nature portfolio

Peer Review File

A global atlas and drivers of antimicrobial resistance in Salmonella during 1900-2023

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This manuscript on providing a global atlas and drivers of antimicrobial resistance in Salmonella is a significant topic and a great place to start on the battle of mitigating AMR as it relates to food safety, climate change, and One Health. The authors presented an approach to address the gaps of AMR patterns globally by using publicly available genomic data of Salmonella isolates from 148 countries/regions between 1900 and 2023. The approach follows the current trends of genomic data mining to look at the big pictures and generate new research directions. The authors provided a lot of data that would serve as great conversation started toward addressing AMR concerns. However, the data was presented in a way that it was hard to follow along, the figures and tables captions did not do a great job at summarizing the results for a quick snapshot of what the authors were trying to convey. There are a lot of great, insightful, and innovative information to take awake from this report, but it is hard to sort out the way it was presented. The authors did not present a firm conclusion, so that the readers can grasp and learn from the report. The tables and figures were great, just needed clearer legends. Overall, a great report, just not broken down for the readers to understand.

Reviewer #2

(Remarks to the Author)
Line 95 – gram-negative instead of Gram-negative;
Line 99 – is 4.75% a high prevalence? The sentence should be rewritten;
Line 287 - I didn't exactly understand this sentence because it talks about acquired genes, however, the authors mention chromosomal genes such as gyrA, parC…
Line 486 – 489 This would be a statement based on what facts?
Line 548 – Salmonella (italic);
Line 622-633 - I would like to know what the authors considered "food". I think it would be better to specify what food is, as foods of animal origin, such as beef, poultry, and pork, were considered animals or foods. Were the animal samples collected from carcasses, or were they fecal samples?
635 – in silico (italic); Salmonella (italic);

General comments:
- What was the number of sequences considered to be considered positive? What value of sequence quality was considered?

- it would be better to use the term "genomes" instead of “isolates” since these are genomes downloaded from a database, not isolates.

- In general, I consider that the results are poorly discussed. The authors should further explore why some serovars exhibit some resistance profiles. Example:
Explore more:
-Serorar x ARG;
- the source of isolate x ARG;
- ST x ARG.
Is there a correlation between these factors?

- I wonder why fluoroquinolones are losing their effectiveness so much. I would like the authors to discuss what led to this absurd increase in the resistance profile. Will it come from human and veterinary medicine? The same for the other genes relating to beta-lactams and phosphomycin. This subject needs to be further clarified.

Reviewer #3

(Remarks to the Author)
The authors present an analysis of global long term trends in AMR using freely available genomic data. They also use social and economic indicators to better understand the drivers of AMR. The approach is straight forward but the analysis presented are not particularly novel. Many papers have attempted to summarize the global AMR picture using genomic data with interpretations differing only slightly depending on the pipeline used and the genomes selected. The more original element of the paper is the correlation analysis, but this lacks much depth and may very well have been better off as a separate manuscript. I have attached an annotated manuscript with my comments. Overall I would suggest edits to the following areas:

1.Provide more detail on the correlation methods
2. Reanalyze (or rewrite) fluoroquinolone resistance, understanding that a finding of and FQ gene does not mean that isolate is resistant
3. Double check figures and tables for errors. Provide clarity on use of color and symbols

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This study was used to perform a comprehensive analysis of global AMR patterns from publicly available genomic data of 208,233 Salmonella genomes in 148 countries/regions between 1900 and 2023, to explore driving indicators of AMR. The summary stated that the geographic distribution of AMR varied depending on the location, source, and serovar.

This study had a lot of information to process, and the revised version of the manuscript is a lot clearer and easier to read. The revision was completed efficiently.

The only concern that does not interfere with the publication of the manuscript is the correlation of genomic data analysis to phenotypic expression. Present of genes does not confer virulence. That part of the manuscript was not clearly stated.

Overall great information to share for future research.

Reviewer #3

(Remarks to the Author)
The authors have adequately addressed my concerns.

Point-by-point response to the Reviewers

We thank the Reviewers for their valuable feedback. Below is a point-by-point response to the Reviewers' comments in blue, and a revised manuscript with track changes highlighted, is also attached. We hope that you will find our revised manuscript suitable for publication.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This manuscript on providing a global atlas and drivers of antimicrobial resistance in *Salmonella* is a significant topic and a great place to start on the battle of mitigating AMR as it relates to food safety, climate change, and One Health. The authors presented an approach to address the gaps of AMR patterns globally by using publicly available genomic data of *Salmonella* isolates from 148 countries/regions between 1900 and 2023. The approach follows the current trends of genomic data mining to look at the big pictures and generate new research directions. The authors provided a lot of data that would serve as great conversation started toward addressing AMR concerns. However, the data was presented in a way that it was hard to follow along, the figures and tables captions did not do a great job at summarizing the results for a quick snapshot of what the authors were trying to convey. There are a lot of great, insightful, and innovative information to take awake from this report, but it is hard to sort out the way it was presented. The authors did not present a firm conclusion, so that the readers can grasp and learn from the report. The tables and figures were great, just needed clearer legends. Overall, a great report, just not broken down for the readers to understand.

Response: We express our sincere thanks to the reviewer for the very thoughtful comments and valuable suggestions, which can help us further improve the quality of this work. We are sorry for the confusing use of color and symbols in figures and tables. Therefore, we have removed misleading colors and symbols from all Tables and Supplementary Tables. In addition, we have made the following three-part modifications and added some discussions to make it more readable and friendly for the readers.

