

## REVIEW OPEN ACCESS

Ruminants

# Multivariate Approach to Antimicrobial Residue Concentrations in Animal-Derived Products: An Analytical Review

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**Received:** 6 August 2024 | **Revised:** 23 January 2025 | **Accepted:** 7 March 2025

**Funding:** This study was supported by the Universidad Autonoma del Estado de Mexico (Grant UAEMex6663/2022SF).

**Keywords:** antimicrobial resistance (AMR) | food chain | livestock | public health

## ABSTRACT

**Background:** Antimicrobial resistance (AMR) represents an alarming global public health concern exacerbated by livestock antibiotic misuse, affecting humans and the environment. However, the precise magnitude of antimicrobial residue concentrations in animal-derived products remains not well understood. This study aimed to quantify antimicrobial residues in animal products through an analytical literature review.

**Methods:** This review covered the scientific articles from 1977 to 2020. The antimicrobials were classified according to the European Medicines Agency (EMA) guidelines into four categories. The final database comprised seven qualitative variables (antibiotic, antibiotic class, region, country, decade, EMA category, animal product and animal species) and one quantitative variable (residue concentration recorded as  $\mu\text{g}/\text{kg}$ ). Due to the number of variables involved in the study, a multivariate analysis approach was used using a Factor Analysis of Mixed Data (FAMD) carried out in R.

**Results:** The highest concentrations of antimicrobial residues were detected in fish samples, followed by egg. Notably, concentrations of ruminant-derived products were lower than to monogastric.  $\beta$ -Lactam was the most prevalent residue followed by aminoglycosides, sulphonamides and quinolones, respectively. Moreover, South America had the highest residues levels, followed by Asia and Europe.

**Conclusions:** The multivariate analysis reveals a possible association between the EMA category, animal species, antimicrobial class and animal product. In conclusion, the concentration of antimicrobial residues in products of animal origin depends mainly on their origin (product, species and geographic region), showing the highest concentrations in products derived from fish and poultry.

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## 1 | Introduction

Globally, the ever-increasing demand for animal-derived products results in extensive use of antimicrobials for disease prevention and growth promotion in food animals (Tiseo et al. 2020; Robles-Jimenez et al. 2024). The widespread and improper application of antimicrobials (including antibiotics, antivirals, antifungals and antiprotozoals) in food animals has led to the emergence and development of antimicrobial resistance (AMR), representing a serious hazard to worldwide public health (WHO 2021; Marutescu et al. 2022). It has been estimated that around 63,151 MT of antimicrobials was used globally in food animal production in 2010, with projections indicating a potential rise of 67% to reach 105,596 MT by 2030 (Ibrahim et al. 2020). Projections also indicate that antimicrobial consumption among humans will witness a surge of up to 15% from 2015 to 2030. This alarming trend mirrors the similar trajectory observed in AMR within food animals throughout this timeframe (Tiseo et al. 2020). Additionally, issues related to the disposal of livestock excrement and antibiotic-contaminated wastewater into the environment have emerged as other challenges frequently highlighted in the literature (Robles-Jimenez et al. 2024; Marutescu et al. 2022; Manyi-Loh et al. 2018). Furthermore, scant attention is given to antibiotic traces found in food products derived from animals, presenting yet another concern worthy of discussion (Bennani et al. 2020).

AMR denotes the phenomenon whereby microbes, including, but not limited to, bacteria, develop heightened resistance against specific antimicrobials to which they had initially exhibited sensitivity; consequently, these persistent bacterial infections can augment the likelihood of interpersonal infection transmissions (Bennani et al. 2020). AMR can be acquired through mutations in preexisting or preceding acquisitions genes, or alternatively through the process of acquisition of new genes from other bacteria known as horizontal gene transfer. The latter is the main mechanism causing AMR (Gonzalez-Ronquillo and Hernandez 2017; Vidovic and Vidovic 2020).

