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Article Review

Antimicrobial Resistance in Animals

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ABSTRACT

The application of antimicrobials has been instrumental in enhancing human health, animal health and food security; however, the application of antimicrobials triggers antimicrobial resistance (AMR) that is posing increasing public health threat to animal health, food security and human health. Producers of food animals hold an exceptional responsibility in the prevention of misuse of antimicrobials in purposes of growth promotion and disease prevention; however, emerging legislations have not elicited a very positive reception in groups of animal farming. There are national, regional, and international strategies for the containment of AMR; however, efforts by the World Health Organization, United Nations and the European Union trying to reduce and control the use of antimicrobials in animals within developing countries have been ineffective.

This means that the One Health approach is appropriate for the issue by providing a multisectoral and multipronged approach. Also, there is a need to carry out a cross-sectional satisfaction survey among food animal producers with a view of measuring their satisfaction levels with the available AMR mitigation policies. The results can be used to guide new policies and awareness campaigns targeted at food animal producers.

INTRODUCTION

The AMR is therefore not only a problem of domestic animals but also wild ones, the ecosystems and the individuals. It involves aspects that are clinical, biological, social political economical and even the environment. For that reason of all factors mentioned above, AMR bacteria present in the environment (**Irfan et al. 2022**). As it was pointed out before, the use of antimicrobials in animals' production systems is one of the factors that foster emergence of AMR and kept receiving more attention on the One Health approach of tack-

ling this challenge. AMR is a convoluted process that is an emergence of multi-faceted factors, namely those concerning antimicrobials used in human medicine and international movement of animals and animal products. AMR is a universal threat which, according to O'Neill (2016), will result in 10 million deaths and economic loss of one hundred and 100 trillion USD by 2050. The 'One Health approach' aims at reducing the use and demand of antimicrobials: it requires both time, energy, and cooperation (**Pokharel et al. 2019**).

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The main types of antimicrobials used in animal agriculture include antibiotics, antifungals, and antiparasitic agents. Antimicrobials are used to treat infections, prevent diseases, and promote growth (Kimera et al. 2020).

Antimicrobials are used to prevent infections and promote growth in animals, with 70% sold for use in food animals in the US and 30 countries in Europe (FDA 2017 and EMA 2017).

The total amount of antimicrobials used for food animals is expected to rise to 200,235 tons by 2030, with 8 mg/PCU in Norway, 318 mg/PCU in China (Van Boeckel et al. 2017).

The usage of antimicrobials in food animals is a serious concern, with evidence suggesting it has contributed to the development of AMR in humans. This commentary reviews relevant literature to examine the current trend its impact and future direction, and potential research, policy, and community implications (Carrique-mas et al. 2020 and Pokharel et al. 2020).

2. Antimicrobial usage in food animals and antimicrobial resistance:

The consumption of meat and dairy products has been steadily rising since the 1950s and has led to the use of antimicrobial agents in agriculture because they are used as treatments and as a preventive measure, as a promoter of growth and as a metaphylaxis (National Research Council, 1980; Irfan et al. 2022).

Wichmann et al. (2014) has also shown that animal waste releases antibiotic and drug-resistant microorganisms into the environment. It as well holds similar features with that found in human that between 30% and 90% of the antibiotics fed to animals are excreted in the urine and faeces (Berendsen et al. 2015). Oxytetracycline, doxycycline, and sulfadiazine were detected as the most often antibiotics, followed by tetracycline, flumequine, lincomycin, and tylosin. According to reports, a third of faeces collections include more than one medicine, with cow and swine faeces both containing up to eight antibiotics (Singer et

al. 2016). Research on a range of species, notably swine, has shown how AMR spreads on farms (Bloom of Resident Antibiotic-Resistant Bacteria in Soil Following Manure Fertilization, 2023).

