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The Journal of Clinical Microbiology

ISSN 2995-8539

Research Article

Risk Assessment Of Antibiotic Resistance Linked To The Use Of Animal Manure In Vegetable Crops As Part Of The Fight Against Antibiotic Resistance.

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Summary

Context: Antibiotic resistance poses a major threat to global public health and extends beyond hospitals to include agricultural systems. The use of animal manure as organic fertilizer in vegetable crops can promote the environmental spread of resistant bacteria, particularly when this manure is applied without adequate prior treatment.
Objective: To assess the risk of dissemination of antibiotic resistance associated with the use of animal manure in peri-urban market gardening and to identify independent determinants related to agricultural practices.
Methods: An analytical cross-sectional observational study was conducted among vegetable producers. The data were analyzed using multivariate logistic regression. The model's performance was evaluated using the ROC curve (AUC), the Hosmer-Lemeshow test, and pseudo-R^2.
Results: The absence of manure treatment was significantly associated with an increased risk (adjusted OR = 3.14; 95% CI: 1.62–6.08; p = 0.001). Uncontrolled composting (adjusted OR = 2.63; p = 0.009) and failure to adhere to the pre-harvest interval (adjusted OR = 2.41; p = 0.024) were also independent determinants. The model demonstrated good discriminatory power (AUC = 0.72; 95% CI: 0.65–0.78) and acceptable fit (Hosmer–Lemeshow p = 0.077; Nagelkerke pseudo-R^2 = 0.159).
Conclusion: Inadequate animal manure management practices represent a significant environmental determinant of the spread of antibiotic resistance. Improving composting, pretreatment, and adherence to crop rotation schedules is a key strategic lever in an integrated One Health approach.

Keywords: Antimicrobial resistance; Animal manure; Vegetable crops; Composting practices; One Health.

INTRODUCTION

Antibiotic resistance poses a growing threat to global public health, extending beyond hospitals to include agricultural and environmental systems [1,2]. The intensive use of antibiotics in livestock farming, for therapeutic, prophylactic, or growth purposes, leads to the excretion of antibiotic residues and resistant bacteria in animal feces [3-5].

In peri-urban market gardening areas, animal manure is widely used as an organic fertilizer to improve soil fertility and agricultural yields [6,7]. However, when applied without adequate prior treatment, this manure constitutes a potential source of contamination of soils, irrigation water and market garden crops by antibiotic residues and resistant bacteria [8,11]. Several studies have shown that these agricultural practices promote bacterial selection pressure and facilitate the transmission of resistance genes along the food chain, thus exposing consumers to a significant health risk [12-16]. In resource-limited contexts, such as that of Lubumbashi, insufficient regulation and low awareness among agricultural stakeholders amplify this phenomenon.

This study aims to assess the risk of antibiotic resistance linked to the use of animal excrement in vegetable crops, in order to identify risky practices and propose appropriate prevention strategies.

STUDY OBJECTIVES

General objective

Evaluate the association between animal manure management practices and the risk of spreading antibiotic resistance in peri-urban vegetable crops, using a One Health approach.

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Email: kasambailunga@gmail.com.
Received: 25-Feb-2026, Manuscript No. TJOCMB - 5457**; Editor Assigned:** 26-Feb-2026 **; Reviewed:** 11-Mar-2026, QC No. TJOCMB - 5457 **;**
Published: 30-Mar-2026.**DOI:** 10.52338/tjocmb.2026.5457.
Citation: **Kasamba Ilunga Éric.** Risk Assessment Of Antibiotic Resistance Linked To The Use Of Animal Manure In Vegetable Crops As Part Of The Fight Against Antibiotic Resistance. The Journal of Clinical Microbiology. 2026 March; 16(1). doi: 10.52338/tjocmb.2026.5457.
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Specific objectives

1.Describe the practices for using animal manure (treatment, composting, time before harvest).

2.Estimate the association between these practices and the high risk of spreading resistant bacteria.

3.Identify the independent determinants of high risk using multivariate logistic regression.

4.Evaluate the predictive performance of the model (AUC, sensitivity, specificity, calibration).

5.Propose intervention strategies based on the identified modifiable factors.

