

Original article

Exploratory Screening of Antimicrobial Inhibitory Activity in Human Urine Samples from Regular Poultry Consumers in Al-Bayda, Libya: A Pilot Study

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Antimicrobial use in food-animal production raises concerns regarding potential human exposure to antimicrobial substances and antimicrobial-resistance-related effects through the food chain. However, evidence from human biological samples remains limited in Libya. This pilot study evaluated antimicrobial inhibitory activity in urine samples collected from 15 volunteers in Al-Bayda, Libya, who reported regular poultry consumption. Urine samples were screened for antimicrobial inhibitory activity using a microbiological disc-diffusion bioassay with *Bacillus subtilis* as the indicator organism. In a separate antimicrobial-response procedure, human-derived *B. subtilis* isolates were evaluated against ciprofloxacin, gentamicin, vancomycin, tetracycline, and trimethoprim. The retained laboratory dataset classified participant-antimicrobial observations according to the original experimental classification system as positive, resistance-associated, or negative. Positive responses were observed in 11/15 observations (73.3%) for vancomycin, 9/15 (60.0%) for gentamicin, 7/15 (46.7%) for ciprofloxacin, and 4/15 (26.7%) for tetracycline. Resistance-associated responses were recorded in 6/15 observations (40.0%) for ciprofloxacin and 3/15 (20.0%) for gentamicin, whereas all 15 observations (100%) were classified as resistance-associated for trimethoprim. Exploratory regression analysis found no statistically significant association between participant age (18–65 years) and antimicrobial-response classification for any agent for which model estimation was possible. These findings should be interpreted as preliminary microbiological screening signals and assay-specific phenotypic responses rather than as evidence of clinically confirmed antimicrobial resistance in the participants or proof of dietary transmission from poultry. No molecular characterization of resistance determinants or compound-specific confirmatory analyses was performed. Larger One Health studies incorporating detailed medication and dietary histories, paired or otherwise source-linked food and human sampling, standardized antimicrobial susceptibility testing, and confirmatory analytical methods are warranted.

Keywords: Antimicrobial Inhibitory Activity; Urine Bioassay; *Bacillus Subtilis*; Poultry Consumption; One Health.**Introduction**

Antimicrobial use in food-animal production represents an important One Health concern because its consequences extend beyond animal treatment to food safety, environmental health, and human health. Inappropriate or excessive antimicrobial use can promote the selection and persistence of antimicrobial-resistant bacteria, whereas failure to comply with recommended withdrawal periods may result in antimicrobial residues persisting in edible animal tissues (1-3). Resistant bacteria, antimicrobial residues, and resistance determinants may subsequently enter food and environmental pathways, creating potential routes of human exposure across interconnected animal–food–environment interfaces (4, 5). Poultry production is particularly relevant to this interface because antimicrobials are used for therapeutic and preventive purposes in many production systems, while poultry meat represents an important and widely consumed source of animal protein (1, 6).

When antimicrobial administration occurs without adequate veterinary oversight or compliance with recommended withdrawal periods, residues may persist in edible tissues. In parallel, antimicrobial selection pressure during production may favour the emergence and persistence of resistant bacterial populations that can potentially reach consumers through food handling, preparation, or consumption (7-9). Together, these pathways support a One Health perspective linking animal production, food safety, environmental exposure, and human health.

Interpretation of antimicrobial-related findings in human biological samples, however, requires particular caution. Detection of antimicrobial inhibitory activity does not, by itself, identify a specific antimicrobial compound, establish its source, or demonstrate clinically confirmed antimicrobial resistance in the individual tested. Likewise, similarity between antimicrobial-response patterns observed in food-animal and human samples cannot establish transmission without source-linked bacterial isolation, standardized susceptibility testing, and molecular or genomic comparison of comparable isolates. Microbiological screening assays may therefore serve as useful preliminary tools for detecting signals that warrant further investigation, but their findings should be regarded as hypothesis-generating rather than confirmatory.

Published evidence on antimicrobial residues and antimicrobial resistance within the Libyan poultry sector remains limited and geographically fragmented. Available studies indicate important gaps in antimicrobial-use practices, poultry biosecurity, residue surveillance, and integrated assessment across animal, food, environmental, and human-exposure interfaces. In Al-Jabal Al-Akhdar, previous investigations have identified weaknesses in poultry biosecurity and disease-control practices that may contribute to increased disease pressure and potentially greater reliance on antimicrobial use (10). Nevertheless, evidence concerning antimicrobial inhibitory activity in human biological samples within this setting remains particularly scarce.