Excessive usage of antimicrobials provokes the resistant genes development (Laxminarayan et al. 2014). According to the processes through which bacteria acquire resistance such as mutation, conjugation and transformation of genes among bacteria and selective pressures occasioned by the use of antibiotics then the evolution and emergence of resistance bacteria is encouraged. Then again, animals have higher mutation rates than humans because animal biomass as postulated by Van Boeckel et al. (2017).

Antimicrobial use in food animals can make contribution to the emergence of AMR, that is positively correlated with the extent of antimicrobial intake (Chantziaras et al. 2014 and Roth et al. 2019). Exposure to sub-therapeutic doses of antimicrobials have been reported to alter the gut microbiomes and promote the acquisition of antimicrobial genes (Loofft et al. 2012).

Food production industries in developing countries are using antimicrobials to promote growth, and the amount of meat consumed by each person has dramatically grown., from 10.2 kg/year in 1964-66 to 25.5 kg/year in 1997-99 with an anticipated increase to 36 kg/year by 2030 (WHO, 2019).

Economic losses for farmers are connected to the use of growth boosters in poultry production. (Graham et al. 2007).

3. Impacts on human and animals:

The impact of AMR on animal health can result in elevated morbidity and mortality in animals, moreover cause economic losses and reduced productivity for farmers. In addition, the impact of AMR on human health has the potential for the spread of resistant bacteria from animals to humans through the environment, food chain, and direct contact (Laxminarayan et al. 2014). Animals and humans share similar strains of resistant bacte-

ria (**Pirola et al. 2019**). Plasmid-mediated resistance to colistin is reported in food animals and humans in many countries (**Thi et al. 2019**).

More than any other widely recognized cause of morbidity and mortality, approximately 3.57 million of 4.95 million deaths globally were linked to antimicrobial resistance (**Murray et al. 2022**). Furthermore, antimicrobial resistance is estimated to cause 10 million deaths by 2050 (**Trotter et al. 2019**) although in fact, we are closer to that figure now than we thought. Pathogenic bacteria have been described to become resistant to novel antibiotic classes in a few of years, making it difficult to reduce the infectious diseases burden as was the case with streptomycin, an aminoglycoside approved by the US FDA to treat tuberculosis, some individuals developed resistance to it within a year of the drug's introduction (**Davies and Davies 2010**), Enterobacteriaceae resistance to carbapenems and colistin has increased globally, making it a priority pathogen although Carbapenems and colistin are used as a last option to treat infections brought on by β -lactamases or multidrug-resistant bacteria (**Meletis, 2010, Du et al. 2016 and Savin et al. 2020**); *Klebsiella pneumoniae* has an alarmingly high resistance rate in neonatal sepsis reached to 83.35% (**Hassuna et al. 2020**).

Furthermore, in 2018 there were around 500,000 new cases of rifampicin-resistant tuberculosis worldwide, with the majority of them had multidrug resistance (**Antimicrobial Resistance, 2023**). AMR is projected to increase dramatically as the standard antibiotic treatment regimen becomes outdated. Because of this, terminally ill-patients need palliative care, but the medications provided are no longer clinically effective. Furthermore, AMR-related outbreaks could lead to a 2%-3.5% decline in global total gross domestic product (GDP) by 2050, costing between \$ 60 and \$ 100 trillion (**Sipahi, 2008, Ashiru-Oredope et al. 2013 and Taylor et al. 2023**).

4. AMR Potential Threats:

The studies on the relations between antimicrobial administration in food-producing animals, biosphere susceptibility, potential

negative effects on welfare of people and animals, and certain associated environmentally related complications as part of the One Health approach is still lacking.

The assessment of AMR threats in the community from antimicrobial administration in veterinary health care to human and livestock welfare appears to be challenging due to the heterogeneity of the problem and the lack of relevant data on the processes and pathways associated with the genomic, biochemical, and community levels.

