang J, Huang J, Chen L, Wu S, Zeng H, Wang J, Chen M, Wu H, Gu Q, Wei, X. Prevalence, bacterial load, and antimicrobial resistance of *Salmonella* serovars isolated from retail meat and meat products in China. Frontiers in Microbiology. 2019; 10:2121.
58. Nisha A. Antibiotic residues-a global health hazard. Veterinary World. 2008; 1:375-377.
59. National Academies of Sciences and Medicine. 2018 accessed on 19.11.2020
60. Qiao M, Ying GG, Singer AC, Zhu YG. Review of antibiotic resistance in China and its environment. Environment International. 2017; 110:160-172.
61. Berendsen BJ.A, Wegh,R.S, Memelink J, Zuidema T, Stolker LAM. The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta. 2015; 132: 258–268.
62. Ashbolt NJ, Amezquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH, Heberer T. Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environmental. Health Perspectives. 2013; 121:993-1001.
63. Ben Y, Fu C, Hu M, Liu L, Wong MH, Zheng C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environmental Research. 2019; 169:483-493.
64. Jernberg C, Lofmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 2010;156:3216-3223.
65. Blaser MJ. Antibiotic use and its consequences for the normal microbiome. Science 2016;352:544-545.
66. Perez-Cobas AE, Gosalbes MJ, Friedrichs A, Knecht H, Artacho A, Eismann K *et al*. Gut microbiota disturbance during antibiotic therapy: A multi-omic approach. Gut 2012;62:1591-1601.
67. Chambers L, Yang Y, Littier H, Ray P, Zhang T, Pruden A *et al*. Metagenomic analysis of antibiotic resistance genes in dairy cow faeces following therapeutic administration of third generation cephalosporin. PLoS One 2015;10:DOI:10.1371/journal.pone.0133764.
68. Markland S, Weppelmann TA, Ma Z, Lee S, Mir RA, Teng L *et al*. High prevalence of cefotaxime resistant bacteria in grazing beef cattle: A cross sectional study. Frontiers in Microbiology. 2019;10:176.
69. Bhushan C, Khurana A, Sinha R, Nagaraju M. Antibiotic resistance in poultry environment: spread of resistance from poultry farm to agricultural field, Centre for Science and Environment, New Delhi 2017; http://www.cseindia.org/userfiles/ Antibioticspercent 20npercent20Chickenpercent2030percent20july.pdf accessed on 10.12.2020.
70. Saba CKS. Antimicrobial resistance pattern in food-producing animals in the northern region of Ghana. Technical Report 2017, 1-19.
71. Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC *et al*. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. Journal of Environmental Quality 2009;38:1086-108.
72. Zhou X, Qiao M, Wang F-H, Zhu Y. Use of commercial organic fertilizer increases the abundance of antibiotic resistance genes and antibiotics in soil. Environmental Science and Pollution Research International 2017;24(1):701-710.
73. Xiong W, Wang M, Dai J, Sun Y, Zeng Z. Application of manure containing tetracyclines slowed down the dissipation of tet resistance genes and caused changes in the composition of soil bacteria. Ecotoxicology and Environmental Safety 2018;147:455-460.
74. Hruby CE, Soupir ML, Moorman TB, Shelley M, Kanwar RS. Effects of tillage and poultry manure application rates on Salmonella and fecal indicator bacteria concentrations in tiles draining Des Moines Lobe soils. Journal of Environmental Management 2016;171:60-69.
75. Pruden A, Arabi M, Storteboom HN. Correlation between upstream human activities and riverine antibiotic resistance genes. Environmental Science Technology. 2012;46(21):11541-11549.
76. Zhang XX, Zhang T, Fang HHP. Antibiotic resistance genes in water environment. Applied Microbiology Biotechnology 2009;82:397-414.
77. Koike S, Krapac IG, Oliver HD, Yannarell AC, Chee-Sanford JC, Aminov RI *et al*. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. Applied Environmental Microbiology 2007;73:4813-4823.
78. Bockelmann U, Dorries HH, Ayuso-Gabella MN, Marcay

MSD, Tandoi V, Levantesi C *et al*. Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Applied and Environmental Microbiology 2009;75(1):154-163.
79. Roberts MC. Resistance to tetracycline, macrolide-lincosamidestreptogramin, trimethoprim, and sulfonamide drug classes. Molecular Biotechnology 2002;20:261-283.
80. Hayes JR, Wagner DD, English LL, Carr LE, Joseph SW. Distribution of streptogramin resistance determinants among *Enterococcus faecium* from a poultry production environment of the USA. Journal of Antimicrobial Chemotherapy 2005;55:123-126.
