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REVIEW ARTICLE

The Under Reported Issue of Antibiotic-Resistance in Food-Producing Animals in Pakistan

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A B S T R A C T

The recent revelation of a plasmid-mediated colistin resistant superbug isolated in China from food-producing animals (FPAs) and epidemic of a pan-drug resistant human typhoid strain in Hyderabad, Pakistan proved the notion that novel mechanisms of antibiotic resistance will emerge as we continue to rely on consistent use of antibiotics. The emergence and dissemination of antibiotic resistance is accelerated up by indiscriminate and incessant usage of antibiotics due to uncontrolled infections in hospital settings, community, FPAs and aquaculture. Unfortunately, a major part of the global antimicrobial usage is utilized in FPAs either for therapeutic purpose and/or as growth promoters raising vital concerns of the emergence of novel multidrug-resistant microorganisms. Pakistan-an agriculture-based country ranking in top ten livestock producing countries- is currently shifting towards modern intense farming practices whereby animals are being supplemented with antibiotics on routine basis. However, unfortunately, monitoring usage of antibiotics in FPAs is lacking. Above all, the absence of systemic surveillance data on the level, frequency and impact of antimicrobial resistance further making it more challenging to devise antimicrobial-resistance-control strategies. We are highlighting this lack of information and hope that this review will help us in getting attention of policymakers for improving infection control measures and implementing laws on restricted use of antibiotics in animals.

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INTRODUCTION

Antimicrobials or antibiotics exhibit killing or growth inhibiting activity against target microorganisms, and are naturally secreted by fungi, actinomycetes, and bacteria. It is thought that antibiotic secretion is triggered to compete for available space and nutrients and is therefore vital for the survival of host organisms. The development of resistance in a target microorganism is the acquisition of elements from the genetic pools of resistance that confer resistance against the selected antimicrobials (Levy, 2002b, 1994). Practically, drug resistance is seen when both components, the resistance conferring elements and antimicrobials, came together in the host or in an environment. Current literature indicates that there are more than 15 different classes of antibiotics (Levy, 2002a) and none has escaped a resistance mechanism (Goossens *et al.,* 2005). Although, understanding biochemical mechanisms of drug resistance is crucial, of equal importance is elucidating the ability to disseminate these drug resistance elements.

Antibiotics are largely used in food-producing animals (FPAs) all over the world. In fact, the current modernization, intensification and efficient production of FPAs realized this partly as a result of incorporation of growth supplements, which includes mainly antibiotics (Price *et al.,* 2005). It has been estimated that the global average annual consumption of antimicrobials per kilogram of animal produced was 45 mg·kg−1, 48 mg·kg−1, and 172 mg·kg−1 for cattle, chicken, and pigs, respectively, for the year 2010. This usage of antibiotics in FPAs has been projected to increase by 67% in 2030 (Van Boeckel *et al.,* 2015). This widespread use of antibiotics in FPAs contributes by means of natural selection- to the emergence of antimicrobial resistance (AMR), which has public health consequences. AMR carrying pathogens can be transmitted to human from environment (Graham *et al.*, 2009; Mohsin *et al.*, 2017) or food products (Price *et al.*, 2005) and thus turns into a public health issue. In Pakistan, food-animals are becoming increasingly important for food security. Pakistan is in the list of top 10 animal producing countries (Page and Gautier 2012) with a recent trend of modern intense farming practices. Unfortunately, this modern animal farming in Pakistan, like in many other countries, largely relies on the consistent use of antibiotics as growth promoters and prevention against many diseases. Sadly, no proper systemic surveillance data of the antimicrobial usage (AMU) or emergence of AMR in the country is available; nonetheless, available literature suggests an overall increase in AMR. Emergence and dissemination of antibiotic-resistant pathogens from FPAs carry major public health consequences as well constituting reservoirs for resistance elements in Pakistan (Mohsin *et al.*, 2017; ur Rahman *et al.*, 2018b) (https://www.dawn.com/news/1328924); hence, controlled and restricted use of antibiotics in livestock is imperative. In this review, we accumulated and organized the available data found in different online resources, newspaper reports and also interviewed few veterinary clinicians regarding practices, the occurrence and nature of antibiotic resistance in FPAs in Pakistan.

Development of AMR and its impact: A resistant bacterium offers drug resistance by one of the main following mechanisms (i) reducing import and increasing export of the antibacterial drugs (genetic alterations in porins, up-regulated efflux pumps), (ii) modification, mainly structural, of the drug target (mutations, enzymatic modification), (iii) over expression or increased biosynthesis of the drug target, (iv) secretion of enzymes modifying, degrading or altering targeted antimicrobials such as ß-lactamase (Ali, Tariq *et al.*, 2018; Normark and Normark 2002; ur Rahman *et al.*, 2018a). In addition, bacteria have evolved secretory systems for secretion of intrinsically manufactured toxins and proteins as signals and virulent factors *etc*. (ur Rahman and van Ulsen 2013; ur Rahman *et al.*, 2014; van Ulsen *et al.*, 2014; Piet *et al.*, 2016) with potential contact-dependent killing of target bacteria.