- First, we modified the section titles in the Results section for better presentation:
  - Construction of a global *Salmonella* genome dataset
  - Longitudinal AMR trends of *S. enterica* worldwide
  - Temporal dynamics of ARGs
  - Differences in ARGs among different geographic locations, sources, and serovars
  - Differences in plasmid replicons across sampling periods, serovars, locations, and sources
  - Emergence of FRGs in the novel variant of *S.* Typhimurium
  - Association between AMR and several driving indicators

- Second, we modified the figures and table captions (and titles) as well as legends

to better summarize the results for a quick snapshot of what we were trying to present.

- **Table 1. Differences in AMR rates in *Salmonella* across geographic locations, sources, and serovars.**

Comparative analysis of AMR rates in *Salmonella* across different countries, continents, sources, and serovars. Note: 1, Geographic distribution of AMR levels across six continents. 2, Differences in AMR rates among eleven major countries. 3, Differences in AMR rates among different sources. 4, Differences in AMR rates among the top twenty serovars.

- **Table 2. Relationships between the number of ARGs in *Salmonella* and 115 indicators.**

Note: eleven major countries across 6 continents. "+": Positive associations with AMR. "-": Negative associations with AMR.

- **Figure 1. Study design and overview of the global high-quality *Salmonella* genome catalog.**

A, Flowchart of global atlas of AMR in *Salmonella* analysis pipeline. A total of 471,617 publicly available *Salmonella* genomes were downloaded (as of June 17, 2023), after screening by both bioinformatic software (including STSTR, SeqSero2, and MLST) and clear metadata, a total of 208,233 genomes were obtained. A total of 708 serovars and 3,006 sequence types (STs) were detected. B, Distribution of *S. enterica* genomes in six subspecies. C, Distribution of *S. enterica* genomes in six continents. D) Distribution of *S. enterica* genomes in different periods. E, Overview of *S. enterica* genomes from different sources. F, Overview of *S. enterica* genomes from different serogroups. G, Top 20 serovars among the global *Salmonella* genome catalog. H, Top 20 STs among the global *Salmonella* genome catalog. I, A global map showing the geographic distribution of the 208,233 *Salmonella* genomes from 148 countries or regions across six continents. The map was generated using the MapChart (https://www.mapchart.net).

- **Figure 2. Temporal changes of AMR in *Salmonella enterica*.**

A, Temporal trends of AMR rates at the global scale. B, Temporal trends of AMR phenotypes across six continents. C, Temporal trends of AMR rates among nine sources. D, Temporal trends of AMR rates among the twenty predominant *Salmonella* serovars.

- **Figure 3. Temporal dynamics and differences in ARGs among different geographic locations, sources, and serovars.**

A, The distribution of numbers of ARGs per genome among the twenty predominant serovars. B, Temporal trends in the prevalence of ARGs. C, Differences in ARGs among different geographic regions. D, Differences in ARGs among different isolation sources. E, Differences in ARGs among different serovars.

- **Figure 4. Temporal dynamics and differences in plasmid replicons across sampling periods, serovars, locations, and sources.**

A, The distribution of numbers of plasmid replicons per genome among the top 20 serovars. A total of 316,327 plasmid replicons classified into 120 types were identified in the global *Salmonella* catalog. A total of 40 dominant plasmid replicons are shown. B, Differences in plasmid replicons across different periods. C, Differences in plasmid

replicons across different geographic regions. D, Differences in plasmid replicons across different isolation sources. E, Differences in plasmid replicons across different serovars.

■ **Figure 5. Genetic characterization and phylogeny of the global MRDRST genomes.**

A, A global map showing the geographic distribution of these MRDRST strains. B, Phylogenetic analysis and functional annotations of 616 global MRDRST strains. From the inner to outer circles are ST, Country, Continent, Source, Year, FRGs, MCRs, *mph(A)*, NDMGs, and *bla*$_{\text{CTX-M}}$ genes. C, A heatmap showing the differences of AMR in MRDRST strains. Temporal, regional, and host distribution of genes that are resistant to fosfomycin and other important antibiotics (including amphenicol, polymyxin, azithromycin, and 3GCs).

➢ Third, we have refined the conclusion to make it easier for readers to understand.

Overall, we found that the geographic distribution of AMR varied depending on the location, source, and serovar. The proportion of AMR levels increased across six continents, especially in serovars Agona, Dublin, I 1,4,[5],12:i:-, Muenchen, Senftenberg, Mbandaka, mainly from chickens, food, wild animals, and the environment, while decreased in Schwarzengrund and Saintpaul, mainly from cattle, pigs, and turkey. We also found that multidrug resistance *S.* Typhimurium exhibited MRDR was detected as early as in 1992 in the USA, earlier than in India (2001) and China (2006). Notably, the majority of MRDRST clones were found in Asia, followed by Europe, and South America. We identified the presence of FRGs in 76 MRDRST genomes, including seventy-five *fosA3* and one *fosA7*. Among these FRGs positive strains, sixty-six, two, and seventy genomes coexisted with *mcr-1*, *mph(A)*, and *bla*$_{\text{CTX-M}}$ genes. Moreover, we identified that antibiotic consumption, agriculture, climate, urban, health, and socioeconomic factors contribute to the development of AMR, in *Salmonella*. We present a globally high-resolution genetic atlas of *Salmonella* within the concept of "One Health" on the planet and also identify some socioeconomic and environmental factors driving the rise of AMR, which can provide valuable information for understanding the transmission dynamics and evolutionary trajectories of *Salmonella*.

All authors appreciate the reviewers again for these invaluable comments and suggestions.

Reviewer #2 (Remarks to the Author):

Response: We thank the reviewer for the useful comments and valuable suggestions, which can help us further improve the quality of this work.