It is well established that antibiotic-resistant bacteria (ARB) are widely found in animal-derived foods (i.e., meat, milk and eggs) facilitating direct transmission to humans via undercooked and raw food ingestion, mishandling of food or cross-contamination with other foods, along with indirect exposure through environmental contact (Okaiyeto et al. 2024). ARB can also be transmitted directly from animals on farms (Vidovic and Vidovic 2020). Research demonstrates that antibiotic residue and ARB endure in manure, ultimately seeping into the soil upon fertilization. After application, both biotic and antibiotic contaminants gradually permeate the soil, eventually reaching groundwater (Robles-Jimenez et al. 2024; Wu-Wu et al. 2023). Although the extensive application of antibiotic in the livestock sector contributes significantly to AMR that could potentially affect humans, the precise magnitude of antimicrobial residue concentrations in animal-derived products remains not well understood. Therefore, this study aims to quantify antimicrobial residues in animal products through an analytical literature review.

## 2 | Materials and Methods

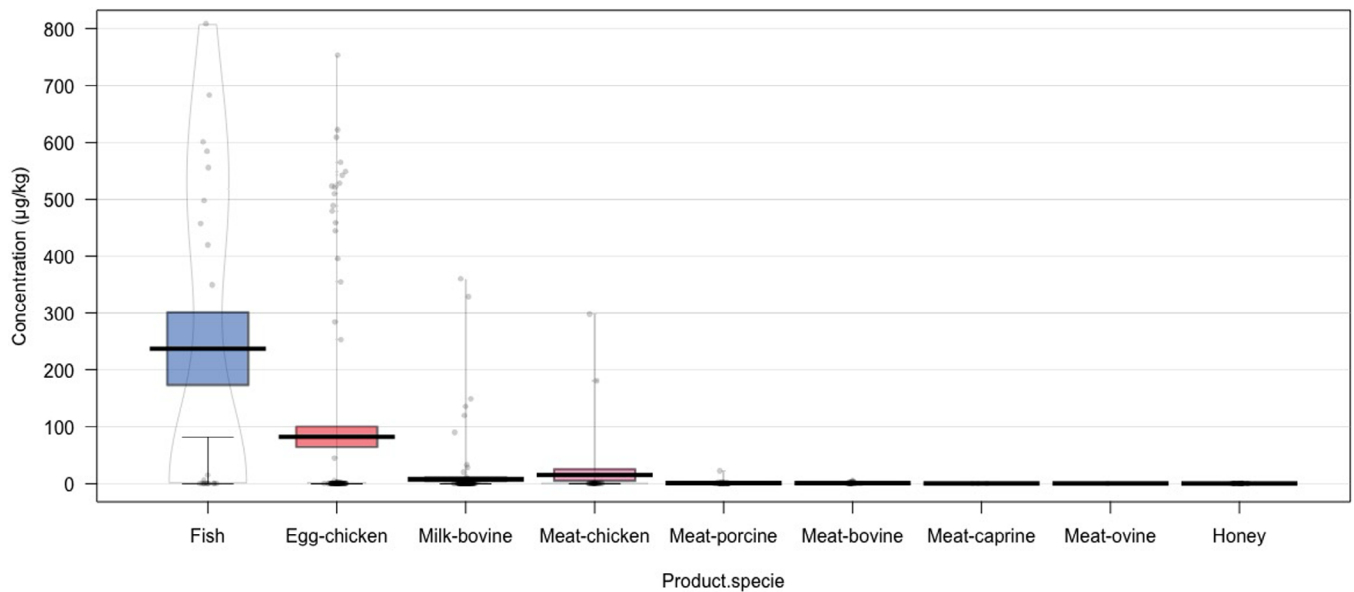
The analytical search of scientific articles focused on studies on veterinary antibiotic residues in animal tissues and prod-

ucts worldwide. For this purpose, a database of publications specifying antibiotic residues worldwide was created, and the articles used covered the years 1977–2020. The publications were obtained from different databases such as ScienceDirect, Scopus, SciELO, PubMed, Redalyc and Google Scholar. The search engine was based on Boolean operators ('and', 'or'). The keywords used were as follows: 'antibiotic residues', 'traceability', 'animal husbandry', 'animal species' (cow, sheep, pig, horse, chicken, rabbit, goat, etc.), 'aquatic and terrestrial animal', 'animal tissues', 'animal products' (milk and eggs) and 'antibiotic concentrations'. The antimicrobials were classified according to the European Medicines Agency (EMA) guidelines into the following categories: A (avoid use in animals), B (limit use in animals), C (use with caution) and D (use with prudence).

