*Journal of Applied and Natural Science*
16(3), 1164 - 1175 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

*Research Article*

# Poultry manure and vegetables as vehicles for antimicrobial resistance determinants distribution in some Farms in Delta State, Nigeria

**Olivia Sochi Egbule**
Department of Microbiology, Delta State University, Abraka
**Edward Ikenna Odum**
Department of Microbiology, Delta State University, Abraka
**Obaro Levinson Oyubu**
Department of Science Laboratory Technology, Delta State University, Abraka
**Sophia Chidinma Odibe**
Department of Microbiology, Delta State University, Abraka
**Benson Chuks Iweriebor***
Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa

*Corresponding author. E-mail: benvida2004@yahoo.com

***Article Info***
https://doi.org/10.31018/jans.v16i3.5725
Received: May 11, 2024
Revised: August 03, 2024
Accepted: August 09, 2024

***How to Cite***
Egbule, O. S. *et al.* (2024). Poultry manure and vegetables as vehicles for antimicrobial resistance determinants distribution in some Farms in Delta State, Nigeria. *Journal of Applied and Natural Science*, 16(3), 1164 - 1175. https://doi.org/10.31018/jans.v16i3.5725

**Abstract**
Antimicrobial resistance has become a major threat to human health globally. One of the reasons for this increase is the abuse and misuse of antibiotics in the poultry industry as a growth promoter. This practice has resulted in the rise of the spread of antimicrobial resistance within the environment as poultry waste is used as manure in the growth of vegetables. With reduced enthusiasm on the part of the pharmaceutical industries to embark on developing new antimicrobial agents in the face of increasing resistance evolution, few options are available in the armamentarium to combat bacterial infections. The study aimed to determine if domestically grown leafy vegetables and soil amended with poultry manure constitute a possible reservoir of antibiotic resistance and assessed their ability to transfer resistance via conjugation experiment. Twenty-seven leafy vegetable samples and poultry manure-enriched soils were collected from some Delta State, Nigeria farms under aseptic conditions. Standard bacteriological methods were used to isolate and identify isolates, then examined for their susceptibility to fifteen antibiotics and potential for resistance transfer via conjugation experiment. Of the 76 bacterial isolates recovered, 52 originated from vegetables, while 24 were from poultry manure-enriched soil. *Escherichia coli* (14.5%) and *Bacillus subtilis* (7.9%) were the most prevalent isolates in vegetables and soil, respectively. The antibiotic resistance profiles of the isolates indicated very high resistance levels in Gram-negative isolates obtained from the soil to all tested antibiotics. The resistance profile of Gram-positive isolates from both vegetables and soil showed ≥ 50% resistance in tetracycline. Also, high level of resistance of between 50% to 100% was detected in *Bacillus* spp. and *Enterococcus* spp. to erythromycin, tetracycline, and chloramphenicol. Multidrug-resistant (MDR) isolates served as donor cells, while standard *Escherichia coli, Pseudomonas aeruginosa, Bacillus* spp. and *Staphylococcus aureus* served as recipient strains. The rate of antibiotic resistance transfer was generally high, particularly for tetracycline (57.1%) and chloramphenicol (61.9%). The high rate of antibiotic resistance transfer observed in this study highlights the risk of MDR spreading through poultry manure use.

**Keywords**: Antibiotics, Bacterial isolates, Poultry manure, Resistance transfer, Soils, Vegetables

## INTRODUCTION

The increasing use of manure-amended soil in vegetable farming represents a proficient medium for antimicrobial-resistant bacteria and gene transmission into humans, and this has been documented as one of the major threats to human health (Berendonk *et al.,* 2015). The transfer of antibiotic-resistance genes (ARGs) from bacteria to human-associated pathogens could occur through the food chain and can be facilitated by mobile genetic elements such as integrons, plasmids and transposons. More so, due to the high levels of transmissible mobile genetic elements (MGEs) in animal manures, they could also play a vital role in the horizontal transfer of ARGs between manure-associated bacteria and resident soil bacteria (Chajęcka-Wierzchowska *et al.,* 2021). Therefore, manured soil has become an environmental reservoir of ARGs and

This work is licensed under Attribution-Non Commercial 4.0 International (CC BY-NC 4.0). © : Author (s). Publishing rights @ ANSF.

MGEs and offers a higher chance of selecting and disseminating resistance into farmland and agricultural produce.