Line 95 – gram-negative instead of Gram-negative;

Response: This has been corrected as suggested.

Line 99 – is 4.75% a high prevalence? The sentence should be rewritten;

Response: We have rewritten this sentence to make it clearer.

Line 135-136: Monte et al. identified 4.75% (26,165) of 550,780 *Salmonella* genomes carried fosfomycin resistance genes (FRGs) and were distributed in 73 distinct serovars.

Line 287 - I didn't exactly understand this sentence because it talks about acquired genes, however, the authors mention chromosomal genes such as gyrA, parC…

Response: We are sorry for the misleading description and have modified these sentences.

Line 389-426: The number of ARGs per genome was the highest in Asia, followed by Africa and South America (Figure S6A). In Asia, the prevalence of ARGs increased observed in *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{LAP-2}}$, and *bla*$_{\text{OXA-10}}$, *mph(A)*, *qnrS1*, decreased in *catA1* and *bla*$_{\text{CARB-2}}$ (Supplementary Table 9). Chromosomal gene mutations, *gyrA* p.D87N, *gyrA* p.S83Y, and *parC* p.S80I, were also showing an increased trend in Asia. In Africa, the prevalence of ARGs increased in *fosA7*, *qnrB19*, and decreased in *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{TEM-1B}}$ (Supplementary Table 10). Moreover, the prevalence of gene mutations increased were observed in *gyrA* p.D87N, *gyrA* p.D87Y, *gyrA* p.S83F, *gyrA* p.S83Y, *parC* p.S80I, and *parC* p.T57S. In Europe, the prevalence of ARGs increased observed in *bla*$_{\text{CMY-2}}$, *bla*$_{\text{TEM-106}}$, *bla*$_{\text{TEM-135}}$, *fosA4*, *mph(A)*, and *qnrB19*, while decreased in *mcr-1.1* (Supplementary Table 11). However, it was worth noting that, unlike in Asia and Africa, *gyrA* p.D87N, and *parC* p.S80I showed a declining trend in Europe. In North America, the prevalence of ARGs increased were observed in *bla*$_{\text{CTX-M-65}}$, *aph(4)-Ia*, *aac(3)-IV*, *fosA3*, and *qnrB19*, decreased in *tet(G)* and *cmlA1* (Supplementary Table 12). In South America, the prevalence of ARGs increased were observed in *bla*$_{\text{CTX-M-65}}$, *mph(A)*, and *qnrB19*, and decreased in *catA1*, *bla*$_{\text{OXA-2}}$, and *aac(6')-Ib-cr* (Supplementary Table 13). We found an increased trend in *parC* p.T57S and a decrease in *gyrA* p.D87N. In Oceania, the prevalence of ARGs increased were observed in *qnrS1*, *fosA7*, *sul3*, *cmlA1*, *bla*$_{\text{CTX-M-55}}$, *mph(A)*, *bla*$_{\text{CTX-M-9}}$, and *mcr-3.1*, decreased in *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{CTX-M-14}}$, *catA1*, *sul1*, and *sul2* (Supplementary Table 14). In addition, we also observed that gene mutations, *parC* p.T57S, and *gyrA* p.D87N, *gyrA* p.D87Y increased while *acrB* p.R717Q decreased in Oceania.

Line 486 – 489 This would be a statement based on what facts?

Response: This description was based on Table 1. We have modified these phrases to make them clearer. Line 644-646: In addition, Oceania had relatively lower AMR prevalence in, for example, amphenicol, beta-lactam, fosfomycin, and tetracycline compared to other continents.

Line 548 – Salmonella (italic);
Response: This has been corrected as suggested.

Line 622-633 - I would like to know what the authors considered "food". I think it would be better to specify what food is, as foods of animal origin, such as beef, poultry, and pork, were considered animals or foods. Were the animal samples collected from carcasses, or were they fecal samples?
Response: The food here referred to conventional food for human consumption, for example, sandwiches, sausage, cooked meat, and orange juice, and the information labeled directly as food recorded in the NCBI BioSample, as well as food of animal origins such as horse meat, hare meat, snail meat, turkey meat, and pork minced meat. The animal samples were mainly collected from both carcasses and fecal samples.

635 – in silico (italic); Salmonella (italic);
Response: These have been corrected as suggested.

General comments:
- What was the number of sequences considered to be considered positive? What value of sequence quality was considered?
Response: We used thresholds for acquired antimicrobial resistance genes, point mutations associated with antimicrobial resistance, and plasmid replicons, which have been shown to work well in previous studies[10,11]. The acquired antimicrobial resistance genes were identified in all genomes using ResFinder 4.1 with coverage ≥90% and identity ≥95%. *Salmonella*-specific point mutations associated with antimicrobial resistance were screened with the PointFinder database with coverage =80% & identity ≥100%. The plasmid replicons were identified in all genomes using PlasmidFinder v2.0.1 with identity ≥ 80% and coverage ≥ 60%.

- it would be better to use the term "genomes" instead of “isolates” since these are genomes downloaded from a database, not isolates.
Response: Thanks for your suggestions. We have checked the full text and changed "isolates" to "genomes."

