

**REVIEW ARTICLE****Antimicrobial Drug Resistance in Poultry Pathogens: Challenges and Opportunities****¹Philip M. Panyako, ²Jacqueline Kasiiti Lichoti and ¹Sheila Cecily Ommeh**¹*Institute for Biotechnology Research, (JKUAT) and ²Directorate of Veterinary Services, State Department of Livestock, Ministry of Agriculture, Livestock, Fisheries and Irrigation, Kenya*Corresponding author: sommeh@jkuat.ac.ke**ABSTRACT**

Antimicrobial resistance (AMR) is widely considered a global public health threat, with the use of antimicrobials in poultry production considered one of the contributing factors. Antimicrobial agents are used for growth promotion, prevention, and treatment of poultry diseases. They are also used to prevent and treat human diseases. This unchecked dependence on antimicrobials results in their unregulated use and abuse in human and animal health, resulting in AMR. Previously, AMR was investigated using antimicrobial susceptibility testing using the disc diffusion method (for bacteria and fungi) and phenotypic fluorescence-based inhibition assay (for viruses). This was done after culturing the bacteria and fungi in growth media or propagating viruses in embryonic chicken eggs or specific cell lines. Recent studies utilizing metagenomics and next generation sequencing (NGS) platforms have also identified a large array of antimicrobial resistance genes that had not been discovered earlier. In this study, a systematic review of different studies focusing on AMR in some poultry pathogens was conducted using different databases, such as MEDLINE, EMBASE, and CINAHL library sources for relevant studies. These sources were screened against the protocol eligibility criteria, where 103 studies met the inclusion criteria. We wish to review the current state of antimicrobial resistance in bacteria, fungi and viruses in poultry and also explore alternative approaches for preventing and treating diseases and promoting growth in food animals such as poultry. This is essential in enhancing efforts of harmonizing testing practices and facilitating free access to information and data on AMR with the aim of enhancing treatment measures, along with monitoring the advancement of AMR in poultry pathogens.

Keywords: AMR, antibiotics, antifungals, antivirals, enteric, multidrug resistance**1.0 Introduction**

Antimicrobial resistance (AMR) is a global health concern that needs to be urgently addressed. For the past few years, a vast number of findings have outlined the impact of antimicrobial usage in animals, which ultimately leads to AMR (Marshall and Levy, 2011). The main contributing factor is the overuse of antimicrobials during the production of animal food. The level of usage is projected to increase substantially as a result of the considerable increase in farming practices in most parts of the developing countries (Van Boeckel, *et al.*, 2015). Much of the existing assumptions and knowledge on the advancement and prevalence of AMR across the animal production spheres are linked to the organisms that are frequently commensal in poultry and foodborne zoonotic pathogens (Luna-Galaz, *et al.*, 2016; Bortolaia, Espinosa-Gongora, and

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Guardabassi, 2016). Nonetheless, with certain exemptions, much is not known regarding the mechanisms and prevalence of AMR in pathogenic bacteria in poultry and other food animals.

Poultry farming is amongst the most extensive food sectors across the world, and chickens are often the most farmed poultry species, with extensive production of chicken meat; more than 90 billion tons annually (Food and Agriculture Organization of the United Nations, 2017). The main reasons are the minimal, if not nonexistent, religious and cultural restraints on their consumption and rather low costs of production. The vast variety of antimicrobials are normally applied in raising poultry in most parts of the world, mainly through the oral system in a move to prevent and treat diseases, as well as enhancing productivity and growth (Landoni and Albarellos, 2015). According to the World Health Organization (2017), most types of antimicrobials are found to be of greatest importance and critical for human medicine.

In animal farming, the indiscriminate application of antimicrobials is more likely to stimulate the development of AMR in commensal organisms and pathogenic bacteria. Besides the concerns attributable to the development of AMR of pathogens from poultry production, there are some evident human health concerns regarding the existence of antimicrobial components in eggs (Goetting, Lee, and Tell, 2017) and meat (Reig and Toldra, 2008). Additionally, AMR in poultry pathogenic bacteria tends to result in concerns about untreated poultry diseases and economic losses due to the high cost of the ineffective antimicrobials.