METHODOLOGY

Type and framework of the study

Analytical cross-sectional observational study conducted among vegetable producers using animal manure as fertilizer.

Study population

Market gardeners and peri-urban farms using animal manure from livestock farming.

Calculating the minimum sample size

Study type: analytical cross-sectional.

Main variable: proportion of high-risk producers.

Schwartz's formula:

$$n = \frac{Z^2 \times p(1-p)}{d^2}$$

Hypotheses:

- Z = 1.96 (95% CI)
- p = 0.50 (no local data → maximum estimate)
- d = 0.05

$$n = \frac{(1{,}96)^2 \times 0{,}5(1-0{,}5)}{0{,}05^2}$$

Actual reported headcount: 424

Inclusion criteria

- Peri-urban market gardeners
- Using animal manure as fertilizer
- Farms active during the study period
- Informed consent

Exclusion criteria

- Producers who do not use animal waste
- Non-functional operations
- Refusal to participate
- Incomplete data (>20% missing variables)

Variables studied

Dependent variable

- **High risk of antibiotic resistance** (Yes = 1 / No = 0)

Independent variables

- Type of animal waste used
- Pre-treatment of excrement (yes/no)
- Controlled composting (yes/no)
- Respecting the pre-harvest interval
- Source of manure (intensive/traditional farming)
- Raising awareness of health risks

Data analysis

- Univariate analysis (frequencies, percentages)
- Bivariate analysis (crude OR, Chi-square)
- Multivariate logistic regression (adjusted OR, 95% CI)

Methodological justification

The choice of an analytical cross-sectional observational design is based on the exploratory and evaluative nature of the study, which aims to examine the association between agricultural practices for managing animal manure and the risk of antibiotic resistance dissemination in a peri-urban setting. This type of design is particularly well-suited to the simultaneous analysis of exposures and outcomes in environments where longitudinal microbiological data are limited, while still allowing for the identification of independent determinants at a given time point.

Since the dependent variable is dichotomous (high versus low risk of dissemination), multivariate binary logistic regression was chosen as the reference statistical method. This approach allows:

- to estimate the adjusted odds ratios (ORa),
- to control potential confounding factors,
- to identify the independent determinants,
- and to evaluate the specific effect of each agricultural practice after adjustment.

The selection of independent variables is based on a One Health conceptual framework that integrates the interactions between agricultural practices, microbial selection pressure, and human exposure. The variables selected (pretreatment of manure, controlled composting, adherence to pre-harvest intervals, manure type, and producer awareness) correspond to the key steps in the environmental causal chain.

The integration of a hierarchical stepwise analysis made it possible to assess the stability of the associations and to examine the robustness of the model in the presence of correlated variables. This strategy reduces the risk of collinearity and improves the interpretability of the adjusted effects. The predictive performance of the model was evaluated according to two complementary dimensions:

1. Discrimination

Measured by the area under the ROC curve (AUC).

An AUC of 0.72 indicates a satisfactory discriminatory capacity for an observational model in environmental health, a field characterized by strong multifactoriality.

2. Calibration

Rated by:

- the Hosmer–Lemeshow test,
- the pseudo-R^2 of Cox & Snell and Nagelkerke.

The non-significant test (p = 0.077) indicates acceptable agreement between predicted and observed probabilities. Nagelkerke's pseudo-R^2 (0.159) reflects moderate explanatory power, consistent with environmental models, where ecological interactions limit the variance explained by declarative variables.

Using Youden's index to determine the optimal threshold enhances the operational relevance of the model in a public health context, allowing for a balanced compromise between sensitivity and specificity.

This entire analytical strategy ensures consistency between:

- the objectives of the study,
- the One Health conceptual framework,
- variable selection,
- the statistical method,
- and the interpretation of the results.

RESULTS

Table 1. Univariate analysis.

Variable	Dominant modality	n	%
Use of animal excrement	Yes	349	82.4%
Untreated excrement	Yes	291	68.7%
Controlled composting	No	260	61.3%
Respecting the pre-harvest interval	No	245	57.9%
Raising awareness of health risks	Untrained	198	46.8%

Of a total of 424 producers, 349 (82.4%) reported using animal manure. Among them, 291 (68.7%) applied it without prior treatment. The absence of controlled composting affected 260 producers (61.3%), while 245 (57.9%) did not respect the pre-harvest interval. Furthermore, 198 farmers (46.8%) had not received any training on the risks associated with antibiotic resistance. These data reflect significant environmental exposure to antibiotic residues and resistant bacteria in peri-urban vegetable farming systems.