81. Lopatto E, Choi J, Colina A, Ma L, Howe A, Hinsa-Leasure S. Characterizing the soil microbiome and quantifying antibiotic resistance gene dynamics in agricultural soil following swine CAFO manure application. PLoS One. 2019;14(8):e0220770.
82. Armalyte J, Skerniskyte J, Bakiene E, Krasauskas R, Siugzdinien R, Kareivien V *et al*. Microbial diversity and antimicrobial resistance profile in microbiota from soils of conventional and organic farming systems. Frontiers in Microbiology 2019;10:892.
83. Alonso A, Maetinez JL. Microbiology multiple antibiotic resistance in *Stenotrophomonas maltophilia*. Antimicrobial Agents and Chemotherapy 1997;41(5):1140-1142.
84. Wang Y, He T, Shen Z, Wu C. Antimicrobial resistance in *Stenotrophomonas* spp. Microbiology Spectrum 2017;6(1): ARBA-0005-2017.
85. Bloch KC, Nadarajah R, Jacobs R. *Chryseobacterium Meningosepticum*: An emerging pathogen among immune compromised adults. Report of 6 cases and literature review. Medicine 1997;76(1):30-41.
86. Anjum MF, Zankari EA, Hasman H. Molecular methods for detection of antimicrobial resistance. Microbiology Spectrum 2017;5(6):1-17.
87. Anjum MF. Screening methods for the detection of antimicrobial resistance genes present in bacterial isolates and the microbiota. Future Microbiology 2015;10(3):317-320.
88. Schwartz T, Kohnen W, Jansen B, Obst U. Detection of antibiotic-resistant bacteria and their resistance genes in waste water, surface water, and drinking water biofilms. FEMS Microbiology Ecology 2003;43:325-335.
89. Fernando DM, Tun HM, Poole J, Patidar R, Li R, Mi R, Amarawansha GEA, Dilantha Fernando WGD, Khafipour E *et al*. Detection of antibiotic resistance genes in source and drinking water samples from a first nations community in Canada. Applied and Environmental Microbiology 2016;82:4767-4775.
90. Fluit ADC, Visser MR, Schmitz FJ. Molecular detection of antimicrobial resistance. Clinical Microbiology Reviews 2001;14(4):836-871.
91. Wang N, Yang X, Jiao S, Zhang J, Ye B, Gao S. Sulfonamide-resistant bacteria and their resistance genes in soils fertilized with manures from Jiangsu Province, Southeastern China. PLoS One 2014;9(11):e112626.
92. Wu N, Zhang W, Liu H, Wang X, Yang F, Zeng M *et al*. Detection of sulfonamide resistant bacteria and resistance genes in soils. Earth and Environmental Science 2017; doi :10.1088/1755-1315/61/1/012075.
93. Mahmud S, Nazir KHMNH, Rahman MT. Prevalence and molecular detection of fluoroquinolone-resistant genes (qnrA and qnrS) in *Escherichia coli* isolated from healthy broiler chickens. Veterinary World 2018;11(12):1720-1724.
94. Majlesi A, Kakhki RK, Nejad ASM, Mashouf RY, Roointan A, Abazari M *et al*. Detection of plasmid-mediated quinolone resistance in clinical isolates of Entero bacteriaceae strains in Hamadan, West of Iran. Saudi Journal of Biological Sciences 2018;25(3):426-430.
95. Agerso Y, Bruun MS, Dalsgaard I, Larsen JL. The tetracycline resistance gene tet(E) is frequently occurring and present on large horizontally transferable plasmids in Aeromonas spp. from fish farms. Aquaculture 2007;266:47-52.
96. Gilbride KA, Lee DY, Beaudette LA. Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control. Journal of Microbiological Methods 2006;66:1-20.
97. Thompson SA, Maani EV, Lindell AH, King CJ, McArthur JV. Novel tetracycline resistance determinant isolated from an environmental strain of *Serratia marcescens*. Applied Environmental Microbiology 2007;73:2199-2206.
98. Frye JG, Lindsey RL, Rondeau G, Porwollik S, Long F, McClelland M *et al*. Development of a DNA microarray to detect antimicrobial resistance genes identified in the national center for biotechnology information database. Microbial Drug Resistance 2010;16(1):9-19.
99. Ghosh A, Mehta A, Khan AM. Metagenomic Analysis and its applications In: Encyclopedia of Bioinformatics and Computational Biology 2019, 184-193.
100. Forsberg KJ, Patel S, Gibson MK, Lauber CL, Knight R, Fierer N *et al*. Bacterial phylogeny structures soil resistomes across habitats. Nature 2014;509:612-616.