- In general, I consider that the results are poorly discussed. The authors should further explore why some serovars exhibit some resistance profiles. Example:
Explore more:
-Serovar x ARG;
- the source of isolate x ARG;
- ST x ARG.
Is there a correlation between these factors?
Response: Thanks for your suggestions. We have added relevant discussion on the relationships between some serovars and specific resistance profiles as well as Supplementary Table 18 to the Discussion section.
Line 728-742: In this work, we found, for example, the *fosA7*-positive *Salmonella*

genomes were mainly ST13 (*S.* Agona), ST15 (*S.* Heidelberg), ST40 (*S.* Derby), *S.* Heidelberg ST15 and *S.* Derby ST40 were mainly isolated from chickens, and pigs, respectively, while *fosA7*-producing *S.* Agona ST13 was isolated from multiple sources, for example, human, pig, chicken, and turkey, etc (Supplementary Table 18). The majority of *fosA3*-positive genomes were ST34 (*S.* I 1,4,[5],12:i:-), ST32 (*S.* Infantis), and ST198 (*S.* Kentucky), which were mainly isolated from humans, chickens, and chickens, respectively. The mobile colistin resistance gene *mcr-1.1* was frequently identified in ST34 (*S.* I 1,4,[5],12:i:- and *S.* Typhimurium) in Asia, which usually exhibited an MDR phenotype[10,22,35]. MCR-1-producing *Salmonella* ST34 links animal foods (for example, pork) to children's community infections[59-61]. The third-generation cephalosporin resistance gene $bla_{\text{CTX-M-65}}$-producing *S.* Infantis ST32 mainly originated from chickens and turkeys in North America and South America. Chromosomal gene mutation *parC* p.S80I mediating fluoroquinolone resistance was mainly identified in ST198 (*S.* Kentucky), ST1 (*S.* Typhi), and ST13 (*S.* Agona) originated from humans.

Reference:

58 Monte, D, Doi Y, Lincopan N. High prevalence and global distribution of fosfomycin resistance genes in *Salmonella* serovars. *Lancet Microbe* 2023, 4(12): e968.

59 Zheng B and Feng Y. MCR-1-producing *Salmonella* Typhimurium ST34 links animal foods to human community infections. *EBioMedicine* 2019; 42: 10-11.

60 Lu X, Zeng M, Xu J, et al. Epidemiologic and genomic insights on *mcr-1*-harbouring *Salmonella* from diarrhoeal outpatients in Shanghai, China, 2006-2016. *EBioMedicine* 2019; 42: 133-144.

61 Luo Q, Wan F, Yu X, et al. MDR *Salmonella enterica* serovar Typhimurium ST34 carrying *mcr-1* isolated from cases of bloodstream and intestinal infection in children in China. *J Antimicrob Chemother* 2020; 5(1): 92-95.

- I wonder why fluoroquinolones are losing their effectiveness so much. I would like the authors to discuss what led to this absurd increase in the resistance profile. Will it come from human and veterinary medicine? The same for the other genes relating to beta-lactams and phosphomycin. This subject needs to be further clarified.

Response: Thanks for your suggestions. We have added relevant discussion on the phenomenon that fluoroquinolones, beta-lactams, and phosphomycin are losing their effectiveness to the Discussion section.

Line 647-661: Between 1991 and 2023, the prevalence increased significantly in fluoroquinolone non-susceptible was observed across six continents, especially in chickens, pigs, cattle, food, wild animals, and humans, which is in line with those from surveillance in the GLASS and has been listed as urgent threat[37]. Globally, 73% of antimicrobials sold are used in food animals for animal protein production[24]. The rapid global expansion of intensive animal production has made a great contribution to the scale-up in demand for animal protein, in the same way that antimicrobials help maintain health and productivity (https://www.fao.org/faostat/en/#home). Notably, significant associations between the increase of ARGs and veterinary antimicrobials

consumption were observed[55]. Excessive use of fluoroquinolones (for example, norfloxacin, ofloxacin, lomefloxacin, and pefloxacin) in animals may have caused much higher resistance rates of these in animals[17]. Similar to previous report[55], chromosomal mutations in *gyrA*, *gyrB*, *parC*, or *parE* were frequently identified in fluoroquinolone resistance isolates. In this work, we found that fluoroquinolone non-susceptible rates were much higher in turkeys, pigs, aquatic animals, chickens, cattle, food, environment, and wild animals than in humans. Previous studies also reported that centralized industrialization for chickens and pigs and global trade for poultry and pork products contribute to the global spread of antimicrobial-resistant *Salmonella*[33,34]. Similar to the fluoroquinolone resistance profile, excessive use of cephalosporins (for example, ceftriaxone) and fosfomycin in animals may result in novel resistance genes and beta-lactam-resistant and fosfomycin-resistant *Enterobacterales* from non-human sources (food animals) to human-associated environment[54]. The previous study reported higher fosfomycin resistance rates in humans than in non-human origins in China[10], here we identified higher fosfomycin resistance rates in chickens, pigs, turkeys, and food than in humans at the global level. The absurd increase in fluoroquinolones, beta-lactam, and fosfomycin resistance may be mainly caused by the excessive use of antimicrobials in veterinary medicine and then spread to food, the environment, and humans.

Line 677-704: In addition to antibiotics, bacteria exposure to non-antibiotic drugs (for example, metals and antibacterial biocides) and plant protection products (such as herbicides) can induce or select for bacteria survival adaptations that result in decreased susceptibility to one or more antibiotics[56,57]. Stricter use of antibiotics in human and veterinary medicine is starting to be a global trend. In order to deal with increasing AMR, many countries/ regions and organizations have made efforts and actions. For example, since 2006, antibiotic growth promoters have been banned in animals by the European Union (Regulation (EC) No. 1831/2003), and recently, the Ministry of Agriculture and Rural Affairs of the People's Republic of China has launched a regulation (No. 194) to prohibit growth-promoting drugs as feed additives after 1 July 2020 (http://www.moa.gov.cn/nybgb/2019/201907/202001/t20200103_6334292.htm). To address AMR threats to humans, animals, plants and environment, a One Health Joint Plan of Action was launched by the Quadripartite – the World Health Organization (WHO), the World Organization for Animal Health (WOAH, founded as OIE), the Food and Agriculture Organization of the United Nations (FAO), and the United Nations Environment Programme (UNEP) in 2022 (https://www.who.int/news/item/17-10-2022-one-health-joint-plan-of-action-launched-to-address-health-threats-to-humans--animals--plants-and-environment).