Although, causalities due to the overuse of antibiotics in animals is hard to establish, however, evidence of close association- between livestock-associated resistant bugs in animals and human (Vieira *et al.*, 2011; Khattak *et al.*, 2018), level of use of antimicrobials and prevalence of resistant bacteria in animals (Aarestrup, 2005), and in humans (Schwarz *et al.*, 2001) exist. For prevention of diseases, antimicrobials are continuously administered in drinking water or added in daily feed for a longer period at lower doses (Wegener, 2003), while growth promoters are supplemented on routine basis in feed or drinking water at low level (Stokstad and Jukes 1950), potentially encouraging growth of selected resistant bacteria (Alexander *et al.*, 2008). There is current understanding that the addition of antibiotics in agriculture and aquaculture are the major sources, besides human use, that help in spreading antimicrobial-resistant microorganisms AMROs) in the surroundings. In this context, two major determinants- the quantity and pattern of antibiotics applied in FPAs-are considered crucial for the emergence and dissemination of resistant bacteria in animal reservoir. Thus, the level and pattern of resistance observed to the great extent reflects the drug usage in animals, nevertheless, other factors may also be important, such as vertical spread of clones resistant to antibacterial drugs and selective and successful adaptation of the resistant strains (Aarestrup *et al.*, 2008).

Presence of antibiotic residues in milk of high producing animals and their spread to the environment is a matter of great concern for public health. A study analyzed marketed dairy milk for the presence of β lactam antibiotic residues, and results indicated that 36.50% of milk samples were found positive for ß-lactam antibiotics residues present at above-permissible level (Khaskheli *et al*., 2008). Other studies suggest a high concentration of different antibiotics in meat, milk, and eggs from various regions of Pakistan (Jabbar, 2013; Solangi *et al*., 2013) suggesting high usage of antibiotics in FPAs.

Resistance elements from animal pool spread through numerous ways, where the food-borne route probably is the most important route. It is particularly worrisome, when the resistance features (elements such as genes) are acquired by pathogens (Geisinger and Isberg, 2017) such as *Salmonella enterica* (Angulo *et al.*, 2000; White *et al.*, 2001), *Campylobacter jejuni*/*coli* (Altekruse *et al.*, 1999; Pezzotti *et al.*, 2003) and *Yersinia entercolitica* (Aarestrup, Frank Møller *et al.*, 1998; Dallal *et al.*, 2010), which are widespread worldwide mainly in industrialized countries (Aarestrup, Frank M *et al.*, 2008). Direct contact between animals and human or human with animal environment may also be a major route of transmission, exemplified by the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) (Lee, 2003; Juhász-Kaszanyitzky *et al.*, 2007;). Furthermore, commensal bacteria with resistance-conferring elements (Wang *et al.*, 2006) as well as the left-over residues of antibiotic mainly in the form of manure (Heuer *et al.*, 2011), where it affects bacteria in soil or wild fauna, thus conveying resistance features to those living in the soil constituting reservoirs of resistance elements (Kemper 2008; Finley *et al.*, 2013).

Besides food-borne route, the spread of zoonotic pathogens harboring AMR features is a big challenge. There are increasing reports of isolation of *Salmonella* and *Escherichia* carrying extended spectrum β lactamase (ESBL) and quinolones resistance (Xia *et al.*, 2010). For more detailed review readers can read referred review article (Aarestrup, *et al.*, 2008). Altogether, there is a clear adverse effect on public-health because of resistant bacteria spread as a result of usage of antimicrobials in veterinary, agriculture, and other non-human contexts. Such consequences like infections that would not have been otherwise happened, recurrent infections due to treatment failure *etc*., are included.

One of the reasons that AMR became a concern is its economic impact on public, patients, ecosystem, food chain, healthcare providers and pharmaceutical industries. AMR increases hospital stay, duration of treatment, dosage of treatment and more importantly, rendering otherwise effective antibiotics ineffective. The institute of medicines estimates the annual cost of treating infections due to antibiotic resistant bacteria in the United States to about 4-5 M USD (Institute of Medicine, 1998) despite the fact that calculation of economic impact always leaves a lot of questions behind, mainly, by not incorporating all the contributing elements with its due impact. Though AMR is increasingly reported in animals and humans, however, limited data is available on the magnitude of economic impact and output on healthcare systems, patients, livestock and agriculture. The issue is even worse in developing countries like Pakistan whereby the national action plan for the confinement of AMR has not yet been implemented, its proposed plans are formulated in line with the WHO.