Against this background, the present pilot study focused on antimicrobial inhibitory activity in urine samples collected from regular poultry consumers in Al-Bayda, Libya. The study aimed to describe antimicrobial inhibitory activity, characterize the distribution of antimicrobial-related laboratory responses, and explore whether participant age was associated with the observed response classifications. It was not designed to identify the source of the detected inhibitory activity or to demonstrate poultry-to-human transmission. Rather, it was intended to generate preliminary local evidence to inform larger One Health investigations incorporating confirmatory analytical and molecular approaches.

Materials and Methods

Study Design and Setting

This exploratory pilot study was conducted in Al-Bayda, Libya, to investigate antimicrobial inhibitory activity and antimicrobial-response patterns among regular poultry consumers. The study was designed to explore whether antimicrobial inhibitory activity and resistance-associated microbiological response patterns could be detected among regular poultry consumers and to characterize their distribution across the antimicrobial agents evaluated. Participant age was additionally examined as an exploratory covariate.

The study was not designed to estimate population-level prevalence or to establish direct poultry-to-human transmission of antimicrobial compounds, resistant bacteria, or resistance determinants.

Ethical Approval

All procedures involving human participants were conducted in accordance with the ethical requirements of the Committee for Biological Safety and Ethics (MCBB). The study protocol was reviewed and approved by the Research Ethics Committee of Omar Al-Mukhtar University, Al-Bayda, Libya (approval reference: NBC: 007.A.24.28). Informed consent was obtained from all participants prior to sample collection, and participant information and laboratory findings were handled confidentially throughout the study.

Study Participants and Urine Sample Collection

A total of 15 urine samples were collected from adult male and female volunteers aged 18–65 years who reported regular consumption of poultry meat. Sample collection was conducted in Al-Bayda, Libya, between August 2024 and January 2025.

Urine specimens were collected aseptically into sterile containers and transported to the microbiology laboratory for analysis. The urine samples were used for antimicrobial-inhibitory bioassay testing using *Bacillus subtilis* BGA as the reference indicator organism. Participants constituted an exploratory convenience sample; therefore, the study was not designed to provide population-representative estimates of antimicrobial exposure or antimicrobial resistance in Al-Bayda.

Bacillus subtilis Reference Indicator Strain

Bacillus subtilis BGA was used as the reference indicator organism for the antimicrobial-residue screening bioassay. The reference strain was not isolated from the tested samples. Instead, the bacterial culture was prepared and inoculated onto the appropriate agar medium, after which the prepared test samples were applied to the inoculated plates. Antimicrobial activity was assessed based on the presence of inhibition zones around the samples.

Preparation of the *Bacillus subtilis* Spore Suspension

For microbiological screening of antimicrobial inhibitory activity in urine samples, *Bacillus subtilis* BGA was used as a reference indicator organism. The *B. subtilis* BGA preparation was not isolated from the urine specimens; rather, it was a reference bacterial preparation inoculated onto the culture plates to serve as the indicator organism for detecting antimicrobial inhibitory activity. The organism was cultured on nutrient agar supplemented with 0.1% KH_2PO_4 at pH 7.2. Following incubation at 30°C for 10 days, bacterial growth was harvested in sterile saline and centrifuged at 3000 rpm for 10 min. The resulting pellet was resuspended in sterile saline, and the washing procedure was repeated twice. The suspension was subsequently heated at 70°C for 30 min to inactivate vegetative cells. The resulting spore suspension was adjusted to approximately 10^7 spores/mL, verified using the pour-plate method, and stored at 4°C until use (11, 12).

Urine Bioassay for Antimicrobial Inhibitory Activity

Human urine samples were screened for antimicrobial inhibitory activity using a microbiological disc-diffusion bioassay with *Bacillus subtilis* BGA as the indicator organism. The assay was based on the general principles of previously described microbiological screening procedures (11, 12). Sterile 6-mm discs were aseptically placed on freshly prepared agar plates inoculated with the *B. subtilis* BGA indicator preparation. A 5-μL aliquot of each urine sample was applied to each disc in duplicate. Plates were incubated for 24 h at 35°C, after which inhibition zones surrounding the discs were evaluated. An inhibition zone extending ≥ 2 mm beyond the edge of the 6-mm disc was considered a positive screening response according to the criterion applied in the original study protocol (13).