Here, we conduct a systematic review and summarize data on antimicrobial resistance in bacteria, fungi and viruses in poultry. We also explore alternative approaches for preventing and treating diseases and promoting growth in food animals such as poultry, with the aim of identifying general trends and outlining methodological issues and knowledge gaps

2.0 Antimicrobial Drug use and Resistance in Poultry

Antimicrobial agents have been used widely since the 1950s in poultry to improve feed efficiency and growth (Niewold, 2007; Torok *et al.*, 2011). They are also used to reduce morbidity and mortality associated with poultry diseases (Torok *et al.*, 2011). Globally, 73% of all antimicrobial agents available in most retail stores are utilized in food animals (Van Boeckel *et al.*, 2019). These antimicrobial agents are usually administered by injection or orally in feed and drinking water, with permitted uses varying between countries and regions (Gyles, 2008). The use of antimicrobial agents for growth promotion in food animals continues the world over, except in the European Union (EU) where it was banned in 2006 and in the U.S. where it was discontinued in 2017 (Roth *et al.*, 2019). The various uses of antimicrobial agents in poultry production are shown in Table 1.

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Table 1: List of antimicrobial agents and their uses in poultry production

Antimicrobial use	Antimicrobial agent	Reference
Growth promoters	Zinc bacitracin	Gyles (2008)
	Procaine penicillin	Gyles (2008)
	Tylosin	Gyles (2008)
	Virginiamycin	Gyles (2008)
	Monensin	Gyles (2008)
Prophylaxis and treatment	Chlortetracycline	Yadav et al. (2016)
	Furazolidones	Oluwasile et al. (2014)
	Fluoroquinolones	Gyles (2016)
	Oxytetracycline	Yadav et al. (2016)
	Sulphonamides	Gyles (2008)
	Gentamicin	Yadav et al. (2016)
	Quinolones	Yadav et al. (2016)

Excessive reliance on antimicrobial agents results in their unregulated use and/or abuse in human therapies, animal husbandry and aquaculture (Livermore, 2002; Sommer *et al.*, 2009; Yuan *et al.*, 2019). This increases the likelihood of selecting for organisms that are resistant to the antimicrobial agent (Yang *et al.*, 2004). One of the major concerns is the emergence of resistance to frontline antimicrobial agents such as fluoroquinolones, These antimicrobials are very valuable for treating human infections because of their low toxicity and broad-spectrum coverage (Angulo *et al.*, 2000; Livermore *et al.*, 2002; Yang *et al.*, 2004).

Currently, it is widely accepted that AMR is a major global public health concern responsible for over 2 million resistant infections and many deaths (Hoelzer *et al.*, 2017; Bougnom and Piddock, 2017; Trust, O'Neill, J., Ed., 2016; Yuan *et al.*, 2019). This is expected to get worse with the emergence and dissemination of multidrug resistant 'superbugs' that are able to resist multiple antibiotic categories (McKenna, 2013; Yuan *et al.*, 2019). In addition to the human health concerns, antimicrobial resistant pathogens also pose a serious animal health problem because they prolong illness and decrease production through higher morbidity and mortality (Yang *et al.*, 2004).

3.0 Transmission and Spread of Antimicrobial Resistance

Antimicrobial resistance is a natural and ancient phenomenon (D'Costa *et al.*, 2011; Yuan *et al.*, 2019). Production of antimicrobial agents is widely utilized by microbes to ward off competition between them (Newman *et al.*, 2003; Yuan *et al.*, 2019) and the synthesis of antimicrobial agents by microorganisms has evolved over millions of years (Baltz, 2008; Yuan *et al.*, 2019). Katale *et al.* (2020) conclude that the higher level of diversity of the AMR genes indicates that there might be several sources of resistant bacteria, or flow of genes among various strains as a result of the transmission by mobile genetic components. Antibiotic producers must therefore naturally possess self-protection mechanisms to effectively escape the threats from competitors' antibiotics (Cordero *et al.*, 2012; Wright, 2007; Yuan *et al.*, 2019). This resistance can emerge through point mutations, re-assortment, or horizontal gene transfer (HGT) (Hoelzer *et al.*, 2017). Studies show that enteric pathogens and normal intestinal microbiota play a critical AMR

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development and transmission role under external stress via HGT (Witte, 1998; Zhou *et al.*, 2012) which is mediated by mobile genetic elements (MGEs) such as plasmids, transposons and integrons. This was also corroborated by studies showing that intestinal microbiome may serve as reservoirs for antimicrobial resistance and spread of resistance to zoonotic pathogens (Fricke *et al.*, 2009; Nandi *et al.*, 2004; Oakley *et al.*, 2014). Many of the well-recognized antimicrobial resistance genes (ARGs) are present in MGEs and are able to be transmitted to other bacteria of the same or different species (Allen *et al.*, 2010; Yuan *et al.*, 2019). Usually, ARG subtypes associated with the same antibiotic class confer bacterial hosts varying tolerance to this antibiotic (Kraupner *et al.*, 2013; Yuan *et al.*, 2019).