Patterns of usage of antibiotics in food producing animals in Pakistan: Unfortunately, in Pakistan, AMU in food-producing animals is unregulated and there is no estimation of annual usage. AMR is one of the major health challenges in Pakistan (Levy, 2002a; Goossens *et al.*, 2005; Ali *et al.*, 2010; Idrees *et al.*, 2011; Khan *et al.*, 2013; Adnan *et al.*, 2017; Mohsin *et al.*, 2017; ur Rahman *et al.*, 2018b). Although, it is hard to find the exact amount of antibiotics being used for treatment, prevention and as growth promoters in FPAs in Pakistan, nevertheless, practices and trends at commercial as well as domestic level indicate that major share of antibiotic usage is aimed for disease prevention and as growth promoters and is offered as supplements on routine basis [https://www.dawn.com/ news/1245840]. Approximately, 50,000 unnecessary registered products and over 600,000 quacksare active in the market (http://www.who.int/ medicines/areas/coordination/ pakistan.pdf) are further exacerbating the situation. A significant number of antimicrobials used in FPAs are important as human medicines (Van Boeckel *et al.*, 2014). Interviewing different farmers and representatives of companies supplying veterinary medicines in Pakistan revealed that numerous types of antibiotics are used as growth promoters in Pakistan. These include mainly lactam antibiotics (comprising mainly penicillins), lincosamides, and macrolides including erythromycin and tetracyclines, oligosaccharide, avilamycin, flavophospholipol. Virginiamycin *etc*. (Table 1). In European Union, monensin is widely used, which is probably one of the safest and most effective antibiotic growth-promoter with regard to human and animal health and associated bacterial-resistance problems. However, monensin is related to pristinamicin and quinupristin, both of which are applied in human medicine and so there are worries that its consistent usage may compromise human therapy (Butaye *et al.*, 2000). Few studies reported antibiotic residues in poultry meat and dairy milk comprising oxytetracycline, gentamicin, neomycin, enrofloxacin, chloramphenicol, amoxicillin, benzylpenicillin, streptomycin, and tylosin (Muhammad *et al.*, 1997; Shahid *et al.*, 2007a; Shahid *et al.*, 2007b), suggesting overuse of these antimicrobials. In the Pakistani context, major contributing factors to the dominantly increasing AMR comprise indiscriminate and overuse of antibiotics in FPAs and all sectors of health, unsuitable selection of antibiotics, poor practices or non-existent institutional antibiotic policies, overwhelming availability of substandard antibiotics in the market, unavailability of appropriate microbiological facilities and research.

Prevalence of antibiotic resistance (AMR) in food-producing animals in Pakistan: For a strategic framework development against AMR, its extent and magnitude must be determined. Unfortunately, national surveillance AR data is lacking, however, fragmented reports have been published mainly focusing on FPAs and agriculture. The data has huge gaps, particularly the extent, quality, quantity and pattern of antibiotic usage is not known, and the overall magnitude of AR is also not determined. However, a few available reports can serve as a point of start for a strategy against AMR. *Escherichia coli* isolated from poultry has been reported to acquire plasmid-mediated colistin (*mcr-1*) resistance in recent studies in Pakistan (Azam *et al.*, 2017; Lv *et al.*, 2018; Aqib *et al.*, 2019). Similarly, an estimated 90% of retail meat samples were found contaminated with multidrug-resistant Campylobacter spp. Majority of these isolates were found resistant to enrofloxacin (79.2%), followed by tylosin (77.6%), ciprofloxacin and amoxicillin (71.2% each), colistin (69.6%), neomycin (32.8%), nalidixic acid (31.2%), gentamicin (25.6%) etc. (Nisar *et al.*, 2017). Multi-drug resistant bacteria harboring antibiotic resistance genes were also found highly prevalent in fish and aquaculture in Pakistan (Shah *et al.*, 2012) (Phillips 2000). Environmental contamination with antibiotic residues may also contribute to drug resistance. Water samples of rivers in Punjab (the largest province of Pakistan) were found containing higher than acceptable level of antibiotic residues, and consistent to this, antibiotic resistance genes were also amplified from water samples (Khan *et al.*, 2013; Mohsin *et al.*, 2017). *E. coli* isolated from chicken and buffalo meat samples

