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A snapshot survey of antimicrobial resistance in food-animals in low and middle-income countries

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ABSTRACT

Antimicrobial resistance remains a threat to global public health. Low-and middle-income countries carry a greater burden of resistance because of higher rates of infection as well as, potentially, location-specific risk factors. Food animals occupy a critical crossover point for the spread of antimicrobial resistance to humans and the environment. However, this domain remains poorly surveilled outside high-income settings. We used point surveillance from 191 studies reporting phenotypic AMR in food animals across 38 African, Middle Eastern, Asian and South and Central American countries to depict antimicrobial resistance trend in food animals. By computing Multiple Antibiotic Resistance indices and finding an overall mean of 0.34 ± 0.16, which is above the 0.2 index associated with multidrug resistance and high risk, we show that multidrug resistance in bacteria from food animal sources is worryingly high. MAR indexes from food animals were overall higher than those previously computed from aquaculture but, unlike aquaculture-computed MAR indices, did not track closely with those of human-associated bacteria in the same countries. Food animals are an important reservoir for rising antimicrobial resistance in bacteria, and hence improved surveillance in this sector is highly recommended.

1. Introduction

While it is ideal to rear animals for food without using antimicrobials today, about 73% of all antimicrobials produced are consumed by animals for food [1]. Countries in Asia, South and Central America and Africa are among the fastest growing consumers of animal food and often favor intensive methods that use or misuse antimicrobials in animal production [2–4]. Antimicrobials are known to be indiscriminately used as growth promoters as well as in disease prophylaxis and metaphylaxis [5,6]. In addition, their use may also be used to mask deficiencies in biosecurity, poor nutrition and hygiene in animal production [7]. The application of antimicrobials in food animal production has been occurring for decades, and early use recorded appreciable commercial returns in terms of growth performance of food animals [6]. However, their use and abuse over the years comes at a high cost in the phenomenon known as antimicrobial resistance (AMR), which threatens both human and animal health, globally [6]. Antimicrobials administered at sub-therapeutic levels select for antimicrobial resistance in exposed microbial populations. Selected resistant organisms may persist and spread to other environmental matrices causing an ever-rising threat to human and animal health [8,9].

Owing to the identification of food animals as important reservoirs for the evolution and dissemination of antimicrobial resistance, several European countries, as well as the United States of America, Mexico and China have imposed different levels of restrictions in the use of antimicrobials in food animals, including the institution of surveillance to monitor antimicrobial resistance [10]. However, in low- and middle-income countries (LMICs), there are few initiatives collating the usage of antimicrobials in food animals [11–13] making it difficult to elucidate their impact in promoting antimicrobial resistance owing to poor surveillance. Food animals can become hosts to resistant bacteria from other animals, their human handlers, and the environment, and can potentially disseminate these bacteria to human consumers [14,15]. According to the FAO [16], several bacterial species of AMR importance in animal production, and of equal importance in transmission of human infections, include *Campylobacter* spp. (resistant to fluoroquinolones),

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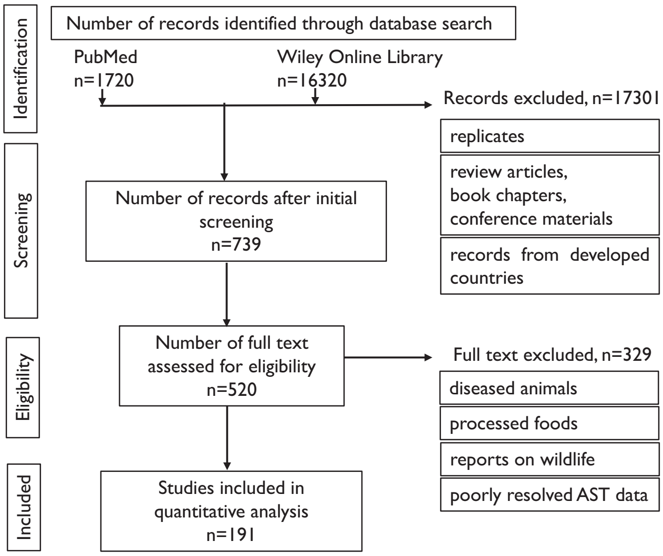

Fig. 1. Article selection process: exclusion and inclusion criteria.