Additionally, little is known about how reception environment affects the fate of antibiotic resistance genes (ARGs), multidrug-resistant bacteria, and AMR. To adequately assess the effects of AMR in the environment, it could be preferable if the relative input of the environment versus the effect of different elements had been examined with reference to the state of AMR (**Irfan et al. 2022**).

In place of national funding for research, the European Council's Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) is using a collaborative approach to combine regional research initiatives in order to better address the threat of AMR.

Network mapping describes a wide range of resources and transmission mechanisms, all likely impacted by the increasing frequency of AMR in ecological, animal, and therapeutic contexts.

Possible treatments for new and possibly effective antibiotic resistance are funded by the Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) for firms developing biopharmaceuticals (**Irfan et al. 2022**).

The public's health is at serious risk due to the rise in antimicrobial exposure and tolerance brought on by the major pharmaceutical companies' reduction in antibiotic research and development (R&D), the misuse of antibiotics, excessive consumption, and the exploitation of antimicrobials as animal growth promoters and in agricultural settings for animal feed (**Garcia**

-Migura et al. 2014).

Animal-associated bacteria may transmit antibiotic-resistant traits to people (Marshall and Levy, 2011). People, cattle, and the ecosystem interact in a complicated way to induce AMR (Finley et al. 2013 and Woolhouse et al. 2015).

Only seven analyses (5% of the total screened publications) revealed a weak correlation between animal antibiotic intake and human resistance, whereas 100 analyses (72%) revealed a significant correlation, according to the most recent analysis of scientific research on antibiotic use in agricultural production (Irfan et al. 2022).

The discovery of veterinary antibiotic metabolites such as ciprofloxacin, enrofloxacin, oxytetracycline, and chlortetracycline in the excrement of feeding cows in eight Chinese provinces in 2010 provides compelling evidence that intensive livestock husbandry is a major vector for the development of antimicrobial resistance (AMR) Zhao and colleagues (2010).

5. Mechanisms of AMR Spread

The exposure of bacteria to subinhibitory doses of antibiotic is one of the primary causes of antimicrobial resistance (AMR), which is mostly caused by improper administration of antibiotics in clinical and agricultural contexts (Levy, 1998 and Capozzi et al. 2013).

Antibiotic-resistant bacteria use several mechanisms include: antibiotic inactivation due to mutations in the enzymatic scaffold or enzyme breakdown, changes in the target of the antibiotic, and changes in the permeability of the cell membrane (Crofts et al. 2017).

One form of resistance that renders an antibiotic ineffective is the enzymatic destruction or alteration of the antibiotic scaffold. The TetX antibiotic-modifying enzymes and β -lactamases are two examples of these enzymes that are commonly used. The chosen target can also be modified, overexpressed, or protected in order to generate antibiotic resistance

(Bush, 2010 and Forsberg et al. 2015). The most well-known example is changing the cell-wall penicillin-binding proteins (PBP) to counteract β -lactam antibiotic action (Arias and Murray, 2012). The employment of efflux pumps or modifications in membrane permeability are two additional resistance mechanisms that prevent antibiotics from the bacterial cells' penetration. Either a multidrug efflux pump (Piddock, 2006) or an exporter unique to an antibiotic, such tetracycline efflux pumps, are produced by bacteria to maintain subinhibitory antibiotic concentrations inside of cells (Roberts, 1996). Contrarily, few bacteria create a more selective porin variation or reduce porin expression to block antibiotic entry into the cell by reducing membrane or wall permeability (Irfan et al. 2022). Through the use of several complimentary mechanisms, certain bacteria can build widespread resistance. Because of a porin mutation that decreases the absorption of carbapenem and boosts the production of chromosomal β -lactamases, clinical isolates of *Enterobacter cloacae* become highly resistant to carbapenems (Babouee Flury et al. 2016).