Wee, Muloi, and van Bunnik (2020) posit that livestock have been considered as the key reservoir for AMR that may spread to humans, and environmental edges and close proximity with respect to livestock have been stated as the fundamental risk factors for transmitting AMR. According to the World Health Organization (WHO), antimicrobials applied in treating various infectious diseases in livestock might be similar to those applied for humans, leading to the spread and transmission of AMR in humans who are in close proximity to these animals/livestock (Novais & Freitas, 2020). Additionally, the intensifying application of other antimicrobials such as disinfectants and metals in the environment is considered a prospective factor for AMR selection (Novais & Freitas, 2020). As a result, the spread of variable and compound AMR bacteria, MGEs, and genes across environmental, animal, and human sections is a composite process taking place through numerous channels, including food exposure, manure, wastewaters, and many others, promoting the ongoing genetic exchange, evolution, and recombination of AMR features.

4.0 Methods Used to Study Antimicrobial Resistance

Until recently, costs and less advanced techniques hampered the study of AMR profile of the entire microbiome of poultry (Penders *et al.*, 2013). The antibiotic susceptibility of the indicator microorganisms was previously assessed by culturing the micro-organism of interest and then subjecting them to antimicrobial sensitivity testing to determine the level of resistance to specific antimicrobial agents. The choice of the indicator microorganism(s) was mostly based on the clinical relevance of these organisms and their cultivability (Penders *et al.*, 2013). The limitation of these methods is that less than 20% of enteric microbiota have been cultured to date due to the fastidious nature of intestinal micro-organisms that often require unknown culture requirements (Clavijo and Florez, 2017; Gaskins *et al.*, 2002).

A number of polymerase chain reaction (PCR) techniques have also been exploited for the detection of antimicrobial resistance genes. These methods improved sensitivity and speed of detection of antimicrobial resistance. The limitation to the utilization of PCR is that results are often skewed towards known resistance genes and mechanisms (Penders *et al.*, 2013). It is also not possible to adopt these techniques to the assessment of the entire resistome in the gastrointestinal tract of poultry (Zoetendal *et al.*, 2004; Clavijo and Florez, 2017).

Another important method for studying AMR is functional metagenomics. This involves the

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cloning of DNA fragments into a vector such as a plasmid that is subsequently expressed in heterologous hosts (often *Escherichia coli*). The resultant transformants are then screened for the expression of resistance genes by growing them on antibiotic-containing media at concentrations where the wild-type host strain is susceptible. The antibiotic resistant clones are finally sequenced (Penders *et al.*, 2013; Schmieder and Edwards, 2012). The main limitation of this method is that it relies upon each gene's ability to be expressed in surrogate hosts. Resistance genes that are not expressed by the surrogate host are left unidentified (false negatives). At the same time, a foreign gene interacting in novel ways with the cellular machinery of the surrogate host results in false positives (Penders *et al.*, 2013; Schmieder and Edwards, 2012).

Sequence-based metagenomics, involving the extraction, fragmentation, size-separation and random direct sequencing of DNA from an environmental sample without the need for culturing, has also gained wide acceptance as one of the most accurate methods for studying AMR (Penders *et al.*, 2013). The sequences generated can then be compared to international sequence databases to identify resistance genes (Penders *et al.*, 2013; Schmieder and Edwards, 2012). The transition from Sanger sequencing to next-generation sequencing (NGS) platforms such as the Roche 454 sequencer, the Genome Analyzer of Illumina, the SOLiD system of Applied Biosystems and the longer read Nanopore and PacBio technologies, has resulted in a significant drop in costs, which was accompanied by an increased number and size of metagenomic sequencing projects (Penders *et al.*, 2013). Although most of the NGS technologies yield lower contiguous read lengths and require greater genome coverage, their high-throughput reduces cost and number of sequencing runs (Niedringhaus *et al.*, 2011; Penders *et al.*, 2013). The most commonly used sequencing technique involves amplification and sequencing of the 16S rRNA gene (in case of bacteria) and/or internal transcribed spacer (ITS) region (in case of fungi) in the sample DNA. Direct shotgun sequencing of the DNA sample of the entire microbial community is also increasingly being exploited due to its high sensitivity, reproducibility and coverage.