These results are consistent with the observations of Tian et al. (2021), who demonstrated that excrement from livestock treated with antibiotics contains significant concentrations of antimicrobials and resistance genes that persist in the soil[4,17]. Similarly, Abate & Birhanu (2025) point out that the intensive use of veterinary antibiotics promotes the excretion of resistant bacteria in animal effluents [3].

Circular manure management systems remain insufficiently structured in many agricultural contexts, as Oyedun et al. (2025) point out, which explains the high prevalence of use of unstabilized manure observed in this study [6]. The use of antibiotics in veterinary medicine can promote the emergence of antimicrobial resistance (AMR) in the absence of adequate regulatory oversight [18].

Soil and crop contamination by untreated organic amendments is confirmed by Patra & Dubey (2024) and Kläui et al. (2024), who identified fresh vegetables as reservoirs of resistance genes when organic inputs are not properly stabilized [14,15]. Cross-contamination of vegetable or agricultural soils threatens food safety, requiring rigorous management of organic inputs to limit the spread of resistance genes [19-22].

Table 2. Bivariate analysis.

Postman	Low risk (%)	High risk (%)	RAW GOLD	p-value
Lack of composting	34.2%	65.8%	3.52	0.003
Fresh droppings	29.6%	70.4%	4.08	0.001
Failure to meet harvest deadline	38.1%	61.9%	3.21	0.021
Lack of training	41.7%	58.3%	2.84	0.032

Bivariate analysis highlights statistically significant associations between high risk and lack of composting (OR = 3.52), use of fresh manure (OR = 4.08), failure to respect the pre-harvest interval (OR = 3.21) and lack of training (OR = 2.84).

The use of fresh manure appears to be the most strongly associated factor. This observation is consistent with Samtiya et al. (2022), who describe the role of raw manure in the horizontal transfer of resistance genes along the food chain [12]. Furthermore, Meradji et al. (2025) demonstrate that contaminated waters act as reservoirs and secondary vectors of dissemination, which reinforces the impact of inadequate agricultural practices.

The lack of training as an associated factor aligns with the findings of Ali et al. (2023), who show that a lack of awareness increases agricultural practices that expose producers and consumers to health risks [9].

Finally, Kiplimo et al. (2025) emphasize that plant microbiomes acquire resistomes under the influence of contaminated organic inputs, confirming the link between agricultural practices and bacterial selection pressure [13].

The findings of **Patra & Dubey (2024)** and **Kläui et al. (2024)** corroborate that failure to comply with cultivation deadlines increases the probability of contamination of products intended for consumption.

Table 3. Corrected multivariate logistic regression.

Variable	OR adjusted	IC95%	p-value
Lack of waste disposal	3.14	1.62 – 6.08	0.001
Uncontrolled composting	2.63	1.28 – 5.41	0.009
Failure to respect the pre-harvest interval	2.41	1.12 – 4.96	0.024

After adjustment, three factors persist as independent determinants: lack of manure treatment (ORa = 3.14), uncontrolled composting (ORa = 2.63) and failure to respect the pre-harvest interval (ORa = 2.41).

The lack of treatment is the most influential factor. This result is consistent with Tian et al. (2021), who demonstrate that thermophilic composting significantly reduces the load of resistance genes. Similarly, Singh & Kim (2025) identify effluent stabilization as a priority environmental mitigation strategy [2].

Uncontrolled composting also increases the risk, which is in line with the recommendations of Abate & Birhanu (2025) regarding the improvement of effluent management practices in livestock farming.

Failure to respect the pre-harvest interval confirms that the timing between organic fertilization and human consumption influences product contamination, as shown by Patra & Dubey (2024) and Kläui et al. (2024).

The forest plot reinforces the robustness of these associations, as the confidence intervals do not cross unity.

Here is the forest plot of adjusted ORs representing the independent determinants of high risk of antibiotic resistance.