Reference:

55 Yang Y, Shen Y, Jiang J, et al. Distinct increase in antimicrobial resistance genes among *Escherichia coli* during 50 years of antimicrobial use in livestock production in China. *Nat Food* 2022; 3(3): 197-205.

56 Wales A, Davies R. Co-selection of resistance to antibiotics, biocides and heavy metals, and its relevance to foodborne pathogens. *Antibiotics* 2015; 4(4): 567-604.

57 Blair J, Webber M, Baylay A, et al. Molecular mechanisms of antibiotic resistance. *Nat*

Rev Microbiol 2015; 13(1): 42-51.

Part of Table 1:

Antimicrobial class		Beta-lactam	Fosfomycin	Fluoroquinolone
Source	Aquatic animals (n=2,610)	10.08%	4.79%	79.12%
	Cattle (n=15,153)	22.59%	6.41%	74.24%
	Chicken (n=31,852)	17.65%	10.48%	73.79%
	Environment (n=16,987)	6.59%	6.78%	82.97%
	Food (n=8,780)	12.49%	11.66%	79.86%
	Human (n=91,141)	19.24%	4.52%	50.81%
	Pig (n=15,721)	25.70%	15.79%	79.14%
	Turkey (n=7,731)	28.53%	15.53%	84.65%
	Wild animal (n=3,810)	15.83%	5.35%	66.22%

All authors appreciate the reviewers again for these invaluable comments and suggestions.

Reviewer #3 (Remarks to the Author):

The authors present an analysis of global long term trends in AMR using freely available genomic data. They also use social and economic indicators to better understand the drivers of AMR. The approach is straight forward but the analysis presented are not particularly novel. Many papers have attempted to summarize the global AMR picture using genomic data with interpretations differing only slightly depending on the pipeline used and the genomes selected. The more original element of the paper is the correlation analysis, but this lacks much depth and may very well have been better off as a separate manuscript. I have attached an annotated manuscript with my comments. Overall I would suggest edits to the following areas:

Response: We express our sincere thanks to the reviewer for the very thoughtful comments with great details and valuable suggestions, which can help us further improve the quality of this work.

1. Provide more detail on the correlation methods

Response: Thanks for your suggestions. We have added a detailed description to the method section and added Supplementary Table 17 about indicator dataset available year to year range from 2000 to 2023. *P* values are shown in Table 2.

Line 1000-1003: The linear regression models were used to measure the association between indicators and AMR genes per genome over time, adjusting for 12 representative country-level data available across six continents ranging from 2000 to 2023. Detailed data from year to year are available in Supplementary Table 17.

2. Reanalayze (or rewrite) fluoroquinolone resistance, understanding that a finding of and FQ gene does not mean that isolate is resistant.

Response: Thanks for your suggestions. We have modified these phrases to make them clearer and precise.

In this work, a total of 136,402 fluoroquinolone non-susceptible genomes were identified, including 118,640 genomes that carried chromosomal gene mutations mediating fluoroquinolone resistance. Of these, a total of 6,229 genomes carried both at least one kind of acquired ARG and chromosomal gene mutations mediating fluoroquinolone resistance. Therefore, we no longer use “fluoroquinolone resistance”, but used “fluoroquinolone non-susceptible” instead in the revised manuscript.

3. Double check figures and tables for errors. Provide clarity on use of color and symbols.

Response: We thank the reviewer for pointing this out. We are sorry for the confusing use of color and symbols in figures and tables. We have removed misleading colors and symbols from all Tables and Supplementary Tables.

Response to specific comments in the annotated manuscript

1. Line 29: “representative indicator”: I have not heard of *Salmonella* referred as an indicator. I would change this phrasing. Indicators are usually easier to detect and

typically non pathogenic.
Response: Thanks for your suggestions. We have changed “representative indicator” to “notorious bacteria”.

2. line 60-61: “The global burden posed by *S. enterica* is partly posed by the emergence and spread of antimicrobial resistance (AMR)”: I don't understand this phrasing.
Response: We are sorry for the misleading description and have modified the sentence.
Line 80-81: “The emergence and spread of antimicrobial-resistant *S. enterica* have increased the global burden posed by *S. enterica*”.

3. line 64: “serve” Do you mean severe?
Response: We have changed “serve” to “severe”.

4. line 70-72: “*S. enterica* is regarded as a reservoir for AMR genes and therefore considered as a representative indicator of antimicrobial-resistant foodborne pathogens”: This is innacurate. True, *Salmonella* carry AMR genes, but they are not ferred to as reservoirs, nor indicators. I would suggest that you remove these phrases.
Response: Thanks for your suggestions. We have deleted this sentence.

5. line 87-88: “AMR animals”: do you mean AMR bacteria from animals?
Response: We have changed “AMR animals” to “AMR bacteria from animals”.

6. line 88-90: “these results provide guidance for policymakers in target interventions in the areas most influenced on the rise of AMR point prevalence surveys.” This phrasing doen't make sense.
Response: We have deleted this sentence.