Table 1: Commonly used antibiotics used in food-producing animals in Pakistan

Antibiotic	Class	Purpose	Type of animals	Comments
Amoxicillin and ampicillin	Aminopenicillins	In aquaculture, treatment and prevention of bovine subclinical mastitis	Bovine and fish	(Butaye *et al.*, 2003) General practice in Pakistan
Zinc bacitracin	Polypeptides	Growth promoters for beef and poultry, prevention of poultry enteritis	Poultry and bovine	Interviewing feed mill experts
Bambermycin	Phosphoglycolipids	Growth promoter for poultry and cattle *etc.*	Poultry and cattle	Structurally similar to vancomycin group
Colistin	Cyclopolypeptides	Used as growth promoter mainly in poultry and rarely in cattle	Poultry and cattle	Structurally similar to and share cross resistance with Polymyxin
Enrofloxacin	Fluoroquinolone	Therapy and prevention of respiratory infections in poultry and cattle	Poultry and bovine	
Erythromycin	Macrolides	Used in aquaculture, therapy for poultry and bovine infections	Bovine and poultry	
Lincomysin	Lincosamide	Growth promoters in poultry,	Poultry and bovine	
Monensin	Ionophores	Growth promoter in poultry, prevention of coccidiosis in poultry	Poultry	
Neomycin	Aminoglycoside	Growth promoter for poultry	Poultry	
Sulfonamides	Sulfonamides	Growth promotes in poultry,	Poultry a	
Tetracycline	Tetracycline	Growth promoter for poultry, prevention of multiple diseases in livestock and poultry	Poultry and livestock	
Virginiamycin	Streptogramins	Growth promoter for poultry (broiler)	Poultry	

were found resistant to sulfamethoxazole-trimethoprim harboring *sul1* and *sul2* genes conferring resistance to sulfonamides (Idrees *et al.,* 2011). A study on chicken meat samples at retail market in Hyderabad Pakistan indicated that 38% meat samples contaminated with *Salmonella* and all of them were found resistant to ampicillin (Soomro *et al.,* 2011). Similarly, of 126 samples from broiler meat 71.4% were found contaminated with *E. coli* and majority of them were resistant to amoxicillin and tetracycline.

The percentage resistance against streptomycin and sulphamethoxazole/trimethoprim was found to be high in backyard poultry (64.2% and 53.5%) as compared to the commercial broiler (28.5% and 35.7%) (Akhtar *et al.,* 2016). A study carried out in Karachi reported that out of 340 samples of meat analyzed for the isolation of ARMOs 66% contained pathogenic bacteria including 33% *Salmonella* isolates that were all found resistant to ampicillin. Furthermore, of these pathogenic bacteria (namely *E. coli, Klebsiella, Enterobacter* species and *Staphylococcus aureus*), 16% were capable of biofilm formation (Ali *et al.,* 2010). Similarly, a study conducted in 2014 in Lahore identified a number of pathogenic species such as *E. coli, Klebsiella, Shigella, Salmonella and Enterobacter* recovered from different market-food items such as dahi bhallay (a spicy appetizer), yoghurt and fruit-chat (combination of different seasonal fruits with sauce). Of these isolates, 46% were found resistant to commonly used antibiotics (Saleem *et al.,* 2014). Similar incidence of AMROs in ready to eat raw food items has also been reported in other parts of the country (Capita and Alonso-Calleja 2013; Nasim *et al.,* 2016; Shah *et al.,* 2012; Siddiqui *et al.,* 2015).

Where Pakistan stands in the perspective of global regulations and what strategy should be adopted: Pakistan has established its national body, Division of Pharmacy Services of the Drug Authority of Pakistan (DRAP) (http://www.dra.gov.pk/), in November 2012, however, unfortunately, the regulations drafted have not yet implemented strictly. Similarly, Pakistan has also established a policy of restricted use of antibiotics in animal husbandry; however, the policy is also not yet been implemented strictly (https://www.cddep.org/wp.../06/abrinlmics_cddep_gelband_and_delahoy_9-14.pdf).
Pakistan has further improved the situation by constituting a national strategic frame work for containment of AR in 2016 (https://drive.google.com/file/d/0B6zZgBqF00pFV0J0a2pqeVFvS3JzR2cyd1NhR0stSTBoVzR3/view), in line with WHO global action plan to fight AMR. As a commitment to the global action plan to tackle AMR, Pakistan has recently developed its national strategic framework for containment of AMR with intention to convert this to an AMR National Action Plan (NAP) by "One-Health" approach (https://www.nih.org.pk/wp-content/ uploads/2018/08/AMR-National-Action-Plan-Pakistan. pdf). As per the guidelines of national action plan for confinement of AMR, non-therapeutic use of antibiotics in FPAs and plants should be discouraged. Establishment and strengthening of AMR surveillance, initiation and encouragement of antibiotic stewardship plans, improving farm biosecurity and awareness against AMR should immediately be adopted in all relevant veterinary, public and private, stakeholders. For high producing animals, other innovative approaches like development of vaccines, alternative treatments like use of phages, and medicinal plants should be encouraged to replace the use of antibiotics. Molecular epidemiological data using One-Health approaches is required to link resistance clones or genes from different sample sources such as, from animals of different origin, human and the environment.