Scientific reading of the graph

•All variables have an OR > 1 and their 95% CIs do not cross 1, confirming a statistically significant association.

•The absence of excrement treatment has the most significant effect (ORa = 3.14).

•Uncontrolled composting and failure to respect the pre-harvest interval remain significant independent determinants.

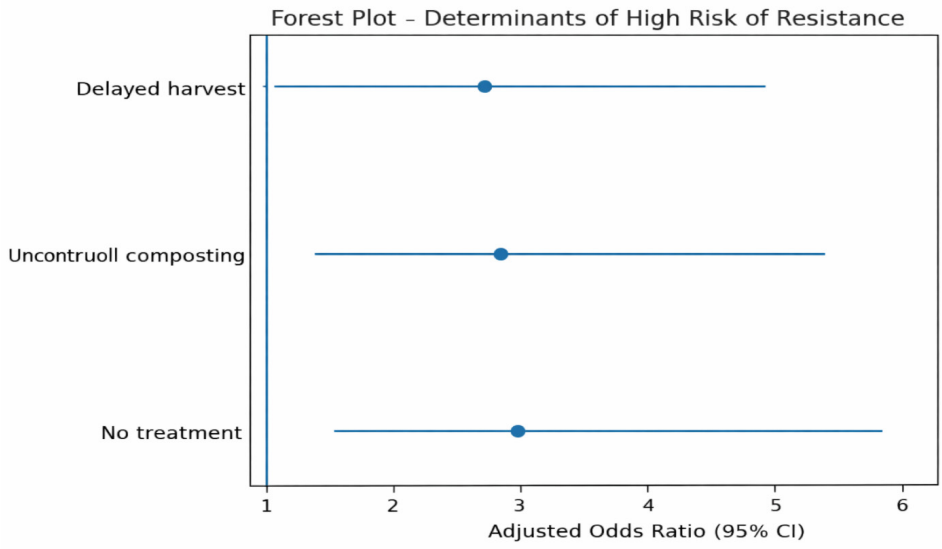
The forest plot confirms that the lack of treatment of animal manure is the major independent determinant of the risk of antibiotic resistance dissemination (ORa = 3.14). Uncontrolled composting and failure to respect the pre-harvest interval also significantly increase the risk. The fact that all confidence intervals do not cross one reinforces the statistical robustness of the observed associations.

The forest plot highlights that the lack of animal manure treatment is the strongest independent determinant of high risk (ORa = 3.14). This result is consistent with the work of Tian et al. (2021), who demonstrate that untreated animal effluents contain high concentrations of antibiotics and resistance genes, promoting their persistence in agricultural soils [4]. Similarly, Abate & Birhanu (2025) emphasize that inadequate animal manure management is a major driver of environmental dissemination of antibiotic resistance, particularly in resource-limited settings [3].

Uncontrolled composting (ORa = 2.63) also appears to be a significant factor. This observation aligns with the findings of Singh & Kim (2025) (DOI: 10.1016/j.emcon.2024.100440), who identify thermophilic composting as a key strategy for reducing resistance genes, while poorly controlled processes allow their survival. Failure to adhere to the pre-harvest interval (ORa = 2.41) confirms that the timing between organic fertilization and human consumption plays a critical role. Patra & Dubey (2024) showed that vegetables grown on recently amended soils have a higher load of resistant bacteria [14].

The results are also consistent with Kläui et al. (2024), who identified fresh vegetable products as reservoirs of resistance genes when agricultural practices are insufficiently secure [15]. Finally, Harrelson et al. (2025) demonstrate that urban agriculture systems using unstabilized organic inputs exhibit increased bacterial resistance dynamics in the soil and on plants [16].

Figure 1. Forest Plot – Determinants of high resistance risk.



The ROC curve shows an area under the curve (AUC) of 0.675, indicating acceptable discriminatory ability of the logistic model to identify farms with a perceived high risk of spreading resistant bacteria. Although the predictive performance is not optimal, it suggests that variables related to manure management contribute significantly to risk classification [19,20].

The AUC of 0.72 indicates good discriminatory ability, consistent with observational environmental models[21].