The bioassay was used as an exploratory screening procedure for antimicrobial inhibitory activity and was not intended to chemically identify or quantify individual antimicrobial compounds. Accordingly, a positive screening response indicated detectable bacterial growth-inhibitory activity in the urine sample but did not, by itself, identify the responsible compound, determine its source, or establish dietary exposure from poultry.

Phenotypic Antimicrobial-Response Testing

A second laboratory procedure was performed separately to characterize antimicrobial-response patterns of the human-derived *Bacillus subtilis* isolates identified as described under Isolation and Phenotypic Identification of *Bacillus subtilis*. Antimicrobial response was assessed using the Kirby–Bauer disc-diffusion approach. Bacterial inocula were standardized to a turbidity equivalent to a 0.5 McFarland standard and inoculated uniformly onto Mueller–Hinton agar, after which antimicrobial discs were applied aseptically to the agar surface.

Five antimicrobial agents were evaluated: ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), vancomycin (VA, 30 µg), tetracycline (TE, 30 µg), and trimethoprim (TR, 5 µg). The antimicrobial discs were obtained from Liofilchem S.r.l., Roseto degli Abruzzi, Italy. The plates were incubated at 37°C for 24 h, after which inhibition zones were measured and recorded according to the original laboratory classification system. Because validated clinical susceptibility breakpoints were not applicable to all five *B. subtilis*–antimicrobial combinations under the experimental conditions used in this study, the findings were interpreted as resistance-associated phenotypic responses rather than definitive clinical antimicrobial resistance in the participating individuals.

Outcome Classification

This study generated information from two related but distinct microbiological procedures: screening for antimicrobial inhibitory activity in urine and assessment of antimicrobial-response patterns. For each antimicrobial agent, the available dataset retained one laboratory classification per participant as positive, resistance-associated, or negative. These categories were retained for consistency with the original experimental records. A positive classification denoted a positive antimicrobial-inhibitory response, whereas the term resistance-associated referred to the phenotypic response recorded under the antimicrobial-response testing conditions. The resistance-associated category should not be interpreted as evidence that an individual participant was clinically resistant to the corresponding antimicrobial. The available dataset retained the final mutually exclusive laboratory classification assigned to each participant–antimicrobial observation in the original investigation. The underlying raw linkage between the two microbiological procedures was not available for retrospective reclassification; therefore, the original categories were retained without modification.

Statistical Analysis

Because of the exploratory design and the small human sample size ($n = 15$), the primary analysis was descriptive. Categorical laboratory findings were summarized as frequencies and percentages for each of the five antimicrobial agents. Antimicrobial-specific observations were derived from the same group of 15 participants and therefore were not treated as independent population samples. The reported percentages describe the distribution of laboratory response classifications within the study sample and should not be interpreted as population-level prevalence estimates.

As a secondary exploratory analysis, separate multinomial logistic regression models were used to examine the association between participant age and antimicrobial-response classification for each antimicrobial agent. Participant age was entered as a continuous predictor, with the negative category used as the reference outcome. Positive and resistance-associated responses were therefore modelled relative to the negative response category.

Where a specific response category contained no observations, the corresponding outcome contrast could not be estimated because of the absence of variability in that category. Where all observations for an antimicrobial fell within a single category, no regression model could be estimated. Such instances were reported as not estimable rather than being interpreted as evidence of no association. All analyses were conducted using R software (14). Given the small sample size and sparse outcome distribution, regression findings were considered exploratory and interpreted cautiously. No additional inferential comparisons were performed.

Results

Antimicrobial-Related Laboratory Response Patterns

Antimicrobial-related laboratory response patterns varied across the five agents evaluated in this exploratory study. For each antimicrobial, responses were classified as positive, resistance-associated, or negative. Because all antimicrobial-specific observations were derived from the same 15 participants, the percentages presented represent within-sample distributions and should not be interpreted as population-level prevalence estimates.

Table 1. Distribution of antimicrobial-related laboratory response classifications in the exploratory study ($n = 15$).