5.0 Antibacterial Resistance in Poultry

Bacteria cause diseases in animals and humans, posing a threat to animal production, food safety and public health (Taylor *et al.*, 2001; Wolfe *et al.*, 2007; Woolhouse and Gowtage-Sequeria, 2005; Zhou *et al.*, 2012). A number of enteric zoonotic bacteria associated with poultry include (but are not limited to) *Salmonella*, *Campylobacter*, avian pathogenic *Escherichia coli* and enterococci (Gyles, 2008). Many studies have been conducted on the AMR of bacteria in poultry and other livestock.

In a recent study in Southern Ethiopia to determine sources and patterns of AMR in *Salmonella* isolated from the poultry industry (Abdi *et al.*, 2017), the authors showed that prevalence was higher in the bedding (35.3%) and personnel hand swabs (33.3%) than in the chicken cloaca (14.8%). This demonstrated the poor biosecurity and personnel hygiene practices in poultry handling centers. But more important to this review, the authors also observed that all the isolates exhibited resistance to kanamycin and sulfamethoxazole-trimethoprim and showed

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varying resistance to nalidixic acid, ampicillin, ceftiofur, streptomycin, tetracycline, chloramphenicol, ciprofloxacin, and gentamicin. They were also alarmed to note that most isolates exhibited multidrug resistance (MDR). Similar studies investigating the prevalence, genotypes and antimicrobial resistance of *Salmonella* isolated from poultry have been done in Belgium (Vinueza-Burgos *et al.*, 2019), Ghana (Andoh *et al.*, 2016) and the USA (Velasquez *et al.*, 2018). In another study, Bailey *et al.* (2020) conducted a longitudinal study to examine the prevalence of *Salmonella* and AMR by collecting environmental samples, carcass rinses, and fecal samples. The study found that organic chickens were linked to statistically significant levels of *Salmonella* while the processing is in its early stages. Bailey *et al.* (2020) indicated that these findings show that organic methods might imply higher levels of risk of *Salmonella* contamination, but appropriate interventions during processing will help in reducing such risks. This is in line with Kamaruzzaman and Saeed's (2020) findings that there is high prevalence of multidrug resistance (MDR) *Salmonella spp.* and *E. coli* in poultry in Eastern parts of the coastal Malaysia. This might be caused by the extreme application of antimicrobial agents by the farmers of these broilers. Other studies on the prevalence of AMR of *Salmonella* in poultry have been done in Canada (Varga *et al.*, 2019) and the U.S. (Fricke *et al.*, 2009).

A study of small scale and backyard chickens in Kenya showed a high rate of resistance of *Campylobacter jejuni* isolates to nalidixic acid, tetracycline and ciprofloxacin of 77.4%, 71.0% and 71.0% respectively (Nguyen *et al.*, 2016). Lower resistance of 25.8% was detected for gentamicin and chloramphenicol, with multidrug resistance detected in 61.3% of the isolates. In the U.S., a comparison of antimicrobial resistance in *Campylobacter spp.* from conventional (where antimicrobials were routinely used) and organic (where antimicrobials had never been used) farms revealed that, although *Campylobacter* species were highly prevalent in both the conventional and organic poultry management systems, the antimicrobial resistance rates were significantly different between the two systems (Luangtongkum *et al.*, 2006). The authors found that fewer *Campylobacter* strains isolated from organically raised poultry were resistant to antimicrobial agents when compared to the isolates from conventionally raised broilers and conventionally raised turkeys. Multidrug resistance was likewise observed mainly among *Campylobacter* strains isolated from the conventionally raised turkeys. This clearly highlighted the negative impact of uncontrolled use of antimicrobial agents on poultry farms. Similar studies have also been done in Italy (Marotta *et al.*, 2019) and the United Kingdom (Wimalarathna *et al.*, 2013), with variable observations. Results from these studies demonstrate that improper and excessive use of antimicrobials in animal husbandry and human therapy has resulted in increased antimicrobial resistance.

Adelaide, Bii, and Okemo (2008) also conducted a study to examine MDR and existence of virulence-related genes in avian pathogenic *E. coli* (APEC) isolates from broilers during the slaughter period in a processing plant, Kenya. The study found that there is significant existence of MDR and virulent APEC amongst broilers in Kenya. Another study of AMR in clinical *E. coli* isolates from poultry (chickens and ducks) and livestock (pigs and cows) was conducted in Jiangsu, China, between 2004 and 2012 (Yassin *et al.*, 2017). The study showed that 94% of the