Vegetables range from seed, root, fruit, stem, and leaf; these determine their soil contact, surface presentation to irrigation, environmental factors such as rain, sun, and wind, as well as their fate at harvest. Although, vegetables are a requisite constituent of a healthy diet, the abundance and transfer of antibiotic-resistant bacteria from manured soils to vegetables often eaten raw or with minimal processing is of great concern as it exposes humans to microorganisms from the soil and contributes to the spread of antimicrobial resistance genes. Vegetables are often exposed to microbial contamination and could be a vehicle for the spread of foodborne disease-causing pathogens. *Escherichia coli, Salmonella, Shigella, Listeria monocytogenes, Clostridium perfringens, Bacillus* spp. and enterococci are predominantly found on the surface of ready-to-eat vegetables and fruits (Laconi *et al.,* 2021; Szott and Friese, 2021; Tan *et al.,* 2020; Gurmessa *et al.,* 2021; Fatoba *et al.,* 2021; Esperón *et al.,* 2020; Black *et al.,* 2021; EARA, 2020). Washing prior to consumption minimizes the microbial load on vegetables but may not be effective enough to remove all microbial contaminants, as consumers may ultimately be exposed directly to the microorganisms present in them (Esperón *et al.,* 2020; Black *et al.,* 2021).

In Nigeria, and especially in the sampling locations, improper practices in poultry farming include using antibiotics in subtherapeutic doses or as growth promoters (Chah *et al.,* 2022). Antibiotics misuse affects the bacterial response to antibiotics and leads to the development of multidrug-resistant (MDR) bacteria, which may be transmitted to the manure used to fertilise soils (Chah *et al.,* 2018; Nwiyi *et al.,* 2018; Nsofor *et al.,* 2013). In addition, recommended or mandated management practices such as ensuring that produce does not come into contact with pathogens carried in raw manure that is used as a fertilizer, disinfecting the manure through composting activities and pre-application of manure in the soil before planting vegetables in a bid to reduce the burden of antibiotic-resistant contaminant, are not carried out. Direct application of animal manure is a common practice in the sampling areas (Chah *et al.,* 2018). Therefore, this study aimed to elucidate whether and to what extent vegetables and poultry-manured soil play a role as a carrier and reservoir of antibiotic-resistant bacteria and their capacity to transfer resistance.

## MATERIALS AND METHODS

### Study area/sample collection

Three different types of indigenous leafy vegetables, namely, pumpkin (*Cucurbita maxima*), water leaf (*Talinum triangulare*) and green leaf (*Defmodium intortum*), commonly eaten by the inhabitants of the Niger Delta region, were collected from different farms fertilizing vegetables with poultry manure in Abraka and Sapele, Delta State Nigeria. Also, soil samples enhanced with poultry manure were obtained from the farms. Twenty-seven vegetables, 9 of each type and 27 soils enhanced with manure were collected aseptically into sterile polyethylene bags and immediately taken to the Delta State University's Microbiology Laboratory for bacteriological analyses.

### Bacteriological examination /identification

Each vegetable sample was macerated using a laboratory mortar and pestle, 10g of each was homogenized in 40 mL of 0.1% peptone water at normal speed for 2 min in a stomacher. About 1mL of homogenized vegetable sample was serially diluted to the tenth factor. For the soil sample, 1g manured-fertilized soil samples were collected from the topsoil (0–20 cm), free from coarse stone or debris and serially diluted to the tenth factor. The bacteria were isolated and identified by standard bacteriologic culture methods (Pezzuto *et al.,* 2016). The samples were inoculated onto appropriate medium for the growth of selected bacteria and some of the media used included bile aesculin azide (ABA) agar, MacConkey agar, blood agar, Bordet–Gengou agar, and Salmonella Shigella agar plates. Plates were incubated at 37$^{o}$C for 24h. This was subsequently followed by biochemical differential tests for bacteria identification (Table 1).