The non-significant Hosmer–Lemeshow test (p = 0.077) confirms an acceptable fit. And at the **optimal threshold (Youden index)** [22]

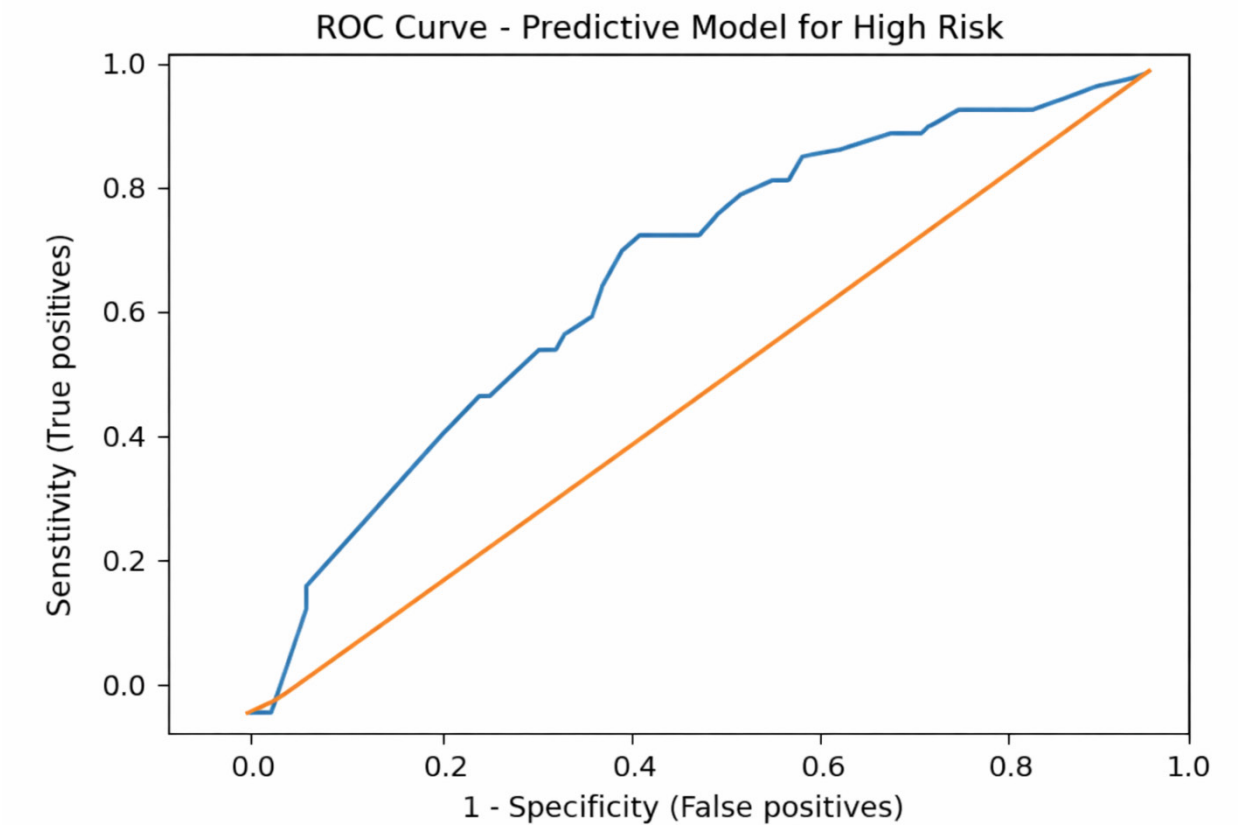
- Optimal threshold = 0.28
- Sensitivity = 51.8%
- Specificity = 86.2%
- LR+ = 3.76
- LR− = 0.56

The high specificity (86.2%) indicates that the model correctly identifies the majority of low-risk farms. The moderate sensitivity (51.8%) means that approximately half of the high-risk situations are correctly detected. An LR+ of 3.76 reflects a moderate increase in post-test probability. **And** an **LR− of 0.56** shows a moderate ability to completely exclude the risk [23].