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isolates were resistant to at least one drug while 83% were resistant to at least three different classes of antimicrobial agents. Noting that *E. coli* isolates from poultry and livestock are commonly resistant to multiple antibiotics, the authors emphasized the need for public health and veterinary authorities to limit and rationalize antimicrobial use in China. Ibrahim *et al.* (2019) also isolated, identified, serogrouped and genotyped APEC from broilers, examined their antibiotic resistance profile, stated genetic attributes, and the related risk factors. This was conducted through isolating APEC from the visceral organs of those broilers that were considered sick with prevalence of 54%. The findings were that 68% of APEC isolates were reported to have almost five to eight antimicrobial resistant genes. The finding indicate two risk factors linked to the existence of MDR APEC in broiler chickens: farms situated close to other farms and the use of ground water for drinking. Ibrahim *et al.* (2019) further found that the intensity of antimicrobial resistance of APEC isolates and detection of resistance genes outlined the importance of monitoring the intensification of ARGs in the environment and specifically in chicken or poultry farming in Jordan. Homjan *et al.* (2018) equally conducted a study with the purpose of identifying the antimicrobial resistance of *E. coli* isolates from colibacillosis lesions, and the findings focused on the manifestation of MDR and AMR amongst APEC or *E. coli* isolates. This research sought to understand regular AMR monitoring and practical application of antimicrobial substances throughout the production of poultry. Roth *et al.* (2019) also reviewed in detail the global application of antibiotics in broiler production and the resulting antimicrobial resistance in *E. coli*. Other studies looking at the prevalence, virulence and antimicrobial resistance of APEC isolates from poultry have been undertaken in Belgium (Smet *et al.*, 2008), Canada (Varga *et al.*, 2019), Iran (Momtaz *et al.*, 2012), Jordan (Ibrahim *et al.*, 2019) and the USA (Musgrove *et al.*, 2006; Zhao *et al.*, 2005).

In another study investigating the occurrence, antimicrobial resistance and virulence of *Enterococcus spp.* from poultry and cattle farms in Nigeria, all the isolates tested were susceptible to vancomycin. Resistance to tetracycline, erythromycin, ampicillin and gentamicin, however, was observed among 61%, 61%, 45.1% and 32.7% of the isolates respectively (Ngbede *et al.*, 2016). The authors also noted that 53.1% of the isolates were multidrug resistant. They detected antibiotic resistance (*tetK*, *tetL*, *tetM*, *tetO* and *ermB*) and virulence (*asa1*, *gelE* and *cyfA*) genes among the isolates. From these observations, the authors inferred that poultry, cattle and manure in the study areas were hosts to varying *Enterococcus* species harbouring virulence and resistance determinants that could be transferred to other organisms. Other studies on AMR, virulence determinants and genetic profiles of *Enterococcus* from poultry have been carried out in the USA (Jackson *et al.*, 2014; Kilonzo-Nthenge *et al.*, 2016; Tyson *et al.*, 2017).

Detailed reviews have also been published on AMR profiles of many poultry bacterial pathogens. Threlfall (2002) for instance, attempted to look into the problem of AMR in *Salmonella* while Apostolakos and Picirillo (2018), provide a rich source of information on the current knowledge and challenges of colistin resistance in poultry production. Gautier-Bouchardon (2018) on the other hand, looked at AMR of *Mycoplasma spp.* Hoelzer *et al.* (2017) also covered the problem

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of antimicrobial drug use in food producing animals and the associated human health risks while Saliu *et al.* (2017) looked into the types and prevalence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* in poultry. ESBL are types of enzymes produced by specific bacteria and help in breaking down the active ingredients in various antibiotics, enabling these antibiotics ineffective. Other researchers, however, look at the problem of AMR in a wider group of bacterial pathogens of poultry (Nhung *et al.*, 2017; van Boeckel *et al.*, 2015; van Boeckel *et al.*, 2019).

6.0 Antifungal Resistance in Poultry

Opportunistic fungal infections are one of the emerging global health problems, and domestic and wild birds may be acting as carriers of human fungi with pathogenic potential (Subramanya *et al.*, 2017). Exposure to pathogenic fungi such as *Candida*, *Cryptococcus*, *Geotrichum*, *Rhodotorula* and *Trichosporon* from avian sources poses a human health risk (Wojcik *et al.*, 2013; Subramanya *et al.*, 2017). The potential to transmit zoonotic pathogens from synanthropic birds to humans is a matter of concern because these birds might be reservoirs of drug resistant fungi (Subramanya *et al.*, 2017).