The data collected from the forms were captured in a spreadsheet for further analysis. The final database comprised eight variables: seven qualitative variables (antibiotic, antibiotic class, region, country, decade, EMA category, animal product and animal species) and one quantitative variable (residue concentration recorded as  $\mu\text{g}/\text{kg}$ ). Due to the number of variables involved in the study, a multivariate analysis approach using factor analysis of mixed data (FAMD) was used. This methodology analyses the similarities between individuals when mixed variables are considered; it also allows for the exploration of the association between quantitative and qualitative variables (Kassambara 2017). The ellipse plot facilitates the interpretation of the FAMD, which uses projections of clouds of individuals to which concentration ellipses corresponding to given characteristics were added (Kassambara 2017). The FAMD was carried out using the FactoMineR package (Le et al. 2008) and Factoextra (Kassambara and Mundt 2017) in R (R Core Team 2016).

## 3 | Results and Discussion

A total of 35 scientific articles were used in this review. A number of 17 of these analysed feeds from ruminants, 16 from poultry, 6 from pigs, 4 from fish and 1 from bees, with some articles analysing more than one animal product. The list of articles used in the current study is provided in the Supporting Information section. The results of the present literature review reveal that the highest concentrations of antimicrobial residues were detected in fish samples ( $237.0 \pm 25.2 \mu\text{g}/\text{kg}$ ), followed by egg ( $82.0 \pm 11.06 \mu\text{g}/\text{kg}$ ), chicken meat ( $14.9 \pm 19.8 \mu\text{g}/\text{kg}$ ) and cow milk ( $7.35 \pm 8.53 \mu\text{g}/\text{kg}$ ). It should be noted that concentrations of most ruminant-derived products were lower compared to monogastric (pigs' meat and egg chicken) (Figure 1). These concentrations are mainly associated with the use of antimicrobials as growth promoters (Gonzalez-Ronquillo and Hernandez 2017). Antibiotic residues refer to the remaining traces of antibiotics in animal products following their therapeutic application (Hassan et al. 2021). A high concentration of antimicrobial residues, including oxytetracycline ( $553.2 \mu\text{g}/\text{kg}$ ), erythromycin ( $41.95 \mu\text{g}/\text{kg}$ ), chloramphenicol ( $1.91 \mu\text{g}/\text{kg}$ ) and sulphonamides ( $7.06 \mu\text{g}/\text{kg}$ ), has been reported in aquaculture products from different countries (Okocha et al. 2018), which confirms the current findings. Qualitative detection of antimicrobial residues in poultry showed the highest prevalence in the liver followed by kidney, breast meat, thigh meats and egg. Tetracycline, a commonly used antibiotic in livestock, was present