101. Schloss PD, Handelsman J. Biotechnological prospects from metagenomics. Current Opinion in Biotechnology 2003;14:303-310.
102. Bag S, Saha B, Mehta O, Anbumani D, Kumar N, Dayal M *et al*. An improved method for high quality metagenomics DNA extraction from human and environmental samples. Scientific Reports 2016;6:26775.
103. Moore AM, Patel S, Forsberg KJ, Wang B, Bentley G, Razia Y *et al*. Pediatric fecal microbiota harbor diverse and novel antibiotic resistance genes. PLoS One 2013;8(11):e78822.
104. Dos Santos DF, Istvan P, Quirini BF, Kruger RH. Functional metagenomics as a tool for identification of new antibiotic resistance genes from natural environments. Microbial Ecology 2017;73(2):479-491.
105. Rituparna D. Metagenomics: aid to combat antimicrobial resistance in diarrhea. Gut Pathogens 2019;11(1):47.
106. Murray PR. What is new in clinical microbiology-microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William beaumont hospital symposium on molecular pathology. Journal of Molecular Diagnostics 2012;14:419-423.
107. Panda A, Kurapati S, Samantaray JC, Srinivasan A, Khalil S. MALDI-TOF mass spectrometry proteomic based identification of clinical bacterial isolates. Indian Journal of Medical Research 2014;140:770-777.
108. Hrabak J, Chudackova E, Papagiannitsis CC. Detection of carbapenemases in Enterobacteriaceae: a challenge for

diagnostic microbiological laboratories. Clinical Microbiology and Infection 2014;20:839-853.
109. Kostrzewa M, Sparbier K, Maier T, Schubert S. MALDI-TOF MS: An upcoming tool for rapid detection of antibiotic resistance in microorganisms. Proteomics Clinical Applications 2013;7:767-778.
110. Frickmann H, Masanta WO, Zautner AE. Emerging rapid resistance testing methods for clinical microbiology laboratories and their potential impact on patient management. BioMedical Research International 2014, 375681.
111. Bauer KA, Perez KK, Forrest GN, Goff DA. Review of rapid diagnostic tests used by antimicrobial stewardship programs. Clinical Infectious Diseases. 2014;59(3):S134-S145.
112. Laws M, Shaaban A, Rahman KM. Antibiotic resistance breakers: Current approaches and future directions. FEMS Microbiology Reviews 2019;43:490-516.
113. Willing BP, Pepin DM, Marcolla CS, Forgie AJ, Diether NE, Bourrie BCT. Bacterial resistance to antibiotic alternatives: A wolf in sheep's clothing? 2018;8(2):38-47.
114. Principi N, Silvestri E, Esposito S. Advantages and limitations of bacteriophages for the treatment of bacterial infections. Frontiers in Pharmacology 2019;10:513.
115. Sulakvelidze A, Alavidze Z, Morris Jr JG. Bacteriophage therapy. Antimicrobial Agents and Chemotherapy 2001;45(3):649-59.
116. Rosen BP. Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. Comparative Biochemistry Physiology Part A. Molecular and Integrative Physiology 2002;133:689-693.
117. Xiu Z, Liu Y, Mathieu J, Wang J, Zhu D, Alvarez PJ. Elucidating the genetic basis for *Escherichia coli* defense against silver toxicity using mutant arrays. Environmental Toxicology and Chemistr 2014;33:993-997.
118. Hans M, Erbe A, Mathews S, Chen Y, Solioz M, Mucklich F. Role of copper oxides in contact killing of bacteria. Langmuir 2013;29:16160-16166.
119. Raffi M, Mehrwan S, Bhatti TM, Akhter JI, Hameed A, Yawar W *et al*. Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. Annals of Microbiology 2010;60:75-80.
120. Gyawali R, Ibrahim SA, Hasfa SHA, Smqadri SQ, Yosef Haik Y. Antimicrobial activity of copper alone and in combination with lactic acid against E*scherichia coli* O157:h7 in laboratory medium and on the surface of lettuce and tomatoes. Journal of Pathology 2011, 650968.
121. Reyes-Jara A, Cordero N, Aguirre J, Troncoso M, Figueroa G. Antibacterial effect of copper on microorganisms isolated from bovine mastitis. Frontiers in Microbiology 2016;7:626.
122. Vahjen W, Pietruszyńska D, Starke IC, Zentek J. High dietary zinc supplementation increases the occurrence of tetracycline and sulphonamide resistance genes in the intestine of weaned pigs. Gut Pathogens 2015;7:23.
123. Ahamed M, Alhadiaq HA, Majeed Khan MA, Karuppiah P, Al-Dhabi NA. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. Journal of Nanomaterials 2014;637858.