### Antimicrobial susceptibility testing

Antibiotic susceptibility testing and interpretation were carried out by the guidelines of the Clinical and Laboratory Standards Institute (Cappacino *et al.,* 2013). From an18-24 hours old pure culture colonies, a 0.5 McFarland standard equivalent, as previously described (Clinical and Laboratory Standards Institute, 2016; Amri and Juma, 2016) was prepared and all culture was subsequently swabbed onto Mueller Hinton agar using sterile cotton swabs. Different antibiotic discs were placed on each plate to test for sensitivity, incubated for 18h at 37$^{o}$C, and the zones of inhibition were measured. The antibiotics tested for susceptibility were ceftazidime, cefuroxime, cefixime, ofloxacin, gentamicin, ciprofloxacin, nitrofurantoin and augmentin for gram-negative isolates; cloxacillin, cotrimoxazole, chloramphenicol, tetracycline, gentamicin, streptomycin, augmentin, and erythromycin for Gram-positive isolates. Multidrug resistance (MDR) was defined as resistance to 3 or more antibiotics.

Plasmid curing of MDR donor isolates using sodium dodecyl sulphate. Plasmid curing was performed on the selected MDR donor isolates as previously described by the method of (Egbule and Yusuf, 2019) using a

**Table 1.** Information on the identification of bacterial tests

| Bacterial species | Laboratory test criteria for identification |
|---|---|
| Escherichia coli | Gram staining (Gram - rod), motile, non-sporing, catalase(+),oxidase(-), MR(+), VP(-),Oxidative/Fermentative (fermentative), indole(+), citrate(-), urease(-), nitrate reduction (+),coagulase (-) and TSI (yellow butt, yellow slant, $H_2S$−, gas-) |
| Staphylococcus aureus | Gram +, nonspore-forming cocci, coagulase (+), catalase (+), oxidase (-), indole (-), MR (+), VP (+), urease (+), glucose (+) and lactose (+) |
| Bacillus sp. | Gram staining (Gram + rod), motile, sporing, anaerobic growth, catalase (+), oxidase (-), MR(-), VP(+), Indole (-), citrate(+),nitrate reduction(+), urease(-), $H_2S$ (+) and glucose(+). |
| Klebsiella pneumoniae | Gram -, non-motile, citrate (+), catalase (+), oxidase (-), indole (-), MR (-), VP(+), urease (+),TSA ($H_2S$−, gas+) |
| Flavobacterium sp. | Gram (-) rod, nonspore-forming, motile, citrate (+), catalase (+), oxidase (-), indole (-), nitrate reduction (+), glucose (+) and $H_2S$ (+) |
| Streptococcus pyogenes | Gram staining (Gram + cocci), motile, sporing, catalase (-), beta-haemolytic, urease (-), MR (+), VP (-), Oxidase (-), indole (-), citrate (+), nitrate reduction (+), coagulase (-) and $H_2S$ (-) |
| Corynebacterium diphtheriae | Gram (+) rod, non-motile, catalase (+), oxidase (-), beta haemolysis, nitrate reduction (+), urease (-) and glucose (+) |
| Arthrobacter sp. | Gram staining (Gram- short thin rod), motile, sporing, catalase (+), MR (+), VP (-), Oxidase (+), indole (-), citrate (+), urease (-), coagulase (-) and $H_2S$ (+) |
| Enterococcus sp. | Gram staining (Gram + cocci), non-motile, non-sporing, catalase (-), oxidase (-), MR (-), VP (+), Indole (-), citrate (-), urease (-), $H_2S$ (-) and coagulase (-) glucose (+) and lactose (+). |
| Alcaligens sp. | Gram staining (Gram- coccobacilli), motile, catalase (+), oxidase (+), MR (-), VP (+), Indole (-), citrate (+), urease (-), $H_2S$ (-), glucose (+) and nitrate reduction (-) |
| Proteus mirabilis | Gram staining (Gram -), motile, catalase (+), oxidase (-), MR (-), VP(-), citrate(+), Indole (-), urease(+), haemolysis on blood agar, $H_2S$ (-) and coagulase (-). |
| Salmonella sp. | Gram staining (Gram), motile, non-sporing, catalase(+),oxidase(-),MR(+), VP(-),Oxidase (-), indole(-), citrate(+), urease(-)and TSI (yellow butt,red slant, $H_2S$−, gas+) |
| Micrococcus sp. | Gram (+) Cocci, non-motile, halotolerant, catalase (+), oxidase (-), coagulase (+), nitrate reduction (+) |

10% sodium dodecyl sulphate (SDS). Briefly, 4.5 mL of nutrient broth was inoculated with overnight broth culture of test isolate, then a 0.5 ml, 10% concentration of SDS was added, and the mixture was incubated at 37° C for 48 h. After incubation, 4.5 mL nutrient broth was supplemented with 0.5 mL of the broth culture of the test isolate and incubated at 37°C for an additional 24 h. After 24 hrs incubation, an antibiotic susceptibility test was carried out on Mueller-Hinton agar plates as described earlier using the same antibiotics. Isolates that lost their resistance markers post-curing were identified as cured plasmid cells, harbouring resistance on plasmids.