**FIGURE 1** | Concentration of antimicrobial residues in food of animal origin according to species and product.

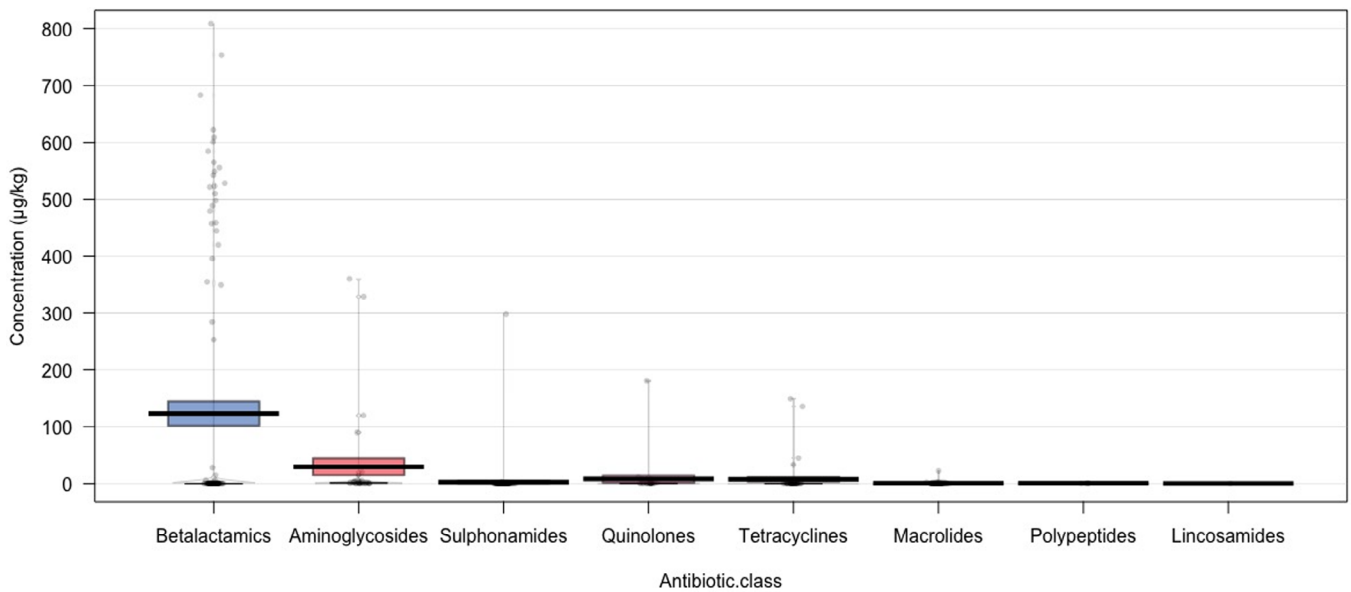
in high levels in the liver of chickens (Mohammadzadeh et al. 2022; Salama et al. 2011). Moreover, in the case of aquaculture food products, a high amount of amoxicillin (683.2 mg/kg) was detected in Tilapia fish (Hassan et al. 2021). With regard to cow's milk samples, Hassan et al. (2021) had reported prevalence rates of amoxicillin, ciprofloxacin and tetracycline residues of 26%, 12.5% and 17.5%, respectively. It has been well established that chicken eggs are often contaminated with high levels of furazolidone (268.25 ng/kg) and sulphonamide (103–230 ng/g) residues (Mund et al. 2017). Similarly, Kabir et al. (2004) reported the presence of several drug residues within eggs, including amprolium, furaltadone, furaprol, oxytetracycline, tylosin, streptomycin and sulfaquinoxaline. In confirmation, it has been well established that when antibiotics are administered, they undergo absorption in the chicken intestine (Mund et al. 2017). Consequently, these compounds reach ovarian structures such as follicles and oviducts, resulting in increased potential for drug residue deposition in both yolks and albumen. A systematic review of antimicrobial residues in food from animal origin reported that antibiotics have been found in the following products: fish, seafood, eggs, poultry, milk and beef (Treiber and Beranek-Knauer 2021). Of note, the international trade of meat, fish and aquaculture products faces setbacks because of antimicrobial residues (McEwen 2006).

Regarding the class of antimicrobials, the highest residue concentrations were shown by  $\beta$ -lactams ( $123.0 \pm 11.0 \mu\text{g/kg}$ ), aminoglycosides ( $29.4 \pm 20.4 \mu\text{g/kg}$ ), sulphonamides ( $2.38 \pm 10.4 \mu\text{g/kg}$ ) and quinolones ( $8.0 \pm 22.1 \mu\text{g/kg}$ ) (Figure 2). In relation to the concentration of antimicrobial residues based on the EMA classification, category D showed the highest concentrations ( $53.85 \pm 7.78 \mu\text{g/kg}$ ), contrary to category A with the lowest levels ( $0.50 \pm 72.3 \mu\text{g/kg}$ ).