7. line 96: “fosfomycin”: Could more context be added here. Fosfomycin resistant *Salmonella* is not on the WHO BPPL that the authors reference. Fosfomycin has, however, moved up on the WHO List of Medically Important Antimicrobials to a high priority critically important antimicrobial because of evidence of emergence and dissemination of plasmid mediated fos resistance genes in food producing animals, and limited therapies for treating CRE infections. Perhaps the authors can include some of this and reference this list.
Response: Thanks for your suggestions. We have added relevant phrases to the Introduction section and cited the reference.
Line 112-116: “moved up to the highest priority critically important antimicrobials on the 2024 WHO list of medically important antimicrobials, because of evidence of the emergence and dissemination of plasmid-mediated fosfomycin resistance genes in food-producing animals, and limited therapies for treating carbapenem-resistant *Enterobacteriaceae* infections[19,54].”
Reference
54: World Health Organization. WHO's list of medically important antimicrobials: a risk management tool for mitigating antimicrobial resistance due to non-human use. Geneva. 2024.

8. line 116: “density positive” had
Response: We have changed “density positive” to “density had positive”.

9. line 130-133: “However, the associations between several socioeconomic, climate, environmental, financial sector, infrastructure, health, poverty, trade, and anthropogenic indicators and country-level AMR in global *Salmonella* have not been fully revealed.”: In an earlier sentence you say that several of these factors showed positive associations with AMR bacteria. Perhaps you should clarify in the earlier section that those associations were assessed on a global scale.
Response: Thanks for your suggestions. This has been modified as suggested.
Line 148-156: In addition to the bidirectional significant associations of antimicrobial consumption and AMR between humans and animals, several socioeconomic factors, environmental indicators, health-related indicators, and cattle density had positive associations with AMR on a global scale specifically in WHO critical priority (carbapenem-resistant *Acinetobacter baumanii*, 3GCs-resistant *Escherichia coli*, 3GCs-resistant *Klebsiella pneumoniae*, carbapenem-resistant *Pseudomonas aeruginosa*) and high priority (oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*) pathogens, while better infrastructure and better governance indicators were associated with lower AMR[26,27].
Line 166-169: However, the associations between socioeconomic, climate, environmental, financial sector, infrastructure, health, poverty, trade, and anthropogenic indicators and country-level AMR in global *Salmonella* have not been assessed on a global scale.

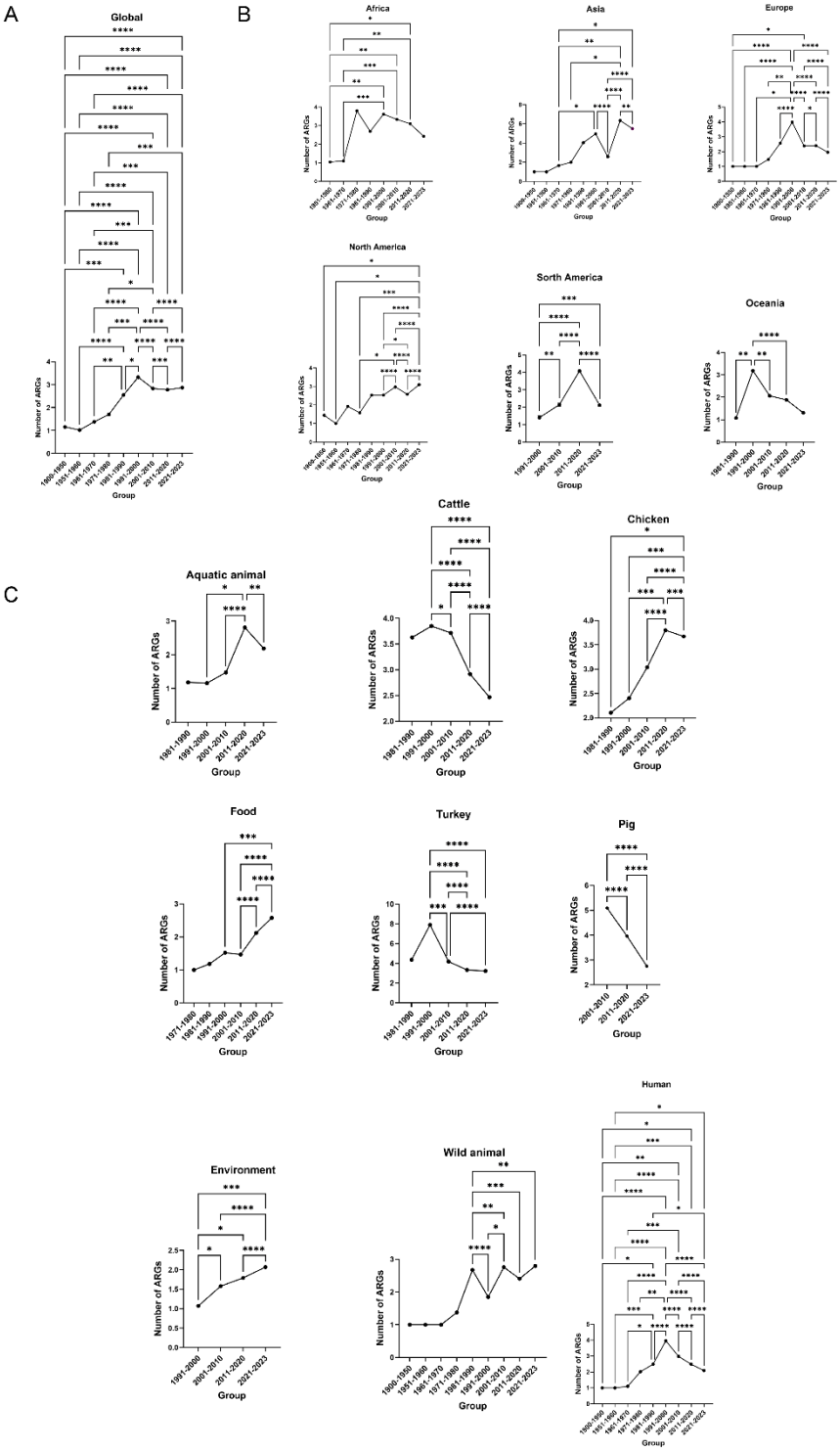
10. line 168-169: “For each drug, we estimated the prevalence of resistance (R%) for all antimicrobial classes based on whole-genome sequencing (WGS) prediction.”:
You have to be careful here. Your fluoroquinolone resistance prediction is artificially high. You cannot call something resistant to fluoroquinolones if it has just one QRDR mutation or PMQR gene. Usually multiple mutations or additional PMDR genes are needed for clinical fluoroquinolone resistance. These isolates most likely have decreased susceptibility to fluoroquinolones, but not full resistance.
Response: We agree with the comments and have modified these phrases to make them clearer and precise. We have changed “resistant to fluoroquinolones” to “fluoroquinolones non-susceptible”.
Line 217-219: The prevalences of tetracyclines resistance and fluoroquinolones non-susceptible were high (R% > 25%) across all tested *Salmonella* genomes at the global scale.