**Bacterial conjugation**

Isolates resistant to ≥ 6 antibiotics and harboured plasmids were used as donors in the conjugation experiment. The recipient strain was *E. coli* ATTC No 25922, *Pseudomonas aeruginosa* ATTC No27853, *Bacillus* spp. ATTC No: 14884 and *S. aureus* ATTC No: 6538. These strains were collected from the Research Laboratory, University of Hare Alice, South Africa. The conjugation experiment was carried out according to the method described by Sijhary *et al.* (1984). The recipient and the donor stains were cultured separately in 2ml of peptone-enhanced medium (peptone with Tryptic soy broth) and incubated at 35°C for 24 hours. Culture of the lactose fermenting donor was mixed with the non-lactose fermenting recipient *(S. aureus* X *Bacillus subtilis*, *Klebsiella pneumoniae* X *E. coli*, *Enterococc*i X *Bacillus spp*., *Corynebacterium diphtheria* X *S. aureus*, *Salmonella typhi* X *E. coli, Streptococcus pyogenes* x *Bacillus* spp. at a ratio of 1:10 (donor: recipient) and incubated at 37°C for 18 hours. Sample of this mixture was plated on a differential medium and MacConkey agar plate, adopting spread plate procedure, plates were incubated at 37°C for 24 hours. Based on the colony appearance, lactose fermenters appeared pink, while non-lactose fermenters appeared colourless. Presumptive transconjugants were isolated, and resistance transferred was confirmed by subjecting isolates to antibiotic susceptibility tests as described earlier.

## RESULTS

**Bacteria isolated from vegetables and soil enriched with poultry manure**

A total of 27 indigenous fresh vegetable leaves of three different types and soil enriched with poultry manure were collected, and organisms were isolated and iden-

tified. These isolates were tested first for their susceptibility to antibiotics and then their ability to transfer resistance.

Out of the 76 bacterial isolates recovered from the different types of leafy vegetables and soils, 52 isolates originated from vegetables, while 24 were from soils. The bacteria isolated in vegetables were similar to those from soil enriched with poultry manure. The identified isolates from the vegetable sample by decreasing order of prevalence were *Escherichia coli* (14.5%), followed by *Staphylococcus aureus*, and *Klebsiella* spp. (10.5%), *Proteus mirabilis* (9.2%), *Enterococcus* spp. (7.9%), *Corynebacterium diphtheria* (5.3%), *Salmonella* spp. (2.6%) *Streptococcus pyogenes*, and *Alcaligens* spp. (1.3%). The prevalence of isolates from soil samples indicates that *Bacillus* spp. was the most frequent organism encountered (7.9%), followed by *Staphylococcus aureus* and *Enterococcus* spp. (5.3%) then *Corynebacterium diphtheria, Alcaligene* spp. and *Micrococcus* (2.6%). *Escherichia coli, Klebsiella, Arthrobacter,* and *Salmonella* (1.3%) were the least isolated (Fig. 1). *Arthrobacter* spp. and *Micrococcus* spp. were not from vegetables sampled, while *Proteus mirabilis* and *Streptococcus pyogenes* were not from the soil.

**Antimicrobial resistance of bacterial isolates**

The analysis of resistance profiles of the isolates under study revealed very high levels of resistance in Gram-negative isolates from the soil to all tested antibiotics. Most of the Gram-negative isolates from the soil were 100% resistant to the antibiotics tested. Even though the levels of antimicrobial resistance amongst the Gram -negative isolates from vegetable samples were high, they were comparatively lower than their counterparts from the soil. For instance, 100% resistance was observed in Gram-negative isolates from the soil to ciprofloxacin, gentamycin, and ofloxacin, while lower levels of resistance to these antibiotics were observed in isolates from vegetable source (Fig. 2). The resistance profile of Gram -positive isolates from both vegetables and soil showed ≥ 50% resistance in tetracycline. Also, high levels of resistance between 50% to 100% was detected in *Bacillus* spp. and *Enterococcus* spp. to erythromycin, tetracycline, and chloramphenicol (Fig. 3).