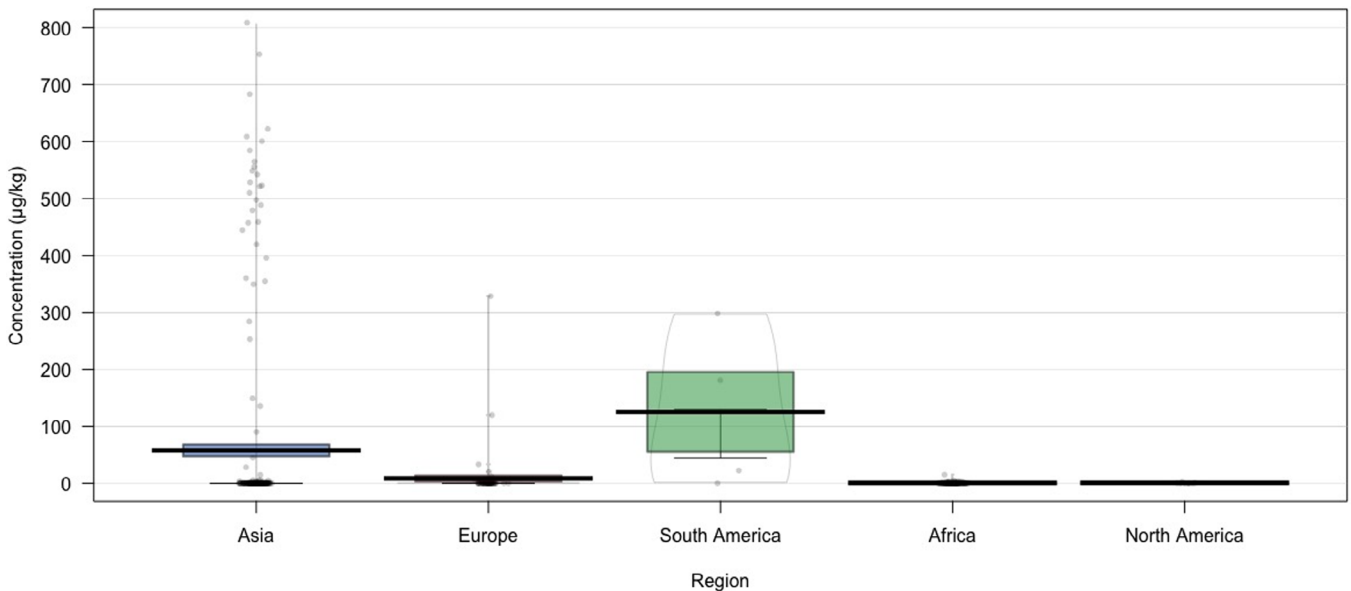
Regarding geographical location, South America showed the highest concentrations ( $125.19 \pm 62.2 \mu\text{g/kg}$ ), followed by Asia ( $58.76 \pm 7.78 \mu\text{g/kg}$ ) and Europe ( $8.41 \pm 15.2 \mu\text{g/kg}$ ) (Figure 3). On the other hand, Africa showed the lowest levels of antimicrobial

residues in food of animal origin ( $0.43 \pm 11.2 \text{ mg/kg}$ ). This is in line with projections that place Brazil as one of the main consumer countries of antimicrobials for use as growth promoters in animals (Gonzalez-Ronquillo and Hernandez 2017).