11. line 175 “(Table 1)”: Double check your histograms in this table. For instance, the bar for China disinfectant resistance looks larger than 10.41%. It looks as though you are setting the bars as a proportion of the highest value in each section of each column. If so, I would not recommend that approach as you are pointing readers to make

comparisons both down the column (e.g, comparing between countries) and across the columns (comparing across antimicrobial classes). The histograms should reflect absolute values. Also you are missing labels for source.
Response: Thanks for your suggestions. We are sorry for the confusing use of color and histograms in Table 1. We have removed misleading colors and histograms from Table 1 and kept the proportions and numbers of genomes.

12. line 190: “different sources”: several of the categories mentioned in this paragrph (cat, duck, egg, etc.) do not appear in the supplementary tables. Also the methods say that your focus was on 9 source categories. Why were these presented differently. Furthermore, many of these percentages are undoubtedly influenced by the number of isolates from those sources that are represented in the database database. It would help to put the total number of isolates (from each continent, country, source, serotype) in each row.
Response: We thank the reviewer for pointing this out. In this study, we mainly focused on all *Salmonella* genomes with clear metadata information combined with quality control by screening STSTR, SeqSero2, and MLST. As for isolation source analysis, we mainly focused on 9 source categories, including chicken, pigs, cattle, turkey, aquatic animals, food, wild animals, humans, and the environment.
To keep the consistency of the presented (focus on 9 main sources) in the manuscript, therefore, we removed the contents outside these 9 categories and added the total number of genomes from each continent, country, source, and serovar in each row to Table 1.

13. line 267: “(Figure S5A),”: It is not clear what the colors are or asterisk are for.
Response: We are sorry that the color of "Supplementary Figure 5A" is confusing and misleading. We have unified the colors into black and added the description marked with *, representing statistically significant differences.



Supplementary Figure 5. The distribution of numbers of ARGs per genome in different sampling periods at a global scale (A), six continents (B), and nine sources. The "*" on the right represents *P* values. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. ****: $P < 0.0001$. No significant differences were not shown.

14. line 268: "Figure 5B": Supplemental

Response: We have changed "Figure 5B" to "Supplementary Figure 5B".

15. line 271: “5C”: Supplemental
Response: We have changed “Figure 5C” to “Supplementary Figure 5C”.

16. line 306-307: “For example, blaVIM-1 was detected in Europe, blaVIM-2 in Oceania, blaKPC-2 in Asia and South America, cfr in Asia and Europe, sul4 in Asia”: Some of these (if not all) are not visible on Fig 3C. You can only tell by looking at the percentages in the supplementary tables and comparing across all tables.
Response: We thank the reviewer for pointing this out. We have added Supplementary Table 15 (Frequency of ARGs and chromosomal gene mutations mediating AMR across continents).

17. line 310: “tet(X6) in”: This is not on the heatmap in Fig 3.
Response: We thank the reviewer for pointing this out. We have added Supplementary Table 16 (Frequency of ARGs and chromosomal gene mutations mediating AMR across nine source categories).

18. line 322: “number of plasmids”: This may not necessarily be an increase in the number of plasmids..only the number of replicons. There are many instances when one plasmid can contain replicons from more than one plasmid type. An increase could be indicative of more plasmid recombination.
Response: We agree with the comments and have modified these phrases to make them clearer and precise. We have modified these phrases, and changed “plasmids” to “replicons” or “plasmid replicons”.

19. line 346: “MRDRST”: Did you use biomarkers to identify these S Typhimurirum as MRDR? If so, please indicate that in the paper and add to the methods.
Response: Yes, we use the reference sequence (a point mutation (−44G>T) in the csgD gene promoter, upregulation of the csgDEFG operons) obtained from the previous study[22], which first reported the “MRDRST” by our colleagues and published in Nature Communications on 20 July 2024. We have added this to the method section (Line 924-926).
Reference:
22 Xiang Y, Zhu K, Min K, *et al.* A novel variant of *Salmonella enterica* serovar Typhimurium with a rough colony morphology and multidrug resistance: A microbiological and genomic epidemiology study. *Nat Commun* 2024; 15(1): 6123.

20. line 359: “4,524”: Are these all the S typhimurium strains from China or a selection. If this is a selection, how did you pick?
Response: As for the 4,524 genomes from China, including 2,312 genomes downloaded from the NCBI and 2,212 genomes reported in the previous study by our colleagues[22]. Among these 208,233 genomes publicly available, a total of 2,312 genomes were serotyped into both *S.* Typhimurium and S. I 1,4,[5],12:i:- from China.