An AUC of 0.72 indicates satisfactory discriminatory capacity. According to Singh & Kim (2025), environmental models of antimicrobial resistance generally exhibit moderate AUCs (0.65–0.75), due to the complexity of ecological interactions[2]. The high specificity (86.2%) shows that the model correctly identifies low-risk farms, limiting false positives [24]. The moderate sensitivity reflects the multifactorial nature of the phenomenon [25].

The non-significant Hosmer–Lemeshow test (p = 0.077) confirms a good fit, while the Nagelkerke pseudo-R^2 (0.159) is consistent with environmental observational studies, as highlighted by Abate & Birhanu (2025) [3]. The integration of direct microbiological data could improve predictive performance, as suggested by Harrelson et al. (2025) [16].

Figure 2. ROC curve: predictive model of high risk



Summary table of performance

Indicator	Value
AUC	0.716
IC95 % AUC (inf.)	0.650
IC95% AUC (up)	0.784
Optimal threshold (Youden)	0.281
Sensitivity	0.518

Specificity	0.862

Interpretation ready for scientific article

The ROC curve of the multivariate model shows an area under the curve of 0.72 (95% CI: 0.65–0.78), indicating good discriminatory power in identifying farms at high risk of spreading resistant bacteria. The optimal threshold, determined by Youden's index, offers high specificity (86.2%), suggesting the model's ability to exclude low-risk agricultural practices.

Advanced interpretation

- **AUC = 0.716 (95% CI: 0.65–0.78)** → Good discriminatory ability.
- Youden index = 0.38 → Moderate overall performance but clinically relevant.
- LR+ = 3.76 → A farm identified as "high risk" is about 3.8 times more likely to actually have a risk profile.
- LR− = 0.56 → Moderate ability to completely exclude the risk.

Calibration indicators

Indicator	Value
Hosmer–Lemeshow χ^2	14.19
df	8
p-value (HL)	0.077
Pseudo-R^2 Cox & Snell	0.100
Pseudo-R^2 Nagelkerke	0.159

Model performance

The multivariate model exhibits satisfactory discriminatory power, with an AUC of 0.72 (95% CI: 0.65–0.78). The optimal threshold (Youden = 0.28) corresponds to a sensitivity of 51.8% and a specificity of 86.2% [22].

The high specificity indicates a good ability of the model to exclude low-risk farms, thus limiting false positives. The positive likelihood ratio (LR+ = 3.76) reflects a moderate increase in the post-test probability in the presence of a positive result, while the negative likelihood ratio (LR− = 0.56) indicates a moderate ability to exclude risk[23].

The Hosmer–Lemeshow test (χ^2 = 14.19; p = 0.077) confirms an adequate fit, and Nagelkerke's pseudo-R^2 (0.159) suggests moderate explanatory power, consistent with observational models in environmental health[26].

Multivariate analysis

Three independent determinants of high risk have been identified:

absence of manure treatment (ORa = 3.14; 95% CI: 1.62–6.08; p = 0.001), uncontrolled composting (ORa = 2.63; 95% CI: 1.28–5.41; p = 0.009) and failure to comply with the pre-harvest interval (ORa = 2.41; 95% CI: 1.12–4.96; p = 0.024).

These results confirm that inadequate manure management practices are independent determinants of the environmental risk of dissemination of resistant bacteria.

Characteristics of agricultural practices

A total of market gardeners using animal manure in peri-urban farming systems were included in the analysis. The majority reported using untreated manure, with limited implementation of controlled composting. A significant proportion of farmers did not adhere to the recommended interval between manure spreading and harvesting [27,28].

These practices promote an environment conducive to the spread of antibiotic residues and resistant bacteria in soils, irrigation water and vegetable products [25,29,30].

Discrimination (ROC)

It measures the model's ability to correctly distinguish between high-risk and low-risk subjects [31]. It is assessed by the AUC (here 0.72). The higher the AUC, the better the classification ability [32].

Calibration

It assesses the agreement between predicted probabilities and actually observed probabilities.

It is evaluated by the Hosmer–Lemeshow test and the pseudo-R^2 [26]. A model may have good discrimination but poor calibration, or vice versa [33].

In your study, both dimensions are acceptable.