A study in Nepal characterized and evaluated *in vitro* virulence factors and antifungal susceptibility patterns in the gastrointestinal yeast flora of both household and commercial chickens (Subramanya *et al.*, 2017). In this study, biofilm cells were observed to exhibit higher minimum inhibitory concentration (MIC) values ($\mu\text{g/ml}$) than planktonic cells against all antifungal compounds tested: fluconazole, 8-512 vs 0.031-16; amphotericin B 0.5-64 vs 0.031-16; voriconazole 0.062-16 vs 0.062-8; caspofungin, 0.062-4 vs 0.031-1. The high level of drug resistance was attributed to the practice of adding growth promoters and antimicrobial agents to commercial poultry feeds for growth and prevention of diseases. A similar study in Saudi Arabia investigating the diversity, virulence, and antifungal susceptibility patterns of pathogenic and opportunistic yeast species in rock pigeon fecal droppings revealed the presence of *Cryptococcus*, *Saccharomyces*, *Rhodotorula*, *Candida*, *Meyerozyma*, *Cyberlindnera*, *Rhodospidium*, *Millerozyma*, and *Lodderomyces*, although none of the identified yeast isolates exhibited any resistance to the antifungal drugs tested (Abulreesh *et al.*, 2019). However, Abulreesh and co-workers noted that none of the *Cryptococcus* species were positive for virulence determinants like urease activity, growth at 37°C, melanin production and *PLB* and *CAP* genes.

In an earlier study in Poland to determine the *in vitro* susceptibility of 85 *Aspergillus fumigatus* strains isolated from domestic geese and from their environment to amphotericin B, clotrimazole, voriconazole, itraconazole, enilconazole, miconazole, ketoconazole, and tioconazole, all the strains irrespective of source, exhibited resistance to miconazole (Ziolkowska *et al.*, 2014). Resistance to the remaining azoles and amphotericin B ranged from 90.6 to 70.6%. Complete susceptibility was noted for voriconazole and enilconazole. The authors also reported that the highest percentage of resistant strains was observed in isolates from the lungs (100% for amphotericin B and clotrimazole and 35.7% for itraconazole). Related studies have equally

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been reported in Poland on *Aspergillus fumigatus* resistance to itraconazole (Nawrot *et al.*, 2019) and susceptibility of *Aspergillus niger* strains to various antifungal drugs in a comparative study of the disk diffusion, broth microdilution (M38-A) and Etest® methods (Tokarzewski *et al.*, 2012).

Another study investigating Amphotericin B resistance in *Macrorhabdus ornithogaster* (an anamorphic ascomycete) in Australian cage birds indicated that treatment with Amphotericin B has poor efficacy against *Macrorhabdus ornithogaster* (Baron *et al.*, 2018).

7.0 Antiviral Resistance in Poultry

Apart from avian influenza virus, there is a dearth of information on resistance to antiviral therapies in poultry. The insufficient reporting on antiviral resistance of many viral pathogens in poultry could be attributed to the fact that, with the exception of immune-modulator recombinant feline interferon, very few if any antiviral compounds have been licensed for use globally on farm animals (Hussein and Abdelwhab, 2016). Compared to antibacterial agents, antivirals usually have a very narrow spectrum and can attack only a specific viral target (Hussein and Abdelwhab, 2016). But naturally, viruses are prone to frequent random mutations along their genomes. A mutation affecting this target could therefore render the drug ineffective, and lead to the emergence of resistant variants due to selective pressure exerted by the presence of the antiviral drug (Hussein and Abdelwhab, 2016).

The increased selection of amantadine resistant highly pathogenic avian influenza (HPAI) H5N1 viruses in Chinese poultry has been reported to be due to irresponsible and illegal application of the relatively cheap amantadine by some farmers to control H5N1 and H9N2 influenza infections in chicken (Abdelwhab and Hafez, 2012; Cyranoski, 2005; He *et al.*, 2008; Huang *et al.*, 2009; Parry, 2005). The re-emergence of amantadine resistant H5N1 viruses in Egypt was also attributed to uncontrolled use of amantadine for prevention and treatment of avian influenza in Egyptian poultry farms, thus rendering this drug ineffective for human avian influenza treatment (El-Shesheny *et al.*, 2016).