Enterococcus spp. (resistant to aminoglycosides, ampicillin, vancomycin), *Escherichia coli* (resistant to quinolones, sulphonamides, trimethoprim), non-typhoidal *Salmonella* spp. (resistant to cephalosporins, quinolones, tetracyclines) and the livestock associated Methicillin-resistant *Staphylococcus aureus* [16].

The dearth of AMR surveillance systems in LMICs presents a blurry picture as to frequency of resistance in bacteria of food animals. A recently published review by van Boeckel et al. [13] used point surveillance to report trends associated with AMR in food animals in developing countries, thereby identifying country-level AMR hotspots [7]. However, there was no synthesis of findings to allow simple comparison of resistance in other domains such as in clinical isolates and aquaculture. Recognizing that food animals sit at a potentially critical crossover point for resistance transmission, we used a systematic review of the literature to garner a snapshot of AMR prevalence in food animal bacteria using point surveillance by examining phenotypic antibiotic resistance reports in bacteria from studies in LMICs and inform on multiple antibiotic resistance index and frequency of antimicrobial resistant phenotypes from examined studies.

2. Methods

2.1. Literature search

A systematic search of peer-reviewed literature within for the period from 1st January 2010 to 1st June 2021 was conducted to view the dynamics of bacterial AMR prevalence in food animals using the combination of keywords [(“antimicrobial resistance”) AND (“Bacteria” OR “Campylobacter” OR “Staphylococcus” OR “Escherichia” OR “Salmonella”) AND (“animal*” OR “poultry” OR “farm” OR “pigs” OR “cattle” OR “goats”) AND (“developing countries” OR “LMICs” OR “Africa” OR “Asia” OR “South America”)] on PubMed and Wiley Online Library. A combined total of 18,040 records were retrieved from the database search and subsequently screened using Rayyan (rayyan.ai).

2.2. Inclusion and exclusion of records

Criteria used in screening records from the databases are summarized in Fig. 1. Five hundred and twenty (520) records with accessible full texts were checked for inclusion eligibility. Full-text studies (n = 329) were excluded due to poor resolution of AMR data such as uninterpretable figures and graphs coupled with no or inaccessible supplementary AMR data, an unresolvable mix of AMR data for animals and other sources unrelated to this study, and the presence of AMR records from diseased animals and processed food animals. The latter was excluded to limit the exaggeration of AMR prevalence (Fig. 1).

Records included in the meta-analysis were studies from LMICs as designated by the World Bank (https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups) that had interpretable antibiotic susceptibility data of isolates tested, and included bacterial isolates that were identified at least to the genus level and were directly associated with supposedly healthy food animals and/or animal carcasses.

2.3. Antibiotic resistance analysis

Multiple antibiotic resistance (MAR) indices were determined using methods described by Reverter et al. [17]. Briefly, MAR was calculated for individual isolates, or group of isolates from a single study belonging to the same species or genus by determining the proportion of the total number of bacterial antibiotic resistance phenotypes and the total number of antibiotics tested (total number of isolates multiplied by the total number of antibiotics tested) (Supplementary Data 1). Country-specific MAR indices were calculated as mean MAR indices weighted by the total number of isolates recorded for that country (Supplementary Data 2). We also determined the frequency of antimicrobial resistance of the bacteria species reported in the datasets to different antibiotics grouped by their antibiotic classes (Supplementary Data 3). Data were analysed on R version 4.1.1 and Microsoft Excel. Transformed data were visualized using the rworldmap and ggplot2 R packages.

2.4. Comparison of resistance in livestock isolates with human clinical and aquaculture data

Reverter et al. [17] recently performed MAR analysis on aquaculture isolates and compared them to data from human clinical isolates. In order to benchmark our findings against isolates from those two sectors, we retrieved the dataset from https://datadryad.org/stash/dataset/doi:10.5061/dryad.dv41ns1tr.