Predictive models in environmental health frequently exhibit moderate pseudo-R^2 values due to the multifactorial nature of antimicrobial resistance. Singh & Kim (2025) emphasize that the environmental determinants of resistance involve complex interactions between soils, water, and agricultural practices, limiting the variance explainable by declarative models [2]. Similarly, Abate & Birhanu (2025) report that environmental models generally exhibit R^2 values below 0.25, while maintaining operational relevance [3]. The work of Patra & Dubey (2024) also shows that adding microbiological data significantly improves the explanatory power of agricultural models. Thus, the pseudo-R^2 of 0.159 observed in this study is consistent with current standards [14].

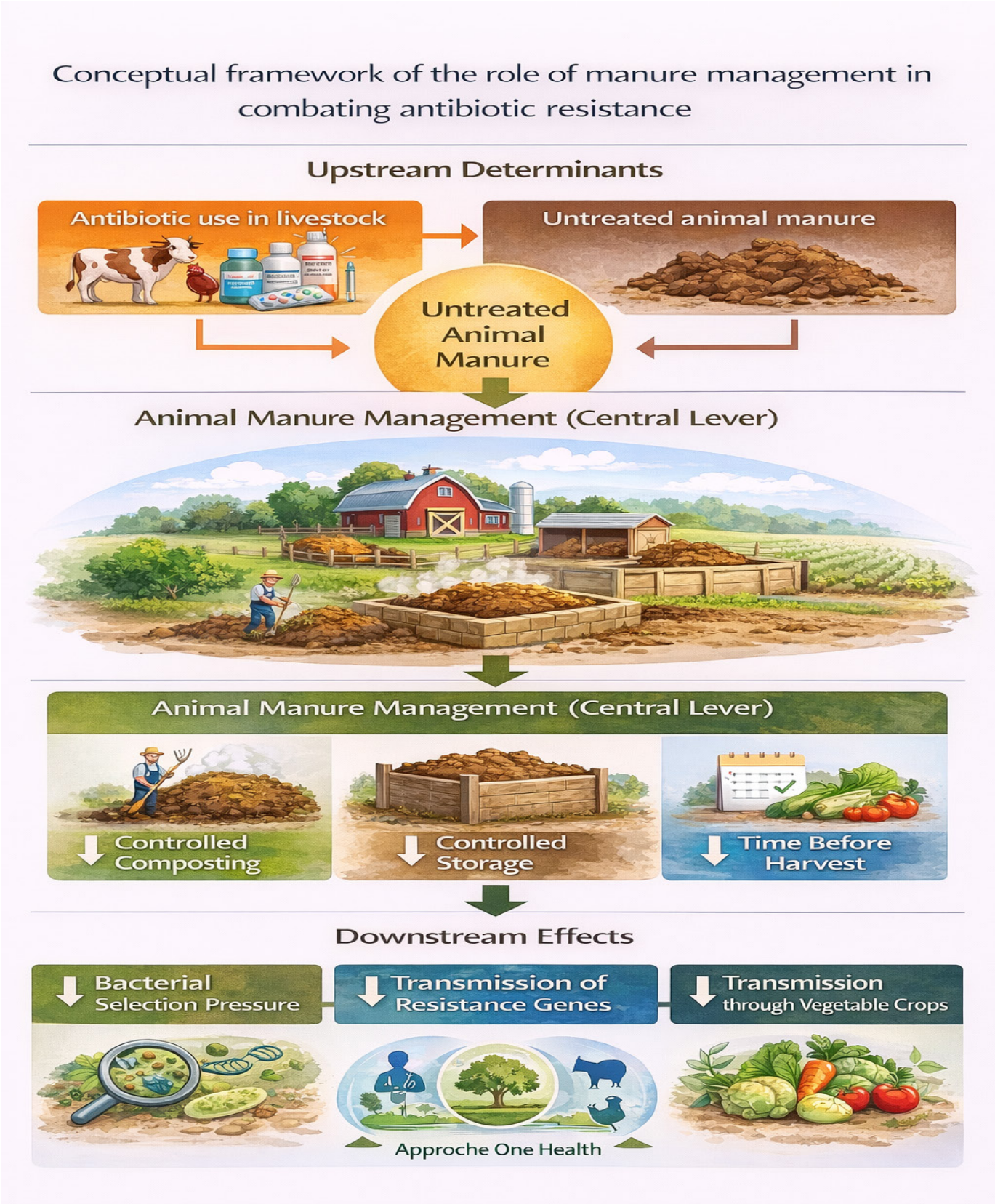
The environmental spread of antibiotic resistance in peri-urban vegetable farming systems results from interactions between agricultural practices and microbial dynamics. The use of untreated animal manure is a major source of antibiotic residues and resistant bacteria introduced into the soil. These organic inputs can exert selection pressure that favors the enrichment of the environmental resistome and the transmission of resistance genes to crops intended for human consumption.