A survey of national policies on antimicrobial use in food animal production reveals that China and Brazil do not have any formal restrictions on antimicrobial use for the purposes of growth promotion that could explain the high levels of antimicrobial residues in animal-derived foods in these regions (Maron et al. 2013). Similarly, Hosain et al. (2021) reported that the developing countries of South and Southeast Asia have high consumption of antimicrobials as a result of intensive farming systems, which make a higher risk of antimicrobial residues in animal products. In contrast, European Union (EU) widely limited antimicrobial use in animal nutrition, primarily to combat the rising threat of AMR. In 2019, the European Parliament voted to prohibit the use of medically important antimicrobials for disease prevention in food-producing animals, a regulation that took effect in 2022 (Wallinga et al. 2022). The United States has faced challenges in enforcing similar restrictions, with approximately 66% of medically important antimicrobials sold in the United States intended for animal agriculture. Although the FDA has made strides by withdrawing non-therapeutic uses of these drugs in feed, the continued approval of their use for disease prevention raises concerns about ongoing AMR (Wallinga et al. 2022; Tiseo et al. 2020). However, in some countries like Mexico, Japan, South Korea and Russia, antimicrobial are semi-restricted (Maron et al. 2013). A recent study (Tiseo et al. 2020) identifying the global trends in antimicrobial use in food animals from 2017 to 2030 shows that Asia is the largest user of antimicrobials, with an expected increase of 10.3% over this time, whereas Africa used the lowest quantities of antimicrobials. It has been well established that numerous factors foster the occurrence of antibiotic residues in animal-derived products, particularly in developing countries, including (1) poor farmer/butcher knowledge about antibiotic breakdown in animals, (2) weakened monitoring from prescription to substance usage and (3)



**FIGURE 2** | Concentration of antimicrobial residues in food of animal origin according to antibiotic class.



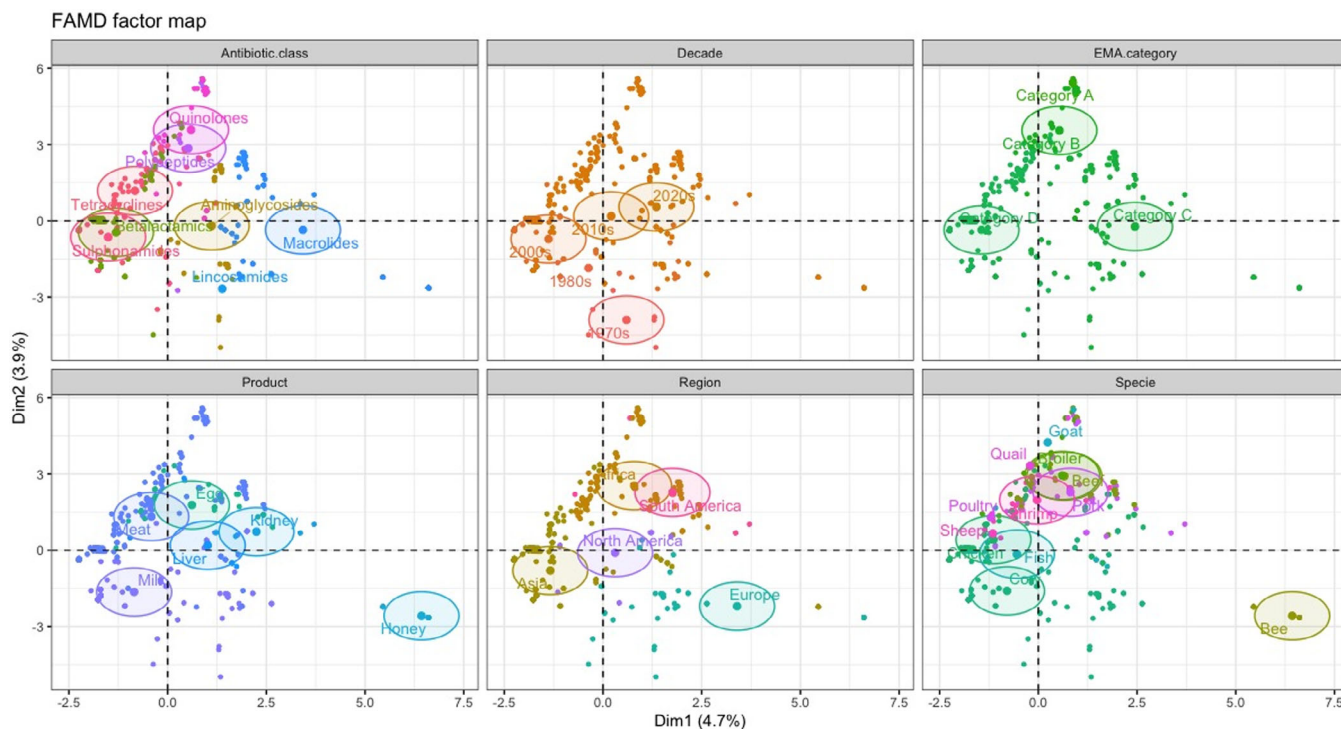
**FIGURE 3** | Concentration of antimicrobial residues in food of animal origin according to region.