Over 70% of the isolates were MDR. However, those selected to serve as donor cells in this study were resistant to ≥ 6 antibiotics. The eligible donors were obtained more from vegetables than soils. Results are presented in Tables 1 and 2. Information on the resistance profile of the MDR Gram positive isolates selected revealed that 6 of the 21 isolates were pan-drug resistant (resistant to all tested antibiotics) and all selected isolates were resistant to tetracycline. The resistance profile of selected donor MDR Gram-negative isolates indicated that the resistance biogram contained at least 2 cephalosporins and a fluoroquinolone.

The resistance profile of the standard recipient strain comprising of *E. coli, P. aeruginosa, Bacillus, S. aureus* is presented in the Table 3. They were all sensitive to the antibiotics. The resistance profile of Gram-positive donor isolates and the transconjugants obtained after the transfer experiment are presented in Table 4. *Bacillus* and *S. aureus* served as recipient strains. Result

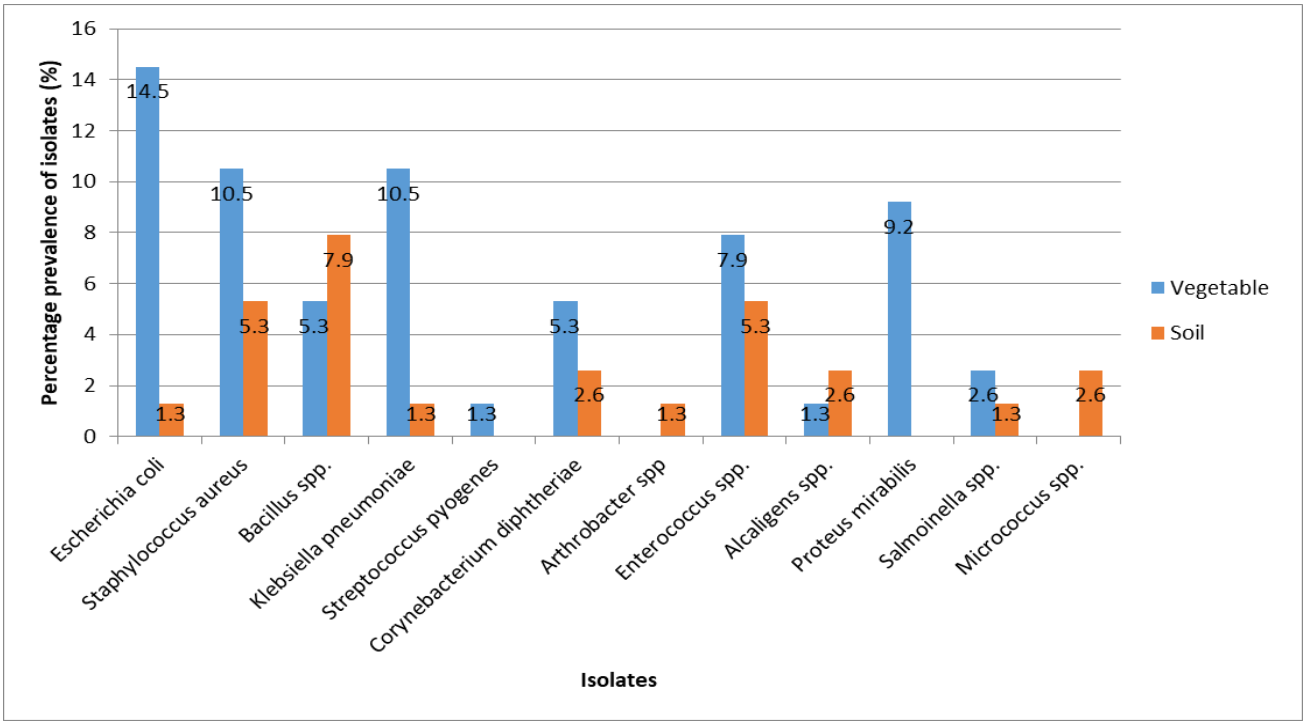


**Fig.** 1. *Percentage prevalence rate of bacterial isolates in vegetables and soil enhanced with poultry manure*

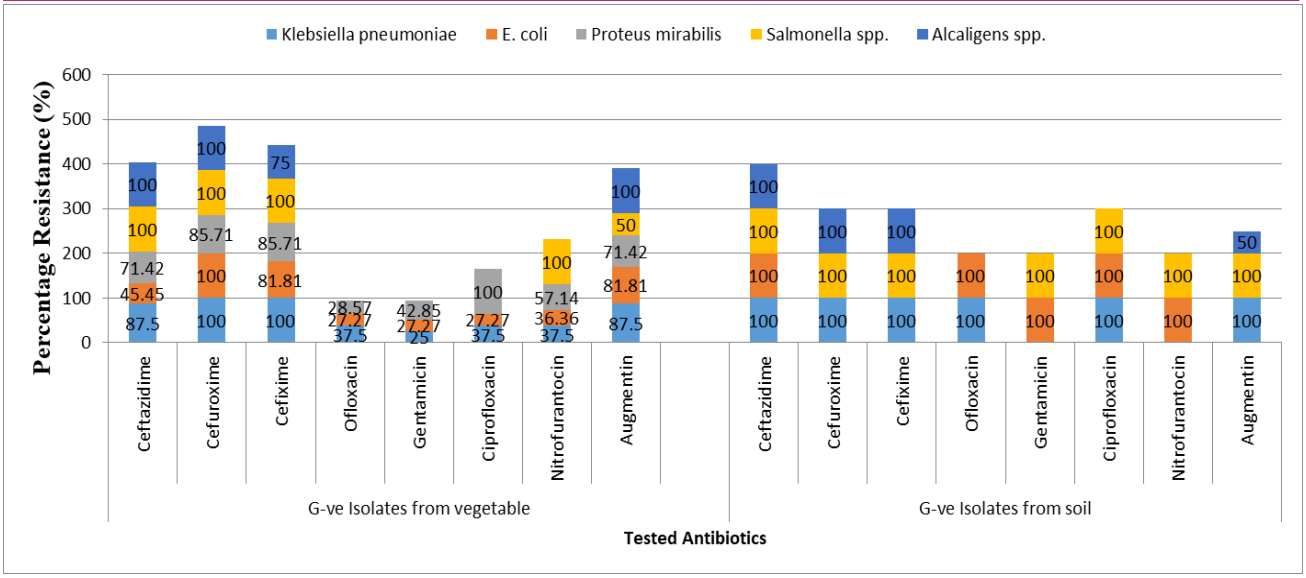


**Fig. 2.** *Antibiotics resistance profile of Gram-negative isolates from vegetables and soil enhanced with poultry manure*