Maximum residual limits have been established by various countries such as Canada, China, Japan and member states of the EU in order to protect consumers from the side effects of drug residues used in production of FPAs (Reig and Toldrá 2008) and EU countries have even withdrawn the use of ionophores in feed (Casewell *et al.,* 2003; Hunter *et al.,* 2010). Unfortunately, despite the implementation of strict guidelines for the use of antimicrobial agents in FPAs world-wide, they are still being used illegally in many developing countries including Pakistan (Byarugaba, 2004). In Sweden, no antibiotic as growth promoters is allowed, while in the US a range of antibiotics are allowed as growth promoters. Overall, the use of antibiotics in different FPAs differ enormously. The World Health Organization (WHO) and Food and Agriculture Organization (FAO) are working together at international level for devising the AMU. The Codex Alimentarius Commission (CAC), under the WHO and FAO, has established standards and recommendations that should be implemented by all countries as a code of practice to minimize and contain AMR (Commission, Codex Alimentarius 2005; Commission, Joint FAO/WHO Codex Alimentarius *et al.,* 2003). In conclusion, challenging and daunting tasks are ahead of Pakistan to fight AMR issue in the near future. Gaps and hurdles include weak health infrastructure and relevant expertise, illiteracy, lack of awareness among the clinicians, farmers and public and lack of access to basic needs, clean water and vaccination. National level strategies including infection control and antibiotic stewardship must urgently be initiated and implemented, where it has already been drafted to improve antibiotic prescription and utilization along with nationwide vaccination to control several epidemic infections. This should further be assisted with regular surveillance programme to determine the extent and trend of AMR.

Factors affecting the spread and dissemination of resistance: To simplify the resistance phenomenon, we must keep working and concentrate on two main factors; the antibiotics itself that provide selective pressure to dominate the second important element- the resistance genes. After all, if for example, either the antibiotics or the resistance genes were not existed, we would not have the phenomenon of resistance. Shortly, a more logical way to look at this problem would be either to avoid selection and/or to stop the dissemination of resistant bacteria. Thus, dissemination of antibiotic resistance can be limited either through management of the selective pressure leading to resistance or through interventions aimed at restricting the spread of the selected resistance traits.

In the United States alone, an estimated 23×10^6 of antibiotics are used annually; half of this are provided to people for use during sickness and the rest is for agricultural use (Harrison and Lederberg 1998). About 7 x 10^6 kg of antibiotics, mainly penicillin and tetracycline, are annually used in FPAs as growth promoters (Harrison and Lederberg 1998). On top of that, an estimated 45 x 10^3 kg of antibiotics, chiefly tetracycline and streptomycin, are sprayed over the fruits and crops annually. Most of these compounds would certainly exist in environment as residues and bacteria would confront them at a point. A pool of genes, thus, conferring resistance against these antibiotics would certainly accumulate/concentrate in the environment in response to selective pressure provided by the existing residues antibiotics in the environment, animal body and food. Such elements could also easily be spread through river water or by wild life and migratory birds to other areas as identified by Raza and his colleagues in Pakistan (Mohsin *et al.,* 2017; Raza *et al.,* 2017). We could not identify the amount of antibiotics used in Pakistan due to unavailability of reports, however, based on the prevailing practices about how easily antibiotics are accessible in the country, it is not hard to estimate that we are currently using absolutely higher amount of antibiotics in clinical settings, agriculture and food animals. Antibiotics in Pakistan are available in the pharmacy stores, and even vendors are selling them out on the streets. This chaotic way of availability of antimicrobials and drug distribution scenario further nurtures emergence of antibiotic resistance. This blind use of antibiotics is thus encouraging new elements of AMR to emerge against antibiotics. Antibiotics are excreted in the environment, water, crops *etc.*, where they keep exerting selective pressure resulting in "post-therapy" environmental selection phase of the antibiotics (Levy, 2001). During such phase, the antibiotic concentration would be less than the therapeutic concentration, which is ideal for selecting ARMOs. Thus, considering this scenario, it may not be the use of consistent high concentration of antibiotics in clinics for treatment (treatment period) rather the slow and persistence release of low amount of antibiotics in the environment (post therapy period) that provide ample amount of time for bacteria to develop resistance. Altogether, we should change our attitude and course of action against the use of antibiotics. First, we should emphasize to implement the shorter course of antibiotics. Secondly, cycling of the antibiotics should be considered, if new antibiotics are available in the market. Thirdly, we should alongside invest in discovering and synthesizing new antibiotics such as improvement of the current tetracycline drugs, understanding the mechanisms of resistance to currently available tetracycline drugs would help improve the efficacy and the fight against resistance. A non-chemical, non-classical approach to reversing the resistance problem would be the revival of the susceptible strain. We need to encourage the growth of the susceptible strain to take over the resistance one.