Antibiotic resistance development in bacteria usually occurs through horizontal and vertical gene transfer. The primary mechanism through which antibiotic-resistance genes are quickly spread across several bacterial species is known as horizontal gene transfer (HGT). Vertical gene transfer mechanism passes genetic information from one generation to the next within a family including any mutations (Munita and Arias 2016). Resistant bacteria are able to flourish and disseminated throughout the environment due a number of distinctive characteristics that they possess. ARGs are stored by native bacteria in the environment and can subsequently be transferred to pathogens via horizontal gene transfer (HGT) (D'Costa et al. 2006 and Vikesland et al. 2017). HGT can also occur through other mechanisms such as transformation, transduction, and conjugation (Vikesland et al. 2019).

Under ideal conditions the processes of HGT are well known, unfortunately, in habitats containing chemical stressors as antibiotics and biocides they are poorly known. Subinhib-

itory and subtherapeutic antibiotic doses would aid development of antimicrobial resistance by developing bacterial natural selection through mutation or horizontal gene transfer leading to increased tolerance to antibiotics.

The situation with regard to AMR is getting worse as a result of a lack of financial incentive, challenges with clinical research, and a lack of scientific innovation, which is exacerbated by the rapid emergence of antibiotic resistance at the molecular level. Novel antibiotic development has reached a wall (Payne et al., 2015), and there aren't many new antibiotics being used in clinical settings.

6. Methods of Monitoring AMR:

AMR in the environment has been widely monitored using a variety of techniques. The next section discusses several of these techniques, including culture-based, molecular-based, and nanotechnology-based ones.

6.1. Culture-Based Methods:

The gold-standard technique for finding bacteria that are resistant to antibiotics has historically been thought of as the solid/semisolid/broth medium in which germs are cultivated and counted in research tests. Techniques based on culture are practical, somewhat exact, and affordable. By including desired antimicrobials in the growth medium for selection, it is possible to successfully obtain antimicrobial-resistant microorganisms from samples. If analogous experiments are carried out without desired antimicrobials, it is possible to determine the percentage of a microbial species that appears to be tolerant. The minimum inhibitory concentration (MIC) broth microdilution test is still regarded as the gold standard for assessing sensitivity for certain antibiotics, such as colistin. Despite being frequently used, this AMR surveillance approach has severe limitations. Samples containing high concentrations of chemicals can result in false-negative findings, and the culture phase requires numerous steps, validation assays, also, time-consuming and requiring prolonged incubation. Many pathogens found in the habitat cannot be cultivated under laboratory conditions. The method used to maintain the spec-

imens and the length of the preservation period may have a big influence on the recovery and counting of particular organisms. The comparatively low output of these methods is without a doubt their main disadvantage (Irfan et al. 2022). The introduction of various culture-based automation technologies has sped up lab cultivation and evaluation. For instance, the Biolog Microbial Identification (**Biolog—Microbial Identification & Characterization, 2023**) (Biolog Inc.), the BD Phoenix™ (Becton, Dickinson and Company) (BD Phoenix™ Automated Identification and Susceptibility Testing System, 2023), the MicroScan-Walkaway system (Beckman Coulter) and the VITEK® system (BioMérieux) (VITEK®2: Healthcare, 2023).

These methods examine grown organisms in tiny chemical-filled growth chambers; any growth or change in color is recognized, and with the use of an algorithm, the strain and its phenotypic traits may be ascertained. At the species level, the outcome is presented as "ID," along with the associated antibiotic-sensitivity profile (AST) (Irfan et al. 2022).

6.2. Molecular Methods:

Molecular techniques were used to genetically analyze populations of commensal and pathogenic microbes. These have been used to identify and keep track of genes that cause antibiotic resistance. Molecular parameters utilized for categorization, such as known ARGs targets include mobile genetic components of genus and species such 16s rRNA, integrons (In), insertion sequences (IS), or plasmid-associated genes, which were often related to HGT (Irfan et al. 2022).