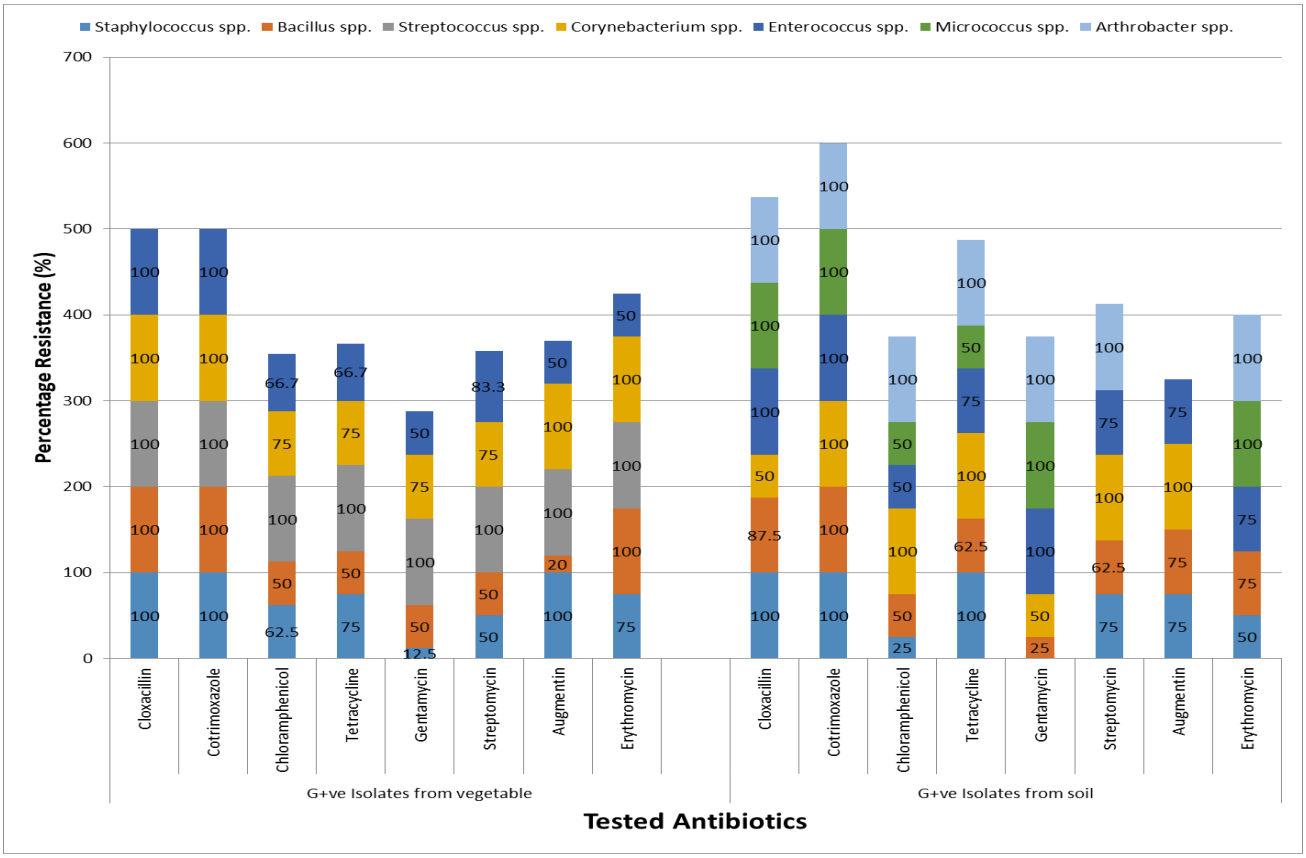


**Fig. 3.** *Antibiotics resistance profile of Gram-positive isolates from vegetables and soil enhanced with poultry manure*

revealed that *S. aureus* had the highest number of resistant transfers. The rate of transfer of the antibiotics was particularly high for tetracycline (57.1%), chloramphenicol (61.9%) and erythromycin (42.9%). Out of five *Bacillus* spp. selected as donor isolates, three transferred tetracycline chloramphenicol and erythromycin. There was also a high transfer rate of gentamycin, chloramphenicol, and tetracycline in Enterococcus spp. In a total of 17 (80.9%) cases, between 4 to 8 antibiotics were transferred in this study (Table 4).

The resistance Profile of both Gram-negative donor Isolates and their transconjugants are shown in Table 5. *E. coli* and *P. aeruginosa* served as recipients. The selected donor isolates included four *Klebsiella pneumoniae*, two *E. coli* and *one Salmonella typhi. One of the Klebsiella pneumoniae* transferred all 8 antibiotics, while no transfer was observed in the *Salmonella typhi* selected (Table 5)

**Table 1.** Resistance patterns of highly multidrug-resistant Gram-positive bacteria (≥ 6 resistances) isolated from vegetables and soil enriched with manure

| Code of isolates | Bacteria strains | Resistance biogram | Resistant marker |
|---|---|---|---|
| BA[1]V | Bacillus spp. | ERY, GEN, AUG, CHL, STR, TET, CIT, CXC | 8 |