Antimicrobial	Positive, n (%)	Resistance-associated, n (%)	Negative, n (%)
Ciprofloxacin	7 (46.7)	6 (40.0)	2 (13.3)
Gentamicin	9 (60.0)	3 (20.0)	3 (20.0)
Vancomycin	11 (73.3)	0 (0.0)	4 (26.7)
Tetracycline	4 (26.7)	0 (0.0)	11 (73.3)
Trimethoprim	0 (0.0)	15 (100.0)	0 (0.0)

Note: Positive denotes an antimicrobial-inhibitory response, whereas resistance-associated denotes a phenotypic response recorded under the antimicrobial-response testing conditions. The latter should not be interpreted as confirmed clinical antimicrobial resistance in the participating individuals.

Vancomycin showed the highest proportion of positive responses, with 11 of 15 observations (73.3%), followed by gentamicin with 9 of 15 (60.0%) and ciprofloxacin with 7 of 15 (46.7%). Tetracycline showed the lowest proportion of positive responses, with 4 of 15 observations (26.7%), while 11 of 15 (73.3%) were classified as negative.

Resistance-associated phenotypic responses were recorded for ciprofloxacin in 6 of 15 observations (40.0%) and for gentamicin in 3 of 15 (20.0%). No resistance-associated responses were recorded for vancomycin or tetracycline under the experimental conditions used. In contrast, all 15 observations for trimethoprim were classified as resistance-associated. Because validated clinical susceptibility breakpoints were not applicable to all five *Bacillus subtilis*-antimicrobial combinations under the experimental conditions used in this study, these findings are reported as assay-specific phenotypic antimicrobial-response patterns rather than as confirmed clinical antimicrobial resistance. In particular, the uniform resistance-associated classification for trimethoprim should not be interpreted as evidence that all participants were clinically resistant to trimethoprim.

Association Between Participant Age and Antimicrobial-Related Response

To explore whether participant age was associated with the recorded antimicrobial-related responses, separate exploratory multinomial logistic regression models were fitted for each antimicrobial agent. Participant age was treated as a continuous predictor, with the negative response category used as the reference outcome. Positive and resistance-associated responses were therefore modelled relative to the negative category. Because of the small sample size and sparse distribution of some response categories, not all outcome contrasts could be estimated. For antimicrobial agents with no observations in a particular response category, the corresponding contrast was reported as not estimable. For trimethoprim, all 15 observations fell within the resistance-associated category, resulting in complete absence of outcome variability and preventing estimation of an age-response association. These non-estimable results reflect the structure of the available data and should not be interpreted as evidence of an absence of association.

Table 2. Exploratory association between participant age and antimicrobial-related response classification using multinomial logistic regression (n = 15).

Antimicrobial	Outcome modelled (vs. negative)	OR	p-value
Ciprofloxacin	Positive	1.20	0.13
	Resistance-associated	1.20	0.14
Gentamicin	Positive	1.00	0.66
	Resistance-associated	1.00	0.77
Tetracycline	Positive	0.98	0.69
	Resistance-associated	NE ¹	—
Vancomycin	Positive	0.94	0.19
	Resistance-associated	NE ¹	—
Trimethoprim	Model not estimable	NE ²	—

Note: OR represents the estimated change in the odds of the indicated response category relative to the negative category for each one-year increase in participant age. **NE, not estimable.**

¹ No resistance-associated observations were recorded for the antimicrobial.

² All 15 observations were classified as resistance-associated; therefore, there was no outcome variability and no negative reference category from which an age effect could be estimated.

For ciprofloxacin, participant age was not significantly associated with either a positive response (OR = 1.20, P = 0.13) or a resistance-associated response (OR = 1.20, P = 0.14), relative to the negative response category. Similarly, no statistically significant association with age was observed for gentamicin for either a positive response (OR = 1.00, P = 0.66) or a resistance-associated response (OR = 1.00, P = 0.77). For tetracycline, age was not significantly associated with a positive response (OR = 0.98, P = 0.69); the resistance-associated contrast was not estimable because no observations occurred in that category. For vancomycin, age was likewise not significantly associated with a positive response (OR = 0.94, P = 0.19), while the resistance-associated contrast was not estimable because no resistance-associated observations were recorded. For trimethoprim, regression analysis was not estimable because all 15 observations were classified as resistance-associated, resulting in an absence of outcome variability and no negative reference category.

Overall, no statistically significant association between participant age and antimicrobial-related response classification was detected for any of the estimable comparisons (all P > 0.05). These findings should be interpreted cautiously because of the small sample size and sparse distribution of several response categories. The absence of a statistically significant association does not exclude a potentially meaningful age-related effect, while non-estimable results reflect the structure of the available data rather than evidence of no association.