limitations in detectability methods and missing certifications for animal-origin food products (Treiber and Beranek-Knauer 2021).

The factor map generated by the factor analysis of mixed data (FAMD) approach (Figure 3) shows the clustering patterns based on antibiotic class, region, decade, EMA category, animal product and animal species. Category A encompasses mainly antibiotics of the quinolone class, and their residues are mainly found in eggs and beef with antibiotic residues allocated to the African continent. Category B includes antibiotics of the polypeptide and tetracycline classes, and their residues are highest in poultry meat with higher concentrations in the 2010s decades and allocated in the South and North American regions. The graph also reveals that the aminoglycoside, lincomycin and macrolide classes are related to category C, which showed higher residue

concentrations in fish and bees and were mainly distributed in Europe. Finally, category D included families such as  $\beta$ -lactams and sulphonamides, with an increase in the frequency of these antimicrobials in poultry meat and cow's milk, with higher concentrations in the 2000s decade and the Asian continent.

In line with our data, a recent meta-analysis (Tao et al. 2022) showed that the highest resistance of most foodborne pathogens was to  $\beta$ -lactams. Similarly, Nguyen et al. (2016) reported that the  $\beta$ -lactams, quinolones and combinations of sulphonamides with trimethoprim were quantitatively the most residue compounds in chicken. A recent meta-analysis (Fatemi et al. 2023) of antimicrobial residues in dairy products in Iran during 2000–2022 showed that the most residues were tetracycline, followed by beta-lactam and sulphonamides. The high stability of quinolones



**FIGURE 4** | Ellipse plots of the factor analysis of mixed data.

and  $\beta$ -lactams constitutes a significant risk to human health because residues of these antibiotics can remain in animal products after heat treatment (Roca et al. 2011).

Analysing the factor map of the FAMD according to the region and decade clustering, we found that the antibiotic residues of the North America region clustered closely to the 2010s, whereas Asia concentration residues clustered closely to the 2000s, as for Africa and South America, data points more recent to the 2020s decades (Figure 4). This finding is in line with the observations of Treiber and Beranek-Knauer (2021) who highlighted that most problems related to antibiotic residues in animal-derived products are found in developing and emerging countries. They further suggest that key factors contributing to the presence of antibiotic residues in these countries include deficiencies in prescription monitoring, inadequate or unavailable antibiotic residue detection methods, and the absence of certification systems for animal-derived foods. Our findings are also consistent with reports that in recent years have estimated that the consumption of antimicrobials will increase over the years for countries in Africa and South America, with Brazil being one of the largest consumers of antibiotics in animal food production (13% of the total) (Van Boeckel et al. 2015). Da Silva et al. (2023) assessed the regulation of antibiotic use in livestock production in the five largest meat-producing countries in South America (Argentina, Brazil, Chile, Colombia and Uruguay). They found that the use of antibiotics as growth promoters is banned in these countries (except for Uruguay and Brazil), based on the World Health Organization's critical classes. However, there are currently no regulatory mechanisms in place in the region regarding the promotion of antibiotics to farmers or the use of financial incentives for prescribers and suppliers (distributors).