| CD[2]V | Corynebacterium diphtheriae | ERY, GEN, AUG, CHL, STR, TET, COT, CXC | 8 |
| SA[3]V | S. aureus | AUG, STR, TET, CHL, COT, CXC | 6 |
| EN[4]V | Enterococci spp. | ERY, AUG, STR, TET, CHL, COT, CXC | 7 |
| SP[5]V | S. pyogenes | ERY, AUG, STR, TET, CHL, COT, CXC | 7 |
| BA[6]V | Bacillus spp. | ERY, AUG, GEN, TET, CHL, COT, CXC | 7 |
| CD[7]V | Corynebacterium diphtheriae | ERY, GEN, AUG, STR, TET, COT, CXC | 8 |
| EN[8]V | Enterococci spp. | ERY, GEN, STR, CHL, COT, CXC | 6 |
| SA[9]V | Staphylococcus aureus | ERY, AUG, GEN, STR, TET, CHL, COT, CXC | 8 |
| EN[10]V | Enterococci spp. | AUG, STR, TET, CHL, COT, CXC | 6 |
| BA[11]S | Bacillus spp. | ERY, AUG, STR, TET, COT, CXC | 6 |
| BA[12]S | Bacillus spp. | ERY, AUG, STR, TET, COT, CXC | 6 |
| EN[13]S | Enterococc spp. | ERY, AUG, STR, CHL, COT, CXC | 6 |
| CD[14]V | Corynebacterium diphtheriae | ERY, GEN, AUG, TET, CHL, COT, CXC | 7 |
| SA[15]V | Staphylococcus aureus | ERY, AUG, STR, TET, CHL, COT, CXC | 7 |
| EN[16]V | Enterococci spp. | ERY, GEN, STR, TET, CHL, COT, CXC | 7 |
| EN[17]S | Enterococci spp. | ERY, GEN, AUG, STR, TET, CHL, COT, CXC | 8 |
| EN[18]S | Enterococci spp. | ERY, GEN, AUG, STR, TET, CHL, COT, CXC | 8 |
| EN[19]S | Enterococci spp. | ERY, AUG, TET, CHL, COT, CXC | 6 |
| BA[20]S | Bacillus spp. | ERY, AUG, STR, TET, COT, CXC | 6 |
| SA[21]S | Staphylococcus aureus | ERY, STR, TET, CHL, COT, CXC | 6 |