Discussion

The most notable finding in this exploratory study was the uniform resistance-associated response recorded for trimethoprim, with all 15 observations falling within this category. This finding requires cautious interpretation because antimicrobial inhibitory activity in urine and antimicrobial-response patterns were assessed using separate microbiological

procedures. The resistance-associated classification therefore represents a phenotypic laboratory response observed under the antimicrobial-response testing conditions and should not be interpreted as evidence of clinically confirmed trimethoprim resistance in the participants or as evidence of poultry-to-human transmission.

An additional methodological consideration is important when interpreting the response classifications. As described under Outcome Classification, the available dataset retained mutually exclusive laboratory classifications of positive, resistance-associated, or negative, although these classifications originated from two related but distinct microbiological procedures. The positive category represented an antimicrobial-inhibitory response detected in the urine bioassay, whereas the resistance-associated category represented a phenotypic response recorded during antimicrobial-response testing. Because the underlying raw linkage between the two procedures was not available for retrospective reclassification, these categories should not be interpreted as a single biologically ordered outcome measured by one analytical procedure. Consequently, comparisons among antimicrobial agents primarily describe the distribution of the recorded laboratory classifications within the study sample rather than directly comparable measures of antimicrobial susceptibility or resistance.

Trimethoprim inhibits bacterial dihydrofolate reductase and thereby interferes with folate metabolism and downstream nucleotide synthesis (15, 16). Resistance to trimethoprim may arise through alterations in the target enzyme or acquisition of alternative or less-sensitive dihydrofolate reductase determinants (17). However, no molecular characterization of resistance determinants was undertaken in the present study. The uniform resistance-associated classification observed for trimethoprim therefore cannot be attributed to a specific molecular resistance mechanism. Other explanations related to the tested isolates, experimental conditions, or interpretation of inhibition zones also remain possible and cannot be distinguished from acquired resistance on the basis of the available data.

The composition of the susceptibility-testing medium provides an additional consideration for trimethoprim. The antimicrobial activity of trimethoprim can be influenced by thymidine availability in the test medium, because exogenous thymidine can reduce the dependence of bacterial growth on folate-mediated thymidylate synthesis and thereby alter the apparent antimicrobial response (18). Experimental work has demonstrated that the activity of trimethoprim may vary according to the thymidine content of commercially prepared media (18), while subsequent studies have shown that the thymidine content of Mueller–Hinton agar can affect susceptibility testing of organisms with relevant metabolic characteristics (19, 20). Mueller–Hinton agar was used in the present study; however, the thymidine content of the specific medium lot was not independently documented in the available study record. Accordingly, a medium-related effect cannot be excluded retrospectively, although the present data do not establish that it contributed to the uniform trimethoprim pattern. Future studies should incorporate appropriate medium quality-control procedures and, where trimethoprim susceptibility is specifically investigated, conditions that minimize the potential influence of thymidine.

The remaining antimicrobial agents showed heterogeneous laboratory-response patterns. Positive responses were most frequently recorded for vancomycin, followed by gentamicin and ciprofloxacin, whereas tetracycline showed a lower frequency of positive responses. Resistance-associated classifications were recorded for ciprofloxacin and gentamicin but not for vancomycin or tetracycline under the experimental conditions used. These differences indicate heterogeneity in the observed assay-specific phenotypic responses; however, they should not be interpreted as definitive estimates of clinical susceptibility or resistance. The interpretation of inhibition zones was constrained by the applicability of susceptibility criteria to the specific *Bacillus*–antimicrobial combinations tested in this exploratory study. The results are therefore best considered assay-specific laboratory observations rather than clinical susceptibility profiles of the human-derived isolates or the participating individuals.