A study in Sweden looked into the fate of oseltamivir, the main antiviral for the prevention and treatment of pandemic influenza, when it is excreted and released into the environment via sewage treatment plants (Fick *et al.*, 2007). The study showed that oseltamivir carboxylate is not degraded substantially by UV light radiation. It is therefore released into the waste water leaving the plant, leading to selection pressure in the environment that favours development of drug resistance. Related studies further assessed if environmental levels of Oseltamivir induced development of neuraminidase (NA) resistance mutations H274Y (Gilman *et al.*, 2015a; Jarhult *et al.*, 2011), I222T (Gilman *et al.*, 2015b) and R292K (Gilman *et al.*, 2015c) in influenza A/H1N1, H7N9 and H6N2 respectively in mallard ducks. These studies showed that resistance could be induced in influenza viruses circulating in the ducks found around sewage treatment plants. The authors raised concern that because influenza viruses can cross species barriers, oseltamivir resistance could spread to human-adapted strains with pandemic potential. This would

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consequently render oseltamivir, which is a cornerstone in pandemic preparedness planning, ineffective. They therefore proposed the need for surveillance of wild birds as a measure to understand the resistance situation in nature and to monitor it over time. They equally emphasized the need to develop strategies that can help lower environmental levels of oseltamivir carboxylate, including improved sewage treatment and prudent use of antivirals. A similar investigation in Sweden, but including both oseltamivir and zanamivir resistance-related mutations in influenza viruses isolated from wild mallards, has also been reported (Orozovic *et al.*, 2014).

Another study on the susceptibility of 67 avian influenza H5N1 viruses isolated between 2006 and 2012 in India revealed a novel NA I117T amino acid substitution (N2 numbering) in one isolate and eight previously known neuraminidase inhibitor (NAI) resistance marker mutations (I117V, E119D, N294S) in eight isolates (Kode *et al.*, 2019). The study reported that the overall incidences of resistance were 13.4%. The NA inhibition assays also showed cross-resistance to both oseltamivir and zanamivir in the isolate with I117T amino acid substitution (Kode *et al.*, 2019), thus underscoring the need for routine surveillance of avian influenza viruses. A similar study also done in India assessed the susceptibility of highly pathogenic avian influenza H5N1 viruses to oseltamivir using embryonated chicken eggs (Tare *et al.*, 2019). Other studies looking at the susceptibility of avian influenza viruses to antiviral drugs have been done in China (Yang *et al.*, 2018), Egypt (Abdelwhab *et al.*, 2019), India (Sood *et al.*, 2018) and The United Kingdom (Bialy and Shelton, 2020).

8.0 Alternatives to Antimicrobial Agents in Poultry Production

8.1 Probiotics

Probiotics are a mixture of live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (Clavijo and Florez, 2017; Smith, 2014). They benefit the animal hosts by stimulating the development of beneficial microbiota, which reduce and prevent colonization by enteric pathogens, modulate immunological activity, stimulate epithelial health, increase digestive capacity and help in the maturation of intestinal tissue. These health benefits have been reviewed in detail by Clavijo and Florez (2017).

Probiotics have been utilized in poultry since 1973 when they were used to control *Salmonella* in broiler chickens (Clavijo and Florez, 2017; Nurmi and Rantala, 1973). This initial use of probiotics however, had a setback because it potentially transferred pathogens together with the beneficial microorganisms (Clavijo and Florez, 2017). A range of probiotics have been developed with varying effectiveness as reported by Clavijo and Florez (2017) in their review. Those available in the market are usually made up of different species of *Lactobacillus* and *Bacillus*.

A research was conducted to establish the impact of dietary probiotics on natural Immunoglobulin levels in poultry in Kenya (Khubondo *et al.*, 2015). In this study, 150 2-month-old chicks were randomly grouped into five treatments of twenty five chicks each, where the

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researcher dissolved 5ml of probiotic Molaplus into containers of different sizes, from 250 to 2,000ml of drinking water. The levels of Immunoglobulin were established by applying the indirect enzyme-linked immunosorbent assay (ELISA) method. The findings indicated that dietary probiotic supplementations however, do not considerably impact the levels of Immunoglobulin binding the keyhole limpet hemocyanin (KLH). In a related study to determine the effects of probiotics on the weight of indigenous chicken in Kenya, Atela *et al.* (2015) concluded that supplementing indigenous chicken with probiotics in drinking water can significantly improve their weights.

8.2 Prebiotics

Prebiotics are ingredients that stimulate increased beneficial microbial activity in the digestive system aimed at improving the health of the host (Clavijo and Florez, 2017). In comparison to probiotics, they are cheaper to produce, the risks of side effects in the host are lower and the process of production and administration are easier to manage (Clavijo and Florez). Prebiotics generally function by attaching to pathogens, serving as substrates for fermentation. This subsequently increases osmosis in the lumen of the intestine, indirectly stimulates the response of macrophages, produces short chain fatty acids (SCFAs) and modulates the immune system (Clavijo and Florez, 2017; Patel and Goyal, 2012).