3. Results and discussion

3.1. Calculated multiple antibiotic resistance indexes from food animal bacteria

In this study, we computed Multiple Antibiotic Resistance (MAR) indices, that is the ratio of antimicrobials to which strains are resistant to the number of antimicrobials tested, for 28,585 bacterial isolates from 191 food animal AMR-specific studies (295 datasets) in 38 LMICs. A total of 6187 (21.6%) of the isolates for which data were retrieved for meta-analyses were from African countries, 3525 (12.8%) from central and south America and 17,631 (78.7%) from Asia (Supplementary Data 1). The bacterial isolates specified a total of 113,049 AMR phenotypes. The calculated mean MAR index was 0.34 (standard error, 0.02), well above the threshold of 0.2 that indicates high risk and multidrug resistance, such as are seen in those from clinical settings [18]. The mean MAR indexes from this study were higher than what was calculated from the global MAR index from aquaculture-related-bacteria 0.25 (SE = 0.01).

In this study, median MAR index was highest in Malaysia (0.69), Philippines (0.62), Pakistan (0.579) and Zambia (0.567), and was lowest in Gabon (0.020), Zimbabwe (0.028), Uganda (0.105) and Bangladesh (0.109) (Fig. 2; Supplementary Data 2). However, high MAR indices were recorded for some settings in countries with lower weighted mean MAR values. For example, MAR indices of 0.95 and 0.84 were recorded for *Salmonella enterica* and *Campylobacter coli* in poultry farms and slaughterhouses, respectively in China [19,20]. Comparably high MAR indices in *Campylobacter* spp. (0.84) and *Escherichia coli* (0.81) from retail markets and chicken farms in the Philippines and Nigeria,

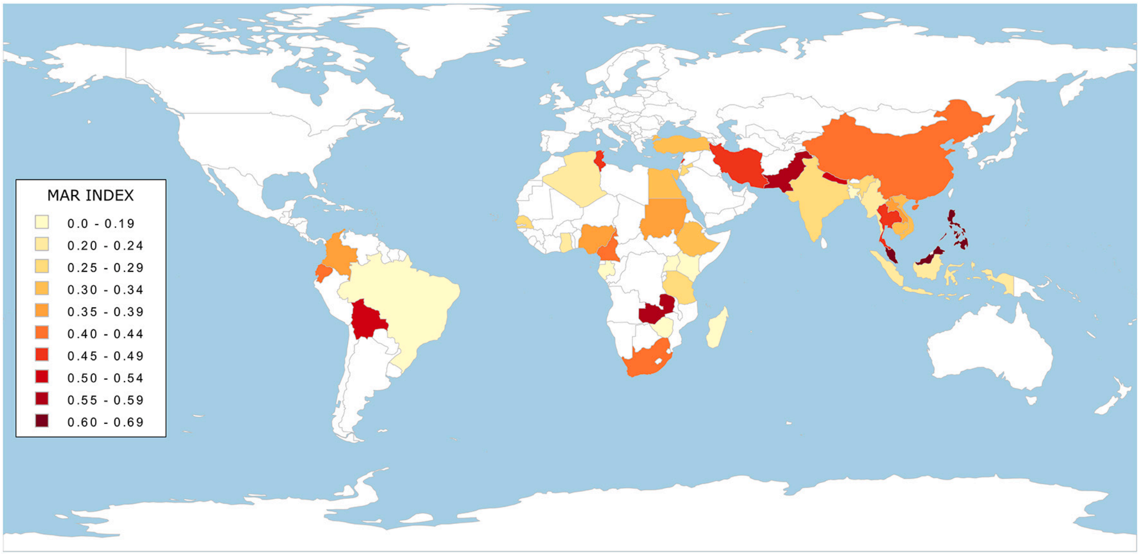

Fig. 2. Calculated multiple antibiotic resistance (MAR) index from food-animal related studies in low and middle-income countries.