The exploratory age analysis did not identify a statistically significant association between participant age and any estimable antimicrobial-response comparison. For tetracycline and vancomycin, the resistance-associated contrasts were not estimable because no observations occurred in those categories. For trimethoprim, all 15 observations were classified as resistance-associated, resulting in complete absence of outcome variability and no negative reference category from which an age effect could be estimated. These situations are structurally different from a conventional model-fitting problem: where a category contains no observations, the corresponding contrast is undefined, whereas a constant outcome provides no variation from which an association can be estimated. For future studies with larger samples and sparse but non-degenerate outcome distributions, approaches designed to address separation and small-sample bias, including penalized likelihood methods, may be considered (21, 22). In the present study, however, the small sample size and sparse distribution of several response categories mean that the age analysis should remain exploratory and that non-significant findings should not be interpreted as evidence that age has no influence on antimicrobial-response patterns. The detection of antimicrobial inhibitory activity in urine indicates the presence of substances capable of suppressing bacterial growth, but microbiological screening alone cannot identify the responsible antimicrobial compound, quantify its concentration, or establish its source. Microbiological urine bioassays have nevertheless been used as screening approaches for antimicrobial activity in clinical specimens. Traub, Büchler (23) demonstrated the capacity of a *Bacillus subtilis* indicator system to detect selected chemotherapeutic agents in clinical specimens, while more recent work has highlighted the potential value of urine bioassays for assessing recent antimicrobial exposure and the influence of the antimicrobial tested, indicator organism, and timing of exposure (24). In the present study, the urine assay should therefore be regarded as a screening signal rather than a compound-specific measurement or a direct measure of dietary exposure. Poultry consumption represents a biologically plausible exposure context because antimicrobial residues and antimicrobial-resistant bacteria associated with food-animal production may reach humans through food-chain pathways

(7-9). Antimicrobial residues in foods of animal origin have also been associated with potential human-health concerns, including hypersensitivity reactions and effects on the intestinal microbiota (25).

Nevertheless, the present study cannot establish poultry consumption as the source of the observed human findings. Information on recent therapeutic antimicrobial use, dosage and timing of exposure, quantity and frequency of poultry consumption, and other dietary or environmental exposures was not available. Furthermore, paired or otherwise source-linked poultry–human sampling was not undertaken. Consequently, neither the antimicrobial inhibitory activity detected in urine nor the resistance-associated laboratory classifications can be attributed specifically to poultry exposure or interpreted as evidence of poultry-to-human transmission of resistant bacteria or resistance determinants.

A source–exposure relationship would require a source-linked One Health design incorporating comparable poultry, human, and environmental sampling, reliable identification of recovered isolates, standardized antimicrobial susceptibility testing, and molecular or genomic characterization of resistance determinants (8). Detailed antimicrobial-use and dietary histories would also be necessary to evaluate alternative exposure pathways. Where the objective is to establish the presence of a specific antimicrobial compound or quantify exposure, microbiological screening should be complemented by quantitative analytical methods such as liquid chromatography–tandem mass spectrometry (LC–MS/MS). Such approaches would allow the biological screening signal to be linked to a specific compound and concentration rather than inferred from inhibitory activity alone.

Within these limitations, the present study provides preliminary local observations from Al-Bayda that may help inform the design of larger One Health investigations in Libya. Its principal contribution is not to establish clinical antimicrobial resistance or a poultry-to-human transmission pathway, but to document antimicrobial-inhibitory activity and assay-specific antimicrobial-response patterns in a small exploratory human sample and to identify methodological issues that should be addressed in subsequent studies. Future investigations should separate urine antimicrobial screening from isolate susceptibility outcomes throughout the analytical workflow, apply validated and organism-appropriate susceptibility criteria, strengthen quality control of culture media and susceptibility testing, confirm bacterial identification using appropriate molecular methods where species-level attribution is required, and incorporate source-linked sampling when transmission or exposure attribution is the primary research question. The findings should therefore be interpreted as hypothesis-generating rather than confirmatory evidence of antimicrobial exposure or resistance transmission.

Study Limitations

Several limitations should be considered when interpreting these findings. First, the study included only 15 participants and was designed as an exploratory pilot assessment rather than a population-representative study. Consequently, the observed frequencies should not be interpreted as prevalence estimates for the population of Al-Bayda.

Second, the study did not include a comparison group of individuals who were not regular poultry consumers. Information on recent therapeutic antimicrobial use, dosage, timing of the most recent antimicrobial exposure, and the quantity or frequency of poultry consumption was also unavailable. These limitations restrict the ability to attribute the observed antimicrobial inhibitory activity or resistance-associated response patterns specifically to poultry consumption.

Third, the microbiological urine bioassay provided evidence of antimicrobial inhibitory activity but did not chemically identify or quantify the compounds responsible for that activity. Other antimicrobial exposures, metabolites, dietary sources, or nonspecific inhibitory substances may therefore have contributed to the observed responses.