The polymerase chain reaction (PCR), on which based the nucleic acid amplification test (NAAT), may determine if a targeted gene is present or absent. A standard PCR experiment may identify ARGs of interest in any sample by utilizing certain DNA probes. Quantitative PCR (qPCR) provides real-time results and precise statistical outputs with faster reaction rates when compared to traditional PCR so qPCR is considered as good tool to examine the efficacy of AMR interventions (Irfan et al.

2022). The third high throughput technique that afford study of whole populations is EpicPCR which incorporates each cell's 16s and ARG, enabling tolerance to be assigned to a specific bacterium (Spencer et al. 2016).

The most sophisticated probe-based PCR technique is metagenomics, it allows a whole DNA sample taken from an ecological sample to be comprehensively analyzed, so it is used for examining the existence of ARGs of interest in a specified sample. This technique has been employed often to identify genes in a range of human and animal faeces samples, including sewage and wastewater effluent (Guo, et al. 2017), medical waste (Munk et al. 2017), and human and animal guts (Thomas et al. 2017).

Even in bacteria that were difficult to grow in labs, PCR-based molecular tools may quickly identify different ARGs compared to culture-based approaches (Luby et al. 2016). Yet, it has been discovered that the target sequence detection frequently denotes resistance, and it is crucial to show that, as shown by diagnostic laboratories, the recognition of the gene is normally not synonymous with tolerance (Performance Standards for Antimicrobial Susceptibility Testing, 2023 and ISO 20776-1:2006—Clinical Laboratory Testing and in Vitro Diagnostic Test Systems, 2023).

For bacterial identification (ID) and ARGs numerous commercially accessible, automated PCR-based techniques are now in use. These come in cartridges that have DNA probes for 16s and several ARGs pre-loaded. It is not necessary to isolate and purify the bacteria for the quick (2–6 h) assays that use direct loading of the samples. These automated devices are expensive, sometimes not designed to handle food and environmental samples, and they need constant manufacturer upgrades to accommodate new ARGs. These technologies include AMPLICOR® (ROCH) and GeneXpert® (Cepheid), both of which rely on multiplex qPCR technology (Molecular Diagnostics, 2023).

Whole-genome sequencing (WGS) is currently the most exact and precise molecular approach for investigating a particular organ-

ism and its ARGs. ARGs, their copy counts, mutations, and new resistance genes can all be examined here, in addition to an organism's whole genome. The most popular WGS systems globally are PacBio® HiFi technology (How HiFi Sequencing Works, 2023) (Pacific Bioscience), (Oxford Nanopore MinION, 2023), (Illumina Inc.), and Nanopore MinION (Sequencing Key Methods and Uses, 2023) (Illumina Inc.). WGS calls for highly qualified experts, pricey infrastructure, and expertise in data processing and manipulation. WGS is the most appropriate for use by national public health agencies and in academic settings or since it may give important information regarding the source of ARGs and their dynamics, while not being the initial step in active AMR surveillance.

6.3. Mass Spectrometry:

Recently by using mass spectroscopy methods, bacterial identification has adopted protein profiling of organisms. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS), a new molecular approach specifically assessed AMR in microbiological specimens. MALDI-ToF can analyze greater quantities and identify bacteria more quickly and accurately than conventional molecular methods. Unfortunately, the initial setup expenditure is high and it requires access to a specialized database and an isolated bacterium. But MALDI-ToF devices have made significant strides in the detection of ARGs, and hospitals all over the world are now adopting the device. Bruker's MALDI Biotyper® (Microbial Identification, 2023) and bioMérieux's VITEK® MS are the two leading manufacturers of MALDI-ToF technology for microbial identification now.

7. Strategies for Combatting Antimicrobial Resistance:

In April 2014, The World Health Organization designated AMR as a "significant universal challenge". The Global Action Plan on Antimicrobial Resistance (World Health Organization 2015) (Global Action Plan on Antimicrobial Resistance, 2023), which encourages member countries to develop identical national action plans by May 2017, was later released by the body that oversees all WHO

member countries; the World Health Assembly. By regulating the proper use of antimicrobial drugs, several national and international representatives have taken actions to minimize the transmission and prevalence of antibiotic resistance. Measures to assess AMR outbreaks were publicly announced by the US Food and Drug Administration (**Huang et al. 2019**). In countries that have put synchronized national policies into place, AMR has been significantly reduced.