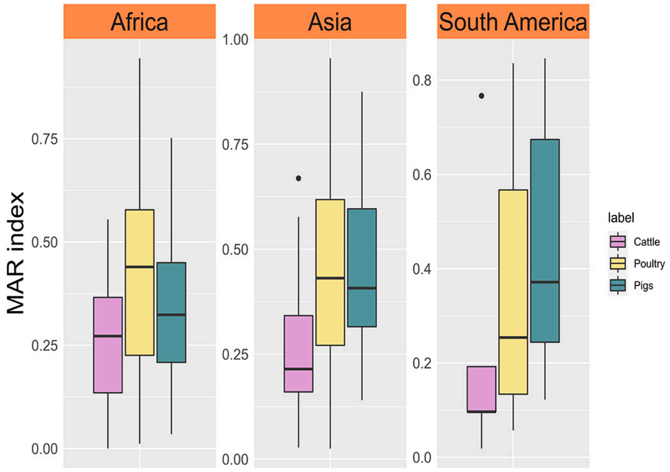

Fig. 3. Multiple antibiotic resistance (MAR) index of food animal (cattle, poultry and pigs) derived bacteria in Africa, Asia and South America.

respectively, were reported [21,22]. In invasive human *E. coli* infections, MAR index (resistance to antimicrobials - aminoglycosides, fluoroquinolones and third generation cephalosporins, obtained from https://resistancemap.cddep.org/ [17] ranged from 0.21 (Malaysia), 0.22 (South Africa), 0.31 (Tunisia) to 0.76 (Nigeria), (0.74) Zambia, 0.60 (Vietnam).

In reference to the food animals being surveilled, majority of the datasets from Africa, Asia and South America analysed in this study were generated from poultry (53.6%), pig (21.4%), and cattle (18.0%) isolates (Supplementary Fig. 1). Median poultry- (0.439) and cattle-associated (0.272) MAR indices were highest in the African studies (Fig. 3). *Escherichia* ($n = 103$, 34.91%), *Salmonella* ($n = 101$, 34.23%), *Staphylococcus* ($n = 37$, 12.57%) and *Campylobacter* ($n = 33$, 11.18%) were the predominant bacterial genera studied ($n = 295$) (Supplementary Fig. 2).

3.2. Drug classes and antimicrobial resistance frequencies

Prevalence of antimicrobial resistance in common food animal bacteria; *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp. and *Staphylococcus* spp. to a combined total of fifteen ($n = 15$) drug classes and subclasses was calculated in this study (Fig. 4, Supplementary Data 3). Results are presented graphically as boxplots showing median antibiotic resistance prevalence (M50), Interquartile ranges (IQR: 25th percentile to 75th percentile) and number (n) of strains tested against antibiotics belonging to the respective drug classes (Fig. 4). Some of the key antimicrobial classes are discussed below.

3.2.1. Aminoglycosides

These are among the oldest classes of antimicrobials in human and animal medicine. In veterinary medicine, they are used in treatment of infections and diseases in all major food animals [23]. In this study, we observed resistance to aminoglycosides were highest among *Staphylococcus* spp. in Africa and Asia with M50 of 33.3% (IQR: 16.2%–60.3%, $n = 639$) and 68.3% (IQR: 15.4–86.05%, $n = 1777$), whereas in South America M50 to aminoglycosides was 28.35% (IQR: 20.81–79.72%, $n = 398$) and 33.81% (IQR: 0.6–86.6%, $n = 76$) for *E. coli* and *Campylobacter* spp., respectively. Similarly higher M50 in *E. coli* (37.5% IQR: 13.1–58.7%, $n = 8437$) and *Campylobacter* spp. (44%, IQR: 10.3–73.5%, $n = 1424$) to this drug class was observed in Asia- based studies (Fig. 4; Supplementary Data 3). Based on their high importance and unavailability of suitable alternatives in treating infections, aminoglycosides are classified as veterinary critically important antimicrobial (VCIA) by the World Organization for Animal Health [24]. In human medicine, these antimicrobials are also regarded as critically important antimicrobials [25].