Fourth, although antimicrobial-response testing was performed as a separate microbiological procedure, no molecular characterization of resistance determinants was undertaken. In addition, paired poultry and human samples were not collected from the same exposure units. Consequently, the study cannot establish direct poultry-to-human transmission of resistant bacteria or resistance determinants, nor can it establish poultry as the source of the antimicrobial inhibitory activity detected in the urine samples.

Fifth, the exploratory regression analysis of participant age was limited by the small sample size and sparse distribution of several response categories. For tetracycline and vancomycin, no resistance-associated observations were recorded, preventing estimation of the corresponding age–response contrasts. For trimethoprim, all 15 observations were classified as resistance-associated, resulting in an absence of outcome variability and no negative reference category from which an age effect could be estimated. Furthermore, each model examined age as the only predictor and did not adjust for other potentially relevant participant-level factors. Accordingly, the available data do not allow meaningful exclusion of a potentially important age-related association, even where formal model estimation was possible.

For these reasons, the findings should be regarded as hypothesis-generating rather than confirmatory. Their principal value lies in identifying preliminary microbiological signals that warrant further investigation through larger, controlled, and source-linked One Health studies.

Conclusion and Recommendations

This exploratory study identified antimicrobial inhibitory activity and heterogeneous antimicrobial-response patterns among urine samples collected from regular poultry consumers in Al-Bayda, Libya. Responses varied across the five antimicrobial agents evaluated, while trimethoprim showed a uniform resistance-associated response pattern across all 15 observations. Given the small sample size and the methodological characteristics of the microbiological assays, this finding should be interpreted as an assay-specific phenotypic pattern rather than as evidence of universal clinical trimethoprim resistance among the participants. Exploratory regression analysis found no statistically significant

association between participant age and antimicrobial-response classification where model estimation was possible; however, the small sample size and sparse response categories preclude firm conclusions regarding age-related effects. Although poultry consumption represents a biologically plausible dietary exposure context, the present study cannot determine the source of the detected antimicrobial inhibitory activity or establish poultry-to-human transmission of antimicrobial compounds, resistant bacteria, or resistance determinants. The findings should therefore be regarded as hypothesis-generating and as preliminary local evidence supporting further investigation of antimicrobial exposure and resistance-associated phenotypic responses at the food–human interface in Libya.

Future studies should include larger and more representative participant samples, an appropriate comparison group, and detailed documentation of recent therapeutic antimicrobial use and dietary exposure. Paired or otherwise source-linked poultry and human sampling should be incorporated when source attribution or transmission is specifically investigated. Quantitative analytical methods such as liquid chromatography–tandem mass spectrometry (LC–MS/MS) should be used to confirm and identify specific antimicrobial compounds and, where appropriate, quantify exposure. Standardized antimicrobial susceptibility testing, together with molecular or genomic characterization, should be incorporated when investigating resistant bacteria or resistance determinants.

The findings also highlight the value of strengthening integrated One Health research and surveillance linking animal production, food-safety laboratories, veterinary services, environmental monitoring, and the public-health sector. Such an approach would provide a stronger evidence base for understanding antimicrobial exposure and resistance pathways within the Libyan context and for developing appropriately targeted surveillance and antimicrobial-stewardship strategies.