Conclusions: Despite lack of a routine nation-wide surveillance, existing literature show that AMR is escalating in Pakistan. An urgent intervention is needed to restrain emergence and spread of AMR. Pakistan should learn from other countries who observed a dramatic decrease in resistance to cephalosporin after stopping use of β-lactam and cephalosporins in poultry production. Proper planning, improved use of antimicrobials, establishment of effective surveillance systems, constituting and enforcing legislature and combined international actions should immediately be taken for the control of antibiotic resistance spread and reversal of resistance. Infection control measures and stewardship programs are vital weapons in controlling the pandemic evolution of multidrug-resistance. In conclusion, AMR is a complex multifactorial phenomenon in context of low-middle income countries where the social behavior and knowledge of farmers and veterinarians towards AMU and AMR is imperative for the design of successful control strategies.

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REFERENCES

Aarestrup FM, Wegener HC and Collignon P, 2008. Resistance in bacteria of the food chain: epidemiology and control strategies. Expert review of anti-infective therapy 6:733-50.

Aarestrup FM, 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin Pharmacol Toxicol 96:271-81.

Aarestrup FM, Bager F, Jensen N, *et al.*, 1998. Resistance to antimicrobial agents used for animal therapy in pathogenic-, zoonotic-and indicator bacteria isolated from different food animals in Denmark: a baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). Apmis 106:745-70.

Aqib AI, Nighat S, Ahmed R, *et al.*, 2019. Drug Susceptibility Profile of Staphylococcus aureus Isolated from Mastitic Milk of Goats and Risk Factors Associated with Goat Mastitis in Pakistan. Pak J Zool 51:307-15.

Jabbar A, 2013. Microbiological evaluation of antibiotic residues in meat, milk and eggs. J Microbiol Biotech Food Sci 2:2349.

Adnan M, Khan H, Kashif J, *et al.*, 2017. Clonal Expansion of Sulfonamide Resistant Escherichia coli isolates Recovered from Diarrheic Calves. Pak Vet J 37:230-2.

Akhtar F, Rabbani M, Muhammad K, *et al.*, 2016. Comparative antibiotic resistance profile of the multidrug resistant e. coli isolated from commercial and backyard poultry. J Anim Poultr Sci 26:1628-32.

Alexander T, Yanke L, Topp E, *et al.*, 2008. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. Appl Environ Microbiol 74:4405-16.

Ali NH, Farooqui A, Khan A, *et al.*, 2010. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J Infect Dev Ctries 4:382-8.

Altekruse SF, Stern NJ, Fields PI *et al.*, 1999. Campylobacter jejuni--an emerging foodborne pathogen. Emerg Infect Dis 5:28.

Angulo FJ, Johnson KR, Tauxe RV *et al.*, 2000. Origins and consequences of antimicrobial-resistant nontyphoidal Salmonella: implications for the use of fluoroquinolones in food animals. Microb Drug Resist 6:77-83.

Azam M, Ehsan I, Saleemi MK, *et al.*, 2017. Detection of the colistin resistance gene mcr-1 in avian pathogenic *Escherichia coli* in Pakistan. J Glob Antimicrob Re 11:152-3.

Butaye P, Van Damme K, Devriese L, *et al.*, 2000. In vitro susceptibility of Enterococcus faecium isolated from food to growth-promoting and therapeutic antibiotics. Int J Food Microbiol 54:181-7.

Byarugaba D, 2004. Antimicrobial resistance in developing countries and responsible risk factors. Int J Antimicrob Agents 24:105-10.

Capita R and Alonso-Calleja C, 2013. Antibiotic-resistant bacteria: a challenge for the food industry. Crit Rev Food Sci Nutr 53:11-48.

Casewell M, Friis C, Marco E, *et al.*, 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 52:159-61.

Commission CA, 2005. Code of practice to minimize and contain antimicrobial resistance. CAC/RCP:61-2005.

Commission JFWCA, Programme JFWFS and Organization WH. Codex Alimentarius: Food hygiene, basic texts: Food & Agr Org, 2003.

Dallal MMS, Doyle MP, Rezadehbashi M, *et al.*, 2010. Prevalence and antimicrobial resistance profiles of Salmonella serotypes, Campylobacter and Yersinia spp. isolated from retail chicken and beef, Tehran, Iran. Food Control 21:388-92.