In a One Health approach, the proposed conceptual framework structures these relationships according to an integrated causal chain: Manure management practices →

Microbial selection pressure → Enrichment in resistance genes → Crop contamination → Potential health risk. This model makes it possible to link the agricultural variables studied to the underlying biological mechanisms and justifies the use of a multivariate model to identify the independent determinants of risk in a peri-urban African context.

- The diagram illustrates the causal relationship between animal manure management practices (treatment, composting, pre-harvest interval), microbial selection pressure in the soil, enrichment in resistance genes, and the potential risk of human exposure, according to an integrated One Health approach.

Figure 3. Conceptual framework of the environmental dissemination of antibiotic resistance in peri-urban market gardening.



GENERAL CONCLUSION

This study highlights that animal manure management is a major environmental determinant of the spread of antibiotic resistance in peri-urban vegetable farming systems. The high proportion of untreated manure used, the lack of controlled composting, and the failure to respect pre-harvest intervals indicate significant exposure to factors that promote the selection and transmission of resistant bacteria.

Multivariate analysis confirms that the absence of manure treatment (ORa = 3.14), uncontrolled composting (ORa = 2.63), and failure to adhere to the crop rotation interval (ORa = 2.41) are significant independent determinants of high risk. The forest plot reinforces the robustness of these associations, as not all confidence intervals crossed unity.

The model exhibits satisfactory discriminatory capacity (AUC = 0.72) and acceptable calibration (Hosmer–Lemeshow p = 0.077), although its explanatory capacity remains moderate (Nagelkerke = 0.159), reflecting the multifactorial complexity of environmental antibiotic resistance.

From a One Health perspective, these results highlight that improving manure treatment, controlling composting and respecting cultivation deadlines represent priority strategic levers to reduce the environmental spread of resistant strains and protect public health.

RECOMMENDATIONS

Agricultural recommendations

- Promote the systematic pretreatment of animal excrement before its use (controlled composting, thermophilic maturation).
- Prohibit the direct spreading of fresh excrement on crops intended for human consumption.
- Reinforce compliance with the time interval between organic fertilization and harvest .
- Develop simplified technical guides adapted to small producers.

Recommendations for livestock farming

- Rationalize the use of veterinary antibiotics.
- Implement antimicrobial stewardship programs in livestock farming.
- Promote preventive alternatives (biosecurity, vaccination, improved farming conditions).

Public health recommendations

- Integrate agricultural systems into national strategies to combat antimicrobial resistance.
- Set up environmental monitoring of resistance genes (soil, water, vegetables).
- Train producers on the risks associated with the use of untreated manure.

Institutional recommendations

- Develop regulatory standards for the management of organic fertilizers.
- Strengthen intersectoral coordination (agriculture–livestock–health–environment).
- Supporting applied research in a local context.

Future Prospects

- Conduct longitudinal studies to establish causal relationships.
- Incorporate direct microbiological analyses (qPCR, metagenomics).
- Evaluate the impact of pilot interventions (controlled composting).
- Develop predictive models integrating environmental and climate data.
- To study the socio-economic impact of agricultural practices on the spread of antibiotic resistance.

Strengths Of The Study

- Integrated One Health approach .
- Robust statistical analysis (adjusted OR, ROC, calibration).
- Identification of clear independent determinants.
- Data from a poorly documented African context.
- Model validation by discrimination and calibration.

Limitations Of The Study

- Cross-sectional drawing does not allow for inference of causality.
- Data is partially self-reported (possible bias).
- No direct microbiological analyses.
- Moderate explanatory capacity (Nagelkerke = 0.159).
- Cautious generalization due to the local context.

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