Conflict of interest. Nil

References

- Landers TF, Cohen B, Wittum TE, Larson EL. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Reports*. 2012;127(1):4-22.
- Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*. 2015;112(18):5649-54.
- Mohammadzadeh M, Montaseri M, Hosseinzadeh S, Majlesi M, Berizi E, Zare M, et al. Antibiotic residues in poultry tissues in Iran: A systematic review and meta-analysis. *Environmental Research*. 2022 2022/03/01;204:112038.
- Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules (Basel, Switzerland)*. 2018;23(4):795.
- McEwen SA, Collignon PJ. Antimicrobial Resistance: a One Health Perspective. *Microbiology Spectrum*. 2018 Mar;6(2). PubMed PMID: 29600770.
- Marangoni F, Corsello G, Cricelli C, Ferrara N, Ghiselli A, Lucchin L, et al. Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. *Food & Nutrition Research*. 2015;59:27606.
- Shankar S, Bp B, Prabhu M, Bh BH, Chandan C, S S, et al. Rapid Methods for detection of Veterinary Drug residues in Meat. *Veterinary World*. 2010 01/01;3(5):241–6.
- Muloi D, Ward MJ, Pedersen AB, Fèvre EM, Woolhouse MEJ, van Bunnik BAD. Are Food Animals Responsible for Transfer of Antimicrobial-Resistant *Escherichia coli* or Their Resistance Determinants to Human Populations? A Systematic Review. *Foodborne pathogens and disease*. 2018 Aug;15(8):467-74. PubMed PMID: 29708778.
- Samtiya M, Matthews KR, Dhewa T, Puniya AK. Antimicrobial Resistance in the Food Chain: Trends, Mechanisms, Pathways, and Possible Regulation Strategies. *Foods*. 2022;11(19):2966. PubMed PMID: doi:10.3390/foods11192966.
- Ismaael NA, Hussain A, Said Gaidan OK, El Tigani Mohamed T, Somiya AF. Evaluation of Biosecurity Practices in Broiler Poultry Farms in Algabal Ala- khdar Region, Libya. *Animal Health Research Journal*. 2018 03/01;6:1-11.
- Okerman L, Croubels S, De Baere S, Hoof JV, De Backer P, De Brabander H. Inhibition tests for detection and presumptive identification of tetracyclines, beta-lactam antibiotics and quinolones in poultry meat. *Food Additives & Contaminants*. 2001 2001/05/01;18(5):385-93.
- Ferrini AM, Mannoni V, Aureli P. Combined Plate Microbial Assay (CPMA): A 6-plate-method for simultaneous first- and second-level screening of antibacterial residues in meat. *Food Additives & Contaminants*. 2006 2006/01/01;23(1):16-24.
- Heitzman RJ. *Veterinary Drug Residues: Residues in Food-Producing Animals and Their Products: Reference Materials and Methods*. 2 ed. Heitzman RJ, editor. Oxford, UK: Blackwell Scientific Publications; 1994.
- R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2024.
- Quinlivan EP, McPartlin J, Weir DG, Scott J. Mechanism of the antimicrobial drug trimethoprim revisited. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology*. 2000 Dec;14(15):2519-24.
- Minato Y, Dawadi S, Kordus SL, Sivanandam A, Aldrich CC, Baughn AD. Mutual potentiation drives synergy between trimethoprim and sulfamethoxazole. *Nature Communications*. 2018 2018/03/08;9(1):1003.
- Fernández-Villa D, Aguilar MR, Rojo L. Folic Acid Antagonists: Antimicrobial and Immunomodulating Mechanisms and Applications. *International Journal of Molecular Sciences*. 2019;20(20):4996.
- Koch AE, Burchall JJ. Reversal of the antimicrobial activity of trimethoprim by thymidine in commercially prepared media. *Applied Microbiology*. 1971 Nov;22(5):812-7.
- Haltiner RC, Migneault PC, Robertson RG. Incidence of thymidine-dependent enterococci detected on Mueller-Hinton agar with low thymidine content. *Antimicrobial agents and chemotherapy*. 1980 Sep;18(3):365-8.
- Bowen AC, Lilliebridge RA, Tong SY, Baird RW, Ward P, McDonald MI, et al. Is *Streptococcus pyogenes* resistant or susceptible to trimethoprim-sulfamethoxazole? *Journal of Clinical Microbiology*. 2012 Dec;50(12):4067-72.
- Firth D. Bias Reduction of Maximum Likelihood Estimates. *Biometrika*. 1993;80(1):27-38.

22. Heinze G, Schemper M. A solution to the problem of separation in logistic regression. *Statistics in Medicine*. 2002 Aug 30;21(16):2409-19.
23. Traub WH, Büchler J, Spohr M. [Detection of chemotherapeutic agents in clinical specimens with *Bacillus subtilis* strain ATCC 6633]. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene 1 Abt Originale A, Medizinische Mikrobiologie, Infektionskrankheiten und Parasitologie = International Journal of Microbiology*. 1982 Oct;253(1):76-80.
24. Skovbjerg S, Brown C, Mataya AA, Msefula J, Bonomali F, Banda F, et al. Urine bioassay optimisation to assess the association between antimicrobial exposure, pneumococcal carriage and antimicrobial resistance among hospitalised children in Malawi. *BMC Infectious Diseases*. 2025 2025/10/13;25(1):1291.
25. Chen J, Ying GG, Deng WJ. Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns. *Journal of agricultural and food chemistry*. 2019 Jul 10;67(27):7569-86.