3.2.2. Beta-lactams and Cephalosporins

Beta-lactams are among most commonly used antimicrobials in the treatment of bacterial infections due to their broad-spectrum activity and safety [26]. Antimicrobials in this drug class are commonly used in animal production in LMICs [27,28]. In this study, frequencies of resistance (M50) to beta-lactams (except carbapenems and cephalosporins) ranged from 32.4% (IQR: 13.3–61.4%, $n = 2002$) in *Salmonella* spp. to 60.7% (IQR: 9.1–89.5, $n = 843$) in *Staphylococcus* spp. in studies from Africa. In Asia based literature, M50 ranged from 50% (IQR: 26.6–95.2, $n = 1786$) in *Staphylococcus* spp. to 53.5% (IQR: 35.5–88.3% $n = 495$) in *Campylobacter* spp., whereas it was 62.5% (IQR: 10.3–85.3, $n = 202$) to 72% (IQR: 30.1–92.0, $n = 221$) in *E. coli* and *Staphylococcus* spp., respectively from South America studies. Antimicrobials in this class are regarded as high priority critically important (Except the 1st and 2nd generation cephalosporins - categorized as highly important antimicrobial agents in veterinary and human medicine respectively) in both veterinary (excluding the carbapenems) and human medicine

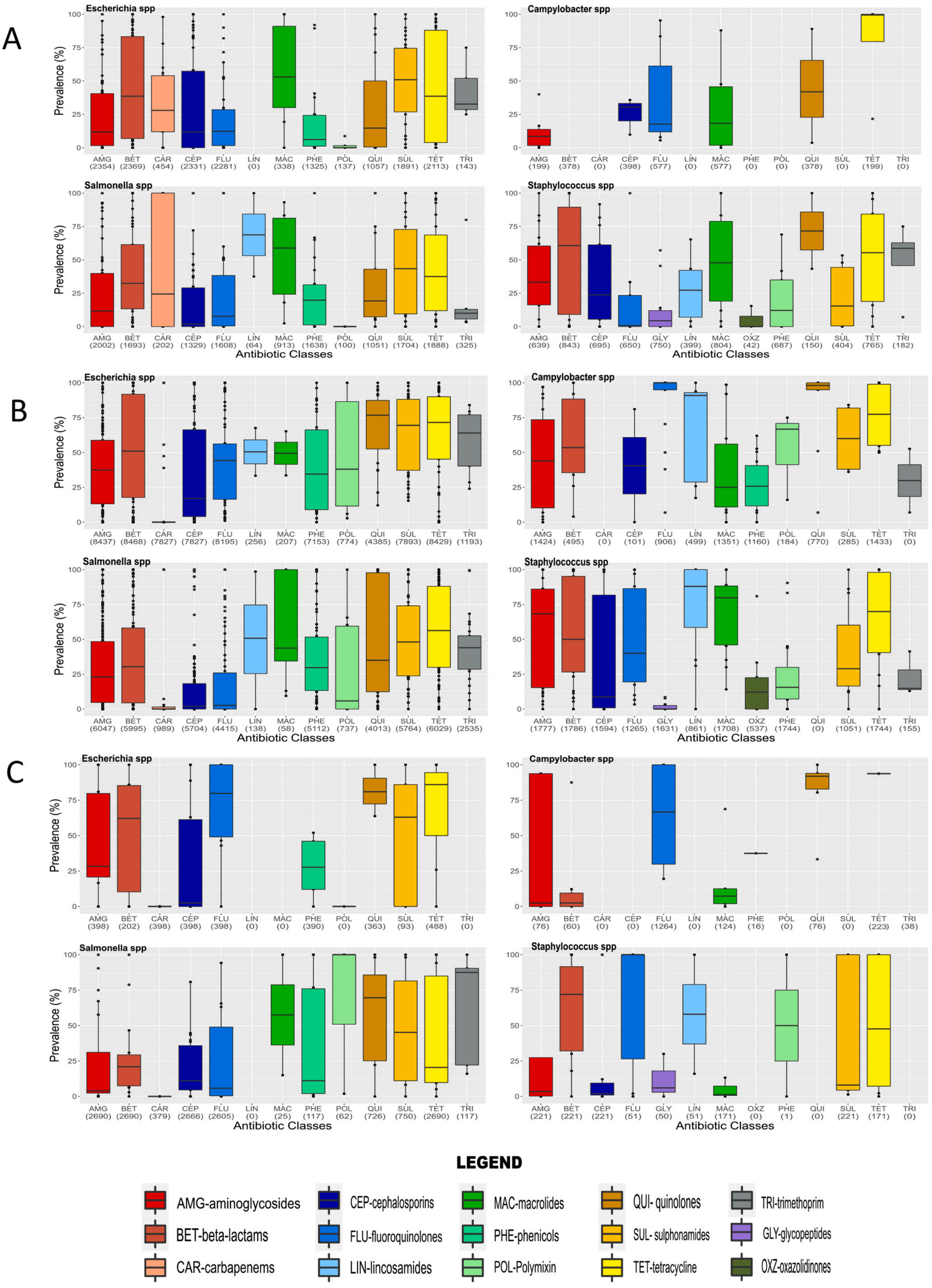

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Fig. 4. Prevalence of antimicrobial resistance (%) in different bacterial Genus from food animal sources in (a) **Africa** (b) **Asia** and (c) **South America** to different classes of antibiotics (number of isolates tested against each agent is indicated in parenthesis).
AMG: aminoglycosides, BET: Beta-lactams, CAR: carbapenems, CEP: cephalosporins, FLU: fluoroquinolones, LIN: lincosamides, MAC: macrolides, PHE: phenicols, POL: polymixins, QUI: quinolones, SUL: sulphonamides, TET: tetracyclines, TRI: trimethoprim, GLY: glycopeptides, OXZ: oxazolidinones.

respectively [24,25]. Furthermore, in this study we observed that M50 to drugs in the Cephalosporins subclass (not delineated by generations) were highest among the *Campylobacter* spp. from studies in Africa (30.5%, IQR: 20.15–33.15%, $n = 398$) and Asia (40.55%, IQR: 20.8–60.8%, $n = 495$). Majority of *Campylobacter* spp. are reportedly considered to harbour mechanisms which aid resistance to beta-lactams and narrow-spectrum cephalosporins [29].

3.2.3. Carbapenems