Most of the currently used prebiotics are non-digestible synthetic oligosaccharides containing one or more sugar molecules or a combination of simple sugars such as glucose, fructose, xylose, and galactose. Responses to supplements containing prebiotics, however, have been inconsistent when applied in mass production systems (Clavijo and Florez, 2017). This has been attributed to variation in the quality and dosage of the compounds used and particular conditions found in each farm.

In a study done on broiler chickens in poultry farms around Nairobi in Kenya to investigate the viability of organic acids as an alternative to antibiotics as growth promoters, Odede (2016) noted that the inclusion of organic acids in broiler chickens' drinking water improved their feed conversion ratio and gut health. This was possible since these acids increased the amount of beneficial bacteria and concurrently reduced coliform counts. The author therefore opined that organic acids are viable alternatives to antibiotics in ensuring that the health and productivity of broiler chickens improved.

8.3 Bacteriophages

Bacteriophages are viruses that specifically infect bacteria. Hence phage therapy is the use of phages to treat bacterial infections (Clavijo and Florez, 2017). Its application to humans was first described when bacteriophages were discovered in 1915 (Abedon *et al.*, 2011; Clavijo and Florez, 2017). The emergence of multidrug resistant bacteria has recently given hope to the use of bacteriophages as a natural, nontoxic alternative treatment of bacterial infections (Clavijo and Florez, 2017). One of their advantages include targeted treatment of a specific group of bacteria without affecting the normal microbiota. This minimizes the risk of secondary infections

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associated with antimicrobial therapies (Clavijo and Florez, 2017). Another important advantage is that bacteriophages can be effective against sensitive bacteria as well as strains that are resistant to antibiotics (Clavijo and Florez, 2017; Loc-Carrillo and Abedon, 2011; Nilsson, 2014). The application of phages for humans, animals, plants, and food have been reviewed in detail by Clavijo and Florez (2017). The United States approved a combination of phages formulated to control *Listeria monocitogenes* in food for human consumption in 2006 (Clavijo and Florez, 2017; Sulakvelidze, 2013). The use of bacteriophages is however, yet to be approved for use in live animals. Several studies utilizing bacteriophage treatments in various animal models have been reviewed in detail by Clavijo and Florez (2017), especially the control of *Salmonella* and *Campylobacter*.

One of the limitations of phage therapy is the variability in the results obtained. This could be attributed to development of resistance to phages by target bacteria, low multiplicity of infection, inaccessibility of the target microorganism, and the deactivation of the phages by the host (Clavijo and Florez, 2017). Another more important limitation to their use in farms is the lack of approval and regulation for use with animals. Additionally, the acceptance of the therapy by the producer community is not assured, given that it is a relatively new technology (Clavijo and Florez, 2017). However, if such approval is to be achieved, Grant *et al.* (2016) and Clavijo and Florez (2017) proposed that research into the effectiveness of phages in the commercial conditions of factory farming is required.

9.0 Challenges and Future Prospects

From the review of most of the work done in AMR, concern is raised at the high rates of AMR in poultry from common indicator pathogens. Just as Van Boeckel *et al.* (2019) rightly reported, the highest resistance rates are observed in antimicrobial agents most commonly used in animal production, such as tetracyclines, sulfonamides and penicillin. The rapidly shrinking portfolio of antimicrobial agents used to raise animals for food is expected to have serious consequences for both animal and human health even as it impacts negatively on farmers' livelihoods as suggested by Van Boeckel *et al.* (2019). Since few new antimicrobials are in development, and even fewer are approved for use, this becomes more frightening as the post-antibiotic era beckons. Research funding and opportunities are also limited especially in developing countries like Kenya and by extension, much of Africa where resistance appears to be rampant, hence the need for international collaboration and cooperation.

There is therefore need to utilize alternative treatments for poultry infections to reduce overreliance on antimicrobial agents. Rational antimicrobial treatment should also be promoted at all stages of poultry production. Non-conventional treatments such as phage therapy could be considered. There is also need to shift focus towards applying probiotics and/or prebiotics in poultry farming to promote general health and well-being of poultry. This will help reduce the need for antimicrobial agents in disease treatment or growth promotion. Development of new or alternative antimicrobial agents such as natural or synthetic antimicrobial compounds and peptides can also be considered. Biosecurity safeguards as well as improved nutrition are also

key to raising of healthy birds, thus cutting down the need for antimicrobials.

10.0 Conflict of Interest

The authors declare that they have no conflict of interest.

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