124. Ansari MA, Khan HM, Khan AA, Sultan A, Azam A. Characterization of clinical strains of MSSA, MRSA and MRSE isolated from skin and soft tissue infections and the antibacterial activity of ZnO nanoparticles. World Journal of Microbiology and Biotechnology 2012;28:1605-1613.
125. Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh A. Nanoparticles: Alternatives against drug-resistant pathogenic microbes. Molecules 2016;21:1-31.
126. Selaledi LA, Hassan ZM, Manyelo TG, Mabelebele M. The current status of the alternative use to antibiotics in poultry production: An African perspective. Antibiotics. 2020;9:595.
127. Rastall RA, Gibson GR. Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Current Opinion in Biotechnology 2015;32:42-46
128. Patel SG, Raval AP, Bhagwat SR, Sadrasaniya DA, Patel AP, Joshi SS. Effects of probiotics supplementation on growth performance, feed conversion ratio and economics of broilers. Journal of Animal Research 2015; 5(1):155-160.
129. Panda AK, Reddy M, Rao SR. Praharaj N. Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influenced by dietary supplementation with probiotic. Tropical Animal Health Production. 2003;35:85-94.
130. Wang JP, Yoo JS, Lee JH, Zhou TX, Jang HD, Kim HJ *et al*. Effects of phenyl lactic acid on production performance, egg quality parameters, and blood characteristics in laying hens. Journal of Applied Poultry Research 2009;18:203-209.
131. Luckstadt C. Effects of dietary potassium diformate on growth and gastrointestinal health in weaned piglets in Vietnam. Conference on International Research on Food Security, Natural Resource Management and Rural Development organized by the Czech University of Life Sciences Prague 2014;17-19.
132. Dittoe DK, Ricke SC, Kiess AS. Organic acids and potential for modifying the avian gastrointestinal tract and reducing pathogens and disease. Frontiers in Veterinary Science 2018;5:216.
133. Luckstadt C, Mellor S. The use of organic acids in animal nutrition, with special focus on dietary potassium diformate under European and Austral-Asian conditions. Recent Advances in Animal Nutrition- Australia 2011;18:123-130.
134. Khan SH, Iqbal J. Recent advances in the role of organic acids in poultry nutrition. Journal of Applied Animal Research 2016;44(1):359-369.
135. Van Immerseel F, Russell JB, Flythe MD, Gantois I, Timbermont L, Pasmans F *et al*. The use of organic acids to combat Salmonella in poultry: A mechanistic explanation of the efficacy. Avian Pathology 2006; 35:182-188.
136. Gheisari AA, Heidari M, Kermanshahi RK, Togani M, Saraeian S. Effect of dietary supplementation of protected organic acids on ileal microflora and protein digestibility in broiler chickens. In: Proceedings of the 16th European Symposium on Poultry Nutrition. Strasbourg, France. 2007, 519- 522.
137. Mansoub NH, Karim R, Leila M, Mohammad AMN, Seyede LZ, Mehdi MK. Effect of different level of butyric acid glycerides on performance and serum composition of broiler chickens. World Journal of

Zoology 2011;6(2):179-182.
138. Naseri KG, Rahimi S, Khaki P. Comparison of the effects of probiotic, organic acid and medicinal plant on Campylobacter jejuni challenged broiler chickens. Journal of Agricultural Science and Technology 2012;14:1485-1496.
139. Franz C, Novak J. Sources of essential oils. In: Baser KHC, Buchbauer G, editors. Handbook of essential oils: Science, Technology, and Applications. Boca Raton: CRC Press/Taylor & Francis Group. 2009, 39-82.
140. Zhai H, Liu H, Wang S, Wu J, Kluenter AM. Potential of essential oils for poultry and pigs. Animal Nutrition 2018;4:179-186.
141. Hammer KA, Carson CF. Antibacterial and antifungal activities of essential oils. In: Thormar H, editor. Lipids and essential oils as antimicrobial agents. Iceland: Wiley 2011, 255-306.
142. Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils. Industrial Crop Products 2014;62:250e64.
143. Vasireddy L, Bingle LEH, Davies MS. Antimicrobial activity of essential oils against multidrug-resistant clinical isolates of the *Burkholderia cepacia* complex. PLoS One 2018;13(8):e0201835.
144. Bassole IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules 2012;17:3989-4006.
145. Manzanilla E, Nofrarias M, Anguita M, Castillo M, Perez J, Martin-Orue S *et al*. Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. Journal of Animal Science 2006;84:2743-2751.
146. Castillo M, Martín-Orue S, Roca M, Manzanilla E, Badiola I, Perez JF *et al*. The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. Journal of Animal Science 2006;84:2725-2734.s