Among members of the beta-lactam drug class, the carbapenems have the widest activity spectrum. In human medicine, these are last-line drug in treatment of severe bacterial infections. Carbapenem-resistant Enterobacteriaceae are priority pathogens for which new antimicrobials are urgently needed for its control [30]. Although animal foods are increasingly recognized as important sources of resistance development, resistance to carbapenems are not commonly reported [31]. Furthermore, unlike in human medicine, the OIE has no categorization for members of this beta-lactam subclass in animal production. In our point surveillance using literature, low M50 of 0 was observed in *Salmonella* and *E. coli* isolates from Asia [*Salmonella* spp. (IQR: 0–1.4%, $n = 989$) and *E. coli* (IQR: 0–0.2%, $n = 7827$)] and South America [*Salmonella* spp. (IQR: nil, $n = 379$) and *E. coli* (IQR: nil, $n = 398$)] (Fig. 4). However, we observed a much higher M50 in *Salmonella* spp. – 24.4% (IQR: 0–100%, $n = 202$) and *E. coli* – 28% (IQR: 12–54%, $n = 454$) isolates from studies in Africa. Although the low frequency of phenotypic carbapenem resistance could be explained by the limited usage of the antimicrobial in animal production [9,32], continuous surveillance of resistance to these important antimicrobials is important and should be closely monitored as we observed much higher M50 in literature from Africa.

3.2.4. Polymixins

Unlike the carbapenems, colistin, an important and common member of the polymyxin drug class is more widely used in food animals for disease treatment and prophylaxis. They are drugs of last resort for the treatment of multidrug resistant Gram-negative bacteria resistant to carbapenems, as such, they are categorized as highest priority critically important antimicrobials in human medicine and also of high importance in veterinary medicine [24,25]. In this study, we observed M50 for polymyxins was 66.7% (IQR: 41.4–70.9&, $n = 184$), 38% (IQR: 11.6–86.6%, $n = 774$) and 5.8% (IQR: 0–59.4%, $n = 737$) in *Campylobacter*, *Escherichia*, and *Salmonella* species, respectively, in Asia (Fig. 4b), but 100% in *Salmonella* from pigs and broilers in Brazil [33] and Ecuador [34] in South America (Fig. 4c). In Africa based studies, resistance to polymyxin was not tested in most of the isolates, and where tested, had a low M50 such as in *E. coli* (M50: 0%, IQR: 0–1.5%, $n = 137$) (Fig. 4a). The development and transmission of plasmid-mediated mobile colistin resistance gene variants seen today has been associated with the heavy usage and misuse of colistin in animals, and colistin resistance determinants are now reported in about 47 countries across six continents [35,36].