Overcoming AMR-related threats seems to depend on a number of factors, including the use of appropriate medications, antimicrobial surveillance through the One Health approach, advancements in medical procedures, the implementation of medical insurance programs, restrictions on drug commercialization, a coordinated epidemic management program, and community management programs.

Rapid diagnostic testing is a significant obstacle in the battle against AMR and a crucial issue, particularly in developing countries where conventional microbiological methods have been often utilized to identify bacteria. Custom therapies for appropriate antimicrobial therapy based on cutting-edge genetic screening technologies might be developed to address these deficiencies. A One-Health approach may be a key way to investigate how people and animals interact and provide new evaluation strategies. Considering the channels of antimicrobial resistance spread throughout the three components—people, animals, and the environment—that are known to be common (**Cantón et al. 2012**), These study fields are particularly important and need to be handled well. However, particularly in low- and middle-income nations, the careless and irrational use of medications is a significant cause of AMR.

There are many reasons why antimicrobial agents are misused, including inpatient use of prescription drugs, lack of knowledge about antibiotics, erroneous diagnosis, especially in developing countries, and the distressing hardships that the pharmaceutical industry causes for clinicians.

The study of the entire AMR problem is hampered by a lack of creative treatments (**Bigdeli et al. 2013**), therefore technical innovation, antibiotic discovery, and combination therapy advancements (**Fischbach, 2011**) are all necessary.

Future studies will focus on identifying the negative impacts of human activity, the role of several key AMR determinants, the impact of resistant strains on humans and animal health, and important technical, cultural, and financial strategies to lower environmental antibiotic resistance. The United States National Action Plan to Combat Antimicrobial Resistant Bacteria (**White House 2015**), the resolution of the 2016 high-level meeting on antimicrobial resistance at the United Nations General Assembly (OPGA/WHO/FAO/OIE 2016), and the FAO/OIE/WHO Tripartite Collaboration are examples of current national and international initiatives to stop the spread of antibiotic-resistant bacteria. Lastly, better management practices for illness prevention are more successful in preserving food animal productivity (**Jonathan et al. 2014**).

8. Conclusion:

Antimicrobial resistance is becoming more widely acknowledged, although there are still big knowledge gaps on its frequency, geographic distribution, and primary causes in the community.

To improve our knowledge of how pervasive AMR is in societies and what population level factors determine its emergence and transmission, epidemiological research approaches need to be contextually relevant. A multidisciplinary framework must be included into a thorough and economical AMR strategy in order to decrease antibiotic usage, enhance monitoring and control the administration of antibiotics.

To learn more about the basic processes behind bacterial evolution, gene transfer, and resistance, additional study is needed. This entails actively investigating the role of persistence and host-pathogen interactions, as well as how they affect the development of antibiotic resistance. Investigating these subjects might result in the new therapeutic and diag-

nostic target identification. Furthermore, it appears that there is a critical need for political will to implement fresh initiatives, develop tools, and assess risk-analysis approaches in order to fully comprehend and confront the emerging risk of AMR.

To address the issue of AMR in animals, the strategies for reducing the use of antimicrobials in animal agriculture includes the use of alternative therapies, such as probiotics and phage therapy and the development of new vaccines. The importance of improved animal husbandry practices such as better hygiene and disease prevention measures is to reduce the need for antimicrobial use. Surveillance and monitoring programs are important to detect and track the spread of AMR in animal populations.

In conclusion, continued efforts are needed to reduce the use of antimicrobials in animal agriculture and mitigate the impact of AMR and researches are needed to explore resistance dynamics and quantify the effects of antimicrobial use in food animals on human and animal health.

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