On the other hand, Europe remains distantly clustered, and its antimicrobial residues are associated with the earliest decades (1970s and 1980s) (Figure 4). This can be explained by the fact that this region has been the most regulated for antimicrobial use for many years (Schmerold et al. 2023). In 1986, Sweden was the first country to ban the use of antibiotics as growth promoters, and it was the farmers themselves who requested the ban, based on reports showing the risks of using antibiotics as growth promoters. This situation reinforces the fact that the main motivation for producers and technicians to implement a more prudent use of antimicrobials must be based on education (Gonzalez-Ronquillo and Hernandez 2017). Later, on 1 January 2006, the EU ban on the use of antibiotics as growth promoters in animal feed came into force (EU 2005). Initially, the EU based the ban on the use of antibiotics as growth promoters on the 'precautionary principle', where precautionary measures are taken when the scientific evidence about a risk to the environment or human health is uncertain and the stakes are high (Bourguignon 2015). Recently, the EU has strengthened its regulation on the use of antibiotics in animal production. In January 2022, the EU limited the use of antibiotics only to the exceptional treatment of individual animals. Moreover, antibiotics can no longer be used to compensate for poor hygiene and animal husbandry practices (EU 2022).

Regulatory frameworks governing the use of antimicrobials in food-producing animals vary significantly across countries, reflecting differing public health priorities and agricultural practices. In the EU, a comprehensive regulatory system is enforced through monitoring protocols that require both producers and third countries exporting to the EU to have approved residue control plans (CVMP 2021; Mulchandani et al. 2023). Conversely, many developing countries adopt a more lenient approach,

often lacking a robust legal framework for antimicrobial use in livestock, which raises concerns about food safety and public health (Mulchandani et al. 2023). Additionally, countries such as Denmark have implemented innovative strategies, including the Yellow Card initiative, which incentivizes farmers to reduce antimicrobial usage by imposing monitoring requirements and penalties for non-compliance (CVMP 2021). These disparities highlight the urgent need for harmonized global standards to protect consumer health from the risks associated with antimicrobial residues in food products.

However, the control of these antimicrobial residues remains a challenge and needs to be further strengthened (ECDC-EFSA-EMA 2024). Therefore, according to these data, it should be considered that the potential risks associated with the administration of antimicrobial agents to food-producing animals, such as bacterial resistance, are a globally interconnected food system that will increase in a 10-year projection.

## 4 | Conclusion

The administration of antimicrobials to food-producing animals, either directly for medical treatment or indirectly via feed for growth promotion, may result in exposure to residues of the antimicrobial and subsequently promote AMR. This article provides insight into the quantification of antimicrobial residues in animal products through an analytical literature review. The results showed that the concentration of antimicrobial residues in products of animal origin mainly depends on their origin (product, species and geographic region), with the highest concentrations in products derived from fish and poultry. For ruminant products, cow's milk shows the highest concentrations. The role of education of all actors in the food production chain is crucial to achieve prudent use of antimicrobials by promoting appropriate hygiene practices, preventive medicine and improved animal husbandry practices. Finally, a global initiative involving policymakers, animal health professionals, food producers and pharmaceutical companies is essential to prioritize the reduction of inappropriate use of antimicrobials in animals.

### Author Contributions

**Juan Carlos Angeles-Hernandez:** conceptualization, investigation, methodology, software, data curation, formal analysis, visualization, validation, project administration, supervision, writing – original draft, resources. **David Alejandro Contreras Caro del Castillo:** methodology, data curation, conceptualization, writing – review and editing. **Astrid Espinosa-Sánchez:** methodology, software, writing – review and editing, investigation, data curation. **Lizbeth E. Robles-Jimenez:** methodology; data curation, conceptualization. **Navid Ghavipanje:** data curation, writing – original draft, writing – review and editing, investigation. **Manuel Gonzalez Ronquillo:** project administration, writing – review and editing, methodology, conceptualization, funding acquisition, writing – original draft, investigation.

### Acknowledgements

This work has been partially funded by the Universidad Autonoma del Estado de Mexico (Grant Reference Number: UAEMex6663/2022SF).

### Ethics Statement

The authors have nothing to report.

### Consent

All authors decided on the publication of this article and held the corresponding author accountable for correspondence during manuscript publishing.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data are available on request from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.