3.2.5. Glycopeptides

Another class of last-resort antimicrobials are the glycopeptides. These are antimicrobials known for the treatment of life-threatening infections caused by Gram-positive bacteria such as *Staphylococcus aureus* [37]. In this study, resistance (M50) to glycopeptides in *Staphylococcus aureus* was under 10% [South America – 6% (IQR: 3–18%, $n = 50$), Africa – 4.34% (IQR: 0–12%, $n = 750$) and Asia – 0% (IQR: 0–2.48%, $n = 1631$)] (Fig. 4A, B and C). The judicious usage of this antimicrobial in food animals is vital to keep low levels of resistance and to avoid a spillover of resistant organisms to other domains such as in human and the environment [38]. Despite their critical value as last line antimicrobials and categorization as highest priority critically important antimicrobials in human medicine, these are currently not present in the OIE's list of veterinary important antimicrobials [24,25].

3.2.6. Quinolones and fluoroquinolones

The quinolones and fluoroquinolones are important antimicrobials used in the treatment of septicemias and other infections by Gram-negative bacteria [24]. They are categorized as veterinary highly important and veterinary critically important antimicrobials, respectively [24]. In this study we observed M50 to fluoroquinolones to be above 60% in *Campylobacter* spp. from Asia (M50: 100%, IQR: 95–100%, $n = 906$) and South America (M50: 66.6%, IQR: 29.9–100%, $n = 1264$), whereas it was 17.7% (IQR: 11.9–61.3%, $n = 398$) in literature pooled from Africa. Among the *E. coli* isolates, M50 to fluoroquinolones ranged from 12.3% (IQR: 1.6–28.5%, $n = 2281$) to 79.8% (IQR: 49.1–100%, n = 398) in studies from Africa and South America, respectively, whereas M50 was 2.65%, 5.75% and 7.7% in *Salmonella* spp. from studies in Asia, South America, and Africa respectively. Resistance to fluroquinolones in *Campylobacter* and *Salmonella* are considered high priority on the WHO's list of priority pathogens for research and development of new antimicrobials [30]. Fluoroquinolones are the drug of choice in treating severe campylobacteriosis and other infections by Gram-negative pathogens [39], hence, the frequencies of resistance seen to this pathogen is worrying. In addition, we observed that the frequency of resistance (M50) to quinolones were highest among *Campylobacter* spp. in Asia (M50: 98.1%, IQR: 95–100%, $n = 770$) and South America (M50: 93.8%, IQR: 85.24–96.05%, $n = 76$) whereas it was 41.9% (IQR: 22.8–65.5%, $n = 378$) in studies from Africa.

3.2.7. Tetracyclines

Tetracyclines are veterinary critically important antimicrobials and highly important in human medicine [24,25]. They exhibit broad-spectrum activity against Gram-negative and Gram-positive bacteria. In this study, M50 was highest in *Campylobacter* spp. among the Gram-negative bacteria (above 70%). In Asia, it was 78.2% (IQR: 55.35–98.43%, $n = 1433$) for *Campylobacter* spp. Higher M50 to tetracycline in *Campylobacter* spp. from poultry were observed in Brazil (93.75%, $n = 15/16$) and the Philippines (94.2%, $n = 194/207$) [22,40]. In South Africa, calculated M50 for tetracycline resistance by Campylobacter spp. poultry production systems were 99.45% (IQR: 79.6–100%, $n = 199$) [41]. Among the Gram-positive bacteria, *Staphylococcus* spp., M50 was above 45%, ranging from 47.7% (IQR: 7.2–100%, $n = 171$) from South America to 70% (IQR: 40.5–98%, $n = 1744$) in Asia. This drug is used as alternatives to fluoroquinolones in treatment of campylobacteriosis [29,42]. Tetracycline resistance is associated with clinical failures of tetracycline to prevent or control Campylobacter abortion storms in sheep [42,43].