Finley RL, Collignon P, Larsson D, *et al.*, 2013. The scourge of antibiotic resistance: the important role of the environment. Clin Infec Dis 57:704-10.

Geisinger E and Isberg RR, 2017. Interplay Between antibiotic resistance and virulence during disease promoted by multidrug-resistant bacteria. J Infec Dis 215:S9-17.

Goossens H, Ferech M, Vander Stichele R, *et al.*, 2005. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 365:579-87.

Graham JP, Evans SL, Price LB *et al.*, 2009. Fate of antimicrobial-resistant enterococci and staphylococci and resistance determinants in stored poultry litter. Environ Res 109:682-9.

Harrison PF and Lederberg J, 1998. Antimicrobial resistance: issues and options: National Academies Press.

Heuer H, Schmitt H and Smalla K, 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 14:236-43.

Hunter PA, Dawson S, French GL, *et al.*, 2010. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. J Antimicrob Chemother 65:i3-17.

Idrees M, Shah MA, Michael S, *et al.*, 2011. Antimicrobial resistant Escherichia coli strains isolated from food animals in Pakistan. Pak J Zool 43:303-10.

Institute of Medicine. Antimicrobial drug resistance: issues and options. Workshop report. Washington: National Academy Press, 1998.

Juhász-Kaszanyitzky É, Jánosi S, Somogyi P, *et al.*, 2007. MRSA transmission between cows and humans. Emerg Infect Dis 13:630-2.

Kemper N, 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecolog Indic 8:1-13.

Khan GA, Berglund B, Khan KM, *et al.*, 2013. Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities–a study in Pakistan. PLoS One 8:e62712.

Khattak I, Mushtaq MH, Ayaz S, *et al.*, 2018. Incidence and Drug Resistance of Zoonotic Mycobacterium bovis Infection in Peshawar, Pakistan.

Khaskheli M, Malik R, Arain M, *et al.*, 2008. Detection of ß-lactam antibiotic residues in market milk. Pak J Nutr 7:682-5.

Lee JH, 2003. Methicillin (oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 69:6489-94.

Levy SB, 2002a. The antibiotic paradox: how the misuse of antibiotics destroys their curative powers: Da Capo Press.

Levy SB, 2002b. Factors impacting on the problem of antibiotic resistance. J Antimicrob Chemother 49:25-30.

Levy SB, 2001. Antibiotic resistance: consequences of inaction. Clin Infec Dis 33:S124-9.

Levy SB, 1994. Balancing the drug-resistance equation. Trends Microbiol 2:341-2.

Lv J, Mohsin M, Lei S, *et al.*, 2018. Discovery of a mcr-1-bearing plasmid in commensal colistin-resistant *Escherichia coli* from healthy broilers in Faisalabad, Pakistan. Virulence pp:1-13.

Mohsin M, Raza S, Schaufler K, *et al.*, 2017. High prevalence of CTX-M-15-Type ESBL-producing *E. coli* from migratory avian species in Pakistan. Front Microbiol 8:2476.

Muhammad G, Anal A, Athar M, *et al.*, 1997. Testing milk and meat for antibiotic residues. Pak J Food Sci 7:35-8.

Nasim A, Aslam B, Javed I, *et al.*, 2016. Determination of florfenicol residues in broiler meat and liver samples using RP-HPLC with UV–visible detection. J Sci Food Agr 96:1284-8.

Nisar M, Ahmad MuD, Mushtaq MH, *et al.*, 2017. Prevalence and antimicrobial resistance patterns of Campylobacter spp. isolated from retail meat in Lahore, Pakistan. Food Control 80:327-32.

Normark BH and Normark S, 2002. Evolution and spread of antibiotic resistance. J Intern Med 252:91-106.

Page S and Gautier P, 2012. Use of antimicrobial agents in livestock. Revue Scientifique et Technique-OIE 31:145.

Pezzotti G, Serafin A, Luzzi I, *et al.*, 2003. Occurrence and resistance to antibiotics of Campylobacter jejuni and Campylobacter coli in animals and meat in northeastern Italy. Int J Food Microbiol 82:281-7.

Phillips M, 2000. The use of chemicals in carp and shrimp aquaculture in Bangladesh, Cambodia, Lao PDR, Nepal, Pakistan, Sri Lanka and Viet Nam. In: Use of Chemicals in Aquaculture in Asia: Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia 20-22 May 1996, Tigbauan, Iloilo, Philippines: Aquaculture Department, SEAFDEC, pp:75-86.

Piet JR, van Ulsen P, Ur Rahman S, *et al.*, 2016. Meningococcal Two-Partner Secretion Systems and Their Association with Outcome in Patients with Meningitis. Infect Immun 84:2534-40.