The 4,524 genomes are not a selection, but the largest number of both *S*. Typhimurium and S. I 1,4,[5],12:i:- genomes publicly available we could use.

Reference:

22 Xiang Y, Zhu K, Min K, *et al.* A novel variant of *Salmonella enterica* serovar Typhimurium with a rough colony morphology and multidrug resistance: A microbiological and genomic epidemiology study. *Nat Commun* 2024; 15(1): 6123.

21. line 370-372: "Our findings provide evidence to guide future fosfomycin-resistant MRDRST epidemic prevention and treatment in Asia. "This should be in the discussion section.

Response: We have removed this to the discussion section in line 773-774.

22. line 375-379: "Taken together, our data provide more insight into the global trends in the increasing co-existing prevalence of the FRGs and mcr-1, blaCTX-M, or mph(A) in novel variants of S. Typhimurium and suggest that prevalence varies widely between different regions, animals, and countries, which needs immediate worldwide attention and to be investigated, especially through enhanced genomic surveillance." : This should be in the discussion.

Response: We have removed this to the discussion section in line 779-784.

23. line 473-475: "Our choice of indicators was also in line with other global/regional surveillance actions, such as the European Union surveillance that uses different *Salmonella* serovars as the proxy": Please consider changing. *Salmonella* are not indicators.

Response: Thanks for your suggestions. We have changed "indicators" to "notable community pathogen".

24. line 633-634: "chicken, pigs, cattle, turkey, and aquatic animals, food, wild animals, humans, and the environment.": Does this mean other sources were discarded or were all 253,872 lumped into one of these 9 categories.

Response: We are sorry for this misleading description and changed the phrases to make it clearer.

A total of 253,872 genomes were discarded because no clear background information on the collection time, isolation source, and sampling sites was simultaneously recorded.

We have moved to Line 919-921: For AMR levels among different sources, we mainly focused on the following categories: chicken, pigs, cattle, turkey, aquatic animals, food, wild animals, humans, and the environment.

25. line 638-639: "Only the prediction result matching between the SeqSero2 and SISTR is used for downstream analysis.": Curious as to why the authors looked for matches between two serotyping pipeline (SISTR and SeqSero2), but only used one AMR gene pipeline.

Response: First, we considered a high-quality *Salmonella* genome that could be

obtained serovar and ST, using both SISTR, SeqSero2, and MLST, according to previous reports[10,11]. Therefore, we used both SISTR and SeqSero2 for serotyping. Notably, 53.31% of 6,035 genomes recorded in the Chinese local *Salmonella* genome database (https://nmdc.cn/clsgdbv2/) missed the serovar or recorded a wrong serovar in the NCBI. Using both SISTR and SeqSero2 for serotyping could meet the criteria for high-quality genome selection.
Second, we mainly focused on acquired AMR genes and (*Salmonella*-specific) chromosomal gene mutations mediating AMR, we used ResFinder 4.1 to identify these marker sequences, which have been shown to perform well in previous studies[10,11].

Reference:

10 Wang YN, Liu Y, Lyu N, *et al.* The temporal dynamics of antimicrobial-resistant *Salmonella enterica* and predominant serovars in China. *Natl Sci Rev* 2023; **10:** nwac269.

11 Wang YN, Xu XB, Zhu BL, *et al.* Genomic analysis of almost 8,000 *Salmonella* genomes reveals drivers and landscape of antimicrobial resistance in China. *Microbiol Spectr* 2023; **11:** e0208023.

26. Line 738: “Regression correlation analysis”: Please provide more details here. What kind of correlation? What was the p value? Was it two tailed? etc? Was analysis done using decade specific datasets or year to year?
Response: Thanks for your suggestions. We have added a detailed description to the method section and added Supplementary Table 17 about indicator dataset available year to year range from 2000 to 2023. *P* values are shown in Table 2.
Line 1000-1003: The linear regression models were used to measure the association between indicators and AMR genes per genome over time, adjusting for 12 representative country-level data available across six continents ranging from 2000 to 2023. Detailed data from year to year are available in Supplementary Table 19.

All authors appreciate the reviewers again for these invaluable comments and suggestions.
We will be happy to edit the text further, based on helpful comments from the reviewers.

Point-by-point response to the Reviewers

We thank the Reviewers for their positive feedback. Below is a point-by-point response to the Reviewers' comments in blue.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

This study was used to perform a comprehensive analysis of global AMR patterns from publicly available genomic data of 208,233 *Salmonella* genomes in 148 countries/regions between 1900 and 2023, to explore driving indicators of AMR. The summary stated that the geographic distribution of AMR varied depending on the location, source, and serovar.

This study had a lot of information to process, and the revised version of the manuscript is a lot clearer and easier to read. The revision was completed efficiently.

The only concern that does not interfere with the publication of the manuscript is the correlation of genomic data analysis to phenotypic expression. Present of genes does not confer virulence. That part of the manuscript was not clearly stated.

Overall great information to share for future research.

Response: Thank you for your time and efforts in handling our manuscript! We have carefully revised our manuscript according to your previous comments. Your insightful comments have greatly improved and strengthened our work.

We agree with the reviewer that the presence of genes may not confer virulence, related work is ongoing on the correlation of genomic data analysis to phenotypic expression by our team, and we hope to share these findings in the next paper. We really appreciate your comments!

Reviewer #3 (Remarks to the Author):

The authors have adequately addressed my concerns.

Response: Thank you for your time and efforts in handling our manuscript! Your previous comments have greatly improved our work.