3.3. One health comparison

We computed MAR from livestock isolates from a total of 14 of the same countries in which Reverter and colleagues [17] computed MAR values for aquaculture pathogens and human clinical pathogens. Comparison is possible because we used the same metric (MAR) and approach. However, the comparison is limited by the fact that aquaculture-relevant bacteria such as *Aeromonas* and *Vibrio* species are not those examined in livestock. However, our livestock isolates overlap

Table 1
Bacterial MAR Indexes from food animals (poultry, cattle, pigs), humans (clinic) and aquaculture bacteria.

country	human (clinic)	poultry	cattle	pigs	aquaculture
Nigeria	0.77	0.39	0.11	0.34	0.42
Zambia	0.74	0.57			0.56
China	0.53	0.44	0.29	0.45	0.33
India	0.60	0.29	0.29		0.36
Jordan	0.61		0.24		0.30
Vietnam	0.61	0.35	0.23	0.30	0.30
Tunisia	0.31	0.53	0.26		0.53
Philippines	0.44	0.78		0.40	0.12
Thailand	0.40	0.46		0.54	0.17
Turkey	0.42	0.32			0.29
Ecuador	0.49	0.66		0.21	0.12
Uganda	0.60	0.18	0.11	0.18	0.08
South Africa	0.22	0.46	0.47	0.41	0.19
Malaysia	0.22	0.70			0.27

better with those that cause clinical human infection. As there are aquaculture and human-related data from fewer countries than we are able to retrieve livestock data in this study, this comparison is only made for fewer countries than what was covered in the systematic review of this study.

When we compared MAR values for the 14 countries, we observed that the weighted MAR indexes from clinical isolates were above 0.6 in seven countries – Nigeria, Zambia, India, China, Jordan, Uganda and Vietnam. In these countries (except Uganda), weighted MAR indices for aquaculture all exceeded 0.3. In contrast, with the exception of Tunisia, for all the countries with weighted clinical MAR below 0.5, aquaculture MAR indices were much lower (Table 1). Poultry and pig MAR indices were similar to those from aquaculture in high clinical MAR index countries (except Uganda), but higher in lower MAR clinical index countries. With the exception of South Africa, cattle MAR indices were lower across the board but still exceeded the MDR threshold in every country except Nigeria. Thus, livestock MAR indices were worryingly high but their magnitude was associated with human clinical MAR indices in the way that aquaculture MAR indices have previously been reported to be [17]. We note that work in the UK has found different bacterial lineages circulating in livestock than in humans [44,45] however the evolution and spread of mobile carbapenamase and colistin resistance genes [46,47], suggest otherwise. A recent meta-analysis of studies in Africa appears to suggest that whether or not resistance genes are transmitted across bacterial host animal species, barrier likely varies with different genes [48]. Thus, genomic surveillance studies in the settings we evaluated are strongly needed to understand how resistance in the livestock sector might impact antimicrobial resistance in the clinic [49].

4. Conclusions

Quantification of AMR in the absence of an effective surveillance system is often very difficult. However, in the absence of an effective surveillance system in LMICs, we have tried to use systematic point surveillance to elucidate the rising AMR in food animals in developing countries. This snapshot, given the limitations of data representation and aggregation, may not portray a perfect picture of the AMR problem in African, Middle Eastern, Asian and South American countries, however, we see that antimicrobial resistance to clinically relevant antimicrobials is rife. Our findings clearly show that bacterial AMR in LMIC food-animal sectors is worrying and therefore deserves closer attention. It also demonstrates that resistance in one subsector is unlikely to be representative of all of them. Once selected, resistance can spread rapidly, and although at lower prevalence of resistance (M50) to some last line antimicrobials in human medicine such as carbapenems and glycopeptides were determined in bacteria species from food-animals in LMICs, closer monitoring of these resistance phenotypes including a review of the OIE's important antimicrobials in veterinary services to reflect these antimicrobials are recommended.

In view of the fallout of AMR, it is pertinent that LMICs replicate efforts by developed countries such as those in the European Union and US which established data generation from food animal surveillance programs to guide policy towards judicious use of antimicrobials. Regulation of antimicrobials and/or complete removal is necessary to curb resistance development and spread.

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Ethical approval

Not Applicable.

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Author's contributions

Odion. O. Ikhimiukor: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Iruka N. Okeke**: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

All data used in this systematic review are presented as Supplementary information.

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