Price LB, Johnson E, Vailes R *et al.*, 2005. Fluoroquinolone-resistant Campylobacter isolates from conventional and antibiotic-free chicken products. Environ Health Persp 113:557.

Raza S, Mohsin M, Madni WA, *et al.*, 2017. First Report of bla CTX-M-15-Type ESBL-Producing Klebsiella pneumoniae in wild migratory birds in Pakistan. Eco Health 14:182-6.

Reig M and Toldrá F, 2008. Immunology-based techniques for the detection of veterinary drug residues in foods. In: Meat Biotechnology: Springer pp:361-73.

Saleem F, Sadiq R, Adalat R, *et al.*, 2014. Prevalence of acid and antibiotic resistant coliform bacteria in yogurt and oriental snacks sampled from markets of Lahore, Pakistan. Biologia 60:81-5.

Schwarz S, Kehrenberg C and Walsh T, 2001. Use of antimicrobial agents in veterinary medicine and food animal production. Int J Antimicrob Agents 17:431-7.

Shah SQ, Colquhoun DJ, Nikuli HL *et al.*, 2012. Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. Environ Sci Technol 46:8672-9.

Shahid MA, Siddique M, Abubakar M, *et al.*, 2007a. Status of oxytetracycline residues in chicken meat in Rawalpindi/Islamabad area of Pakistan. Asian J Poult Sci 1:8-15.

Shahid MA, Siddique M, Rehman U, *et al.*, 2007b. Evaluation of a microbiological growth inhibition assay as a screening test for the presence of antibiotic residues in poultry meat. Amer J Food Technol 2:457-61.

Siddiqui TR, Bibi S, Mustufa MA, *et al.*, 2015. High prevalence of typhoidal *Salmonella enterica* serovars excreting food handlers in Karachi-Pakistan: a probable factor for regional typhoid endemicity. J Health Popul Nutr 33:27.

Solangi AR, Memon SQ, Mallah A, *et al.*, 2009. Quantitative separation of oxytocin, norfloxacin and diclofenac sodium in milk samples using capillary electrophoresis. Biomed Chromat 23:1007-13.

Soomro AH, Khaskheli M, Bhutto MB, *et al.*, 2011. Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry meat in Hyderabad, Pakistan. Turk J Vet Anim Sci 34:455-60.

Stokstad E and Jukes TH, 1950. Further observations on the "animal protein factor". Proceedings of the Soc Experim Biol Med 73:523-8.

ur Rahman S, Ali T, Ali I, *et al.*, 2018a. The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. BioMed Res Int pp:14.

ur Rahman S, Ali T, Muhammad N, *et al.* 2018a. Characterization and mechanism of dissemination of extended spectrum beta lactamase producers Escherichia Coli in food producing animals in Pakistan and China. In: Applied Sciences and Technology (IBCAST), 15th International Bhurban Conference on: IEEE pp:203-14.

ur Rahman S, Ahmad S, Khan I *et al.*, 2018b. Incidence of ESBL-Producing-Escherichia coli in Poultry Farm Environment and Retail Poultry Meat.

ur Rahman S, Arenas J, Ozturk H, *et al.*, 2014. The polypeptide transport-associated (POTRA) domains of TpsB transporters determine the system specificity of two-partner secretion systems. J Biol Chem 289:19799-809.

ur Rahman S and van Ulsen P, 2013. System specificity of the TpsB transporters of coexpressed two-partner secretion systems of Neisseria meningitidis. J Bacteriol 195:788-97.

Van Boeckel TP, Brower C, Gilbert M, *et al.*, 2015. Global trends in antimicrobial use in food animals. Proc Nat Acad Sci 112:5649-54.

Van Boeckel TP, Gandra S, Ashok A, *et al.*, 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infec Dis 14:742-50.

van Ulsen P, Rahman S, Jong WS, *et al.*, 2014. Type V secretion: from biogenesis to biotechnology. Biochim Biophys Acta 1843:1592-611.

Vieira AR, Collignon P, Aarestrup FM, *et al.*, 2011. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: an ecological study. Foodborne Pathog Dis 8:1295-1301.

Wang HH, Manuzon M, Lehman M, *et al.*, 2006. Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. FEMS Microbiol Lett 254:226-31.

Wegener HC, 2003. Antibiotics in animal feed and their role in resistance development. Curr Opin Microbiol 6:439-45.

White DG, Zhao S, Sudler R, *et al.*, 2001. The isolation of antibiotic-resistant Salmonella from retail ground meats. New Engl J Med 345:1147-54.

Xia LN, Li L, Wu CM, *et al.*, 2010. A survey of plasmid-mediated fluoroquinolone resistance genes from *Escherichia coli* isolates and their dissemination in Shandong, China. Foodborne Pathog Dis 7:207-15.