**Table 2.** Resistance patterns of highly multidrug-resistant Gram-negative bacteria (≥ 6 resistances) isolated from vegetables and soil enriched with manure.

| Code 0f isolates | Bacteria strains | Resistance biogram | Resistance marker |
|---|---|---|---|
| KP[22]V | Klebsiella pneumoniae | CRX, GEN, CPR, CAZ | 8 |
| EC[23]V | E. coli | CRX, GEN, OFL, AUG, NIT COR, CAZ | 7 |
| KP[24]V | Klebsiella pneumoniae | CRX, GEN, CXM, AUG, NIT, CAZ | 6 |
| EC[25]V | E. coli | CRX, GEN, CXM, OFL, AUG, NIT, CAZ | 7 |
| KP[26]S | Klebsiella | CRX, CXM, OFL, AUG, CPR, CAZ | 6 |
| SM[27]S | Salmonella typhi | CRX, GEN, CXM, AUG, NIT, CPR, CAZ | 7 |
| KP[28]V | Klebsiella pneumoniae | CRX, CXM, OFL, AUG, CPR, CAZ | 6 |

Key: STR= Streptomycin; CXM= Cefixime; CHL= Chloramphenicol ; CAZ= Ceftazidime; TET= Tetracycline ; CXC= Cloxacillin; AUG= Augmentin; NIT= Nitrofurantoin; GEN= Gentamycin; OFL= Ofloxacin; CPR= Ciprofloxacin; COT= Co-trimoxazole; CRX= Cefuroxime

**Table 3.** Antibiogram of recipient strains

| Gram negative | CAZ | CRX | CXM | OFL | AUG | GEN | NIT | CPR |
|---|---|---|---|---|---|---|---|---|
| *E. coli* ATTC No:25922 | S | S | S | S | S | S | S | S |
| *Pseudomonas aeruginosa* ATTC No: 27853 | S | S | S | S | S | S | S | S |
| Gram positive | ERY | GEN | AUG | STR | TET | CHL | COT | CXC |
| *Bacillus* ATTC No: 14884 | S | S | S | S | S | S | S | S |
| *S. aureus* ATTC No: 6538 | S | S | S | S | S | S | S | S |

**Table 4.** Transfer of resistance in Gram positive isolates

| Code of isolates | Isolates | Resistant profile of the donor | Resistant profile of trans-conjugants | No of transfer resistance |
|---|---|---|---|---|
| BA[1]V | *Bacillus* spp. | ERY, GEN, AUG, CHL, STR, TET, COT, CXC | GEN, AUG, STR, CXC | 4 |
| CD[2]V | *Corynebacterium Diphtheriae* | ERY, GEN, AUG, CHL, STR, TET, COT, CXC | CXC | 1 |
| SA[3]V | *S. aureus* | AUG, STR, TET, CHL, COT, CXC | ERY, GEN, AUG, STR, TET, CXC | 6 |
| EN[4]V | *Enterococci* spp. | ERY, AUG, STR, TET, CHL, COT, CXC | GEN, AUG, CHL, COT, CXC | 5 |
| SP[5]V | *S.pyrogenese* | ERY,AUG, STR, TET, CHL, COT, CXC | ERY, GEN, STR, CHL, COT, CXC | 6 |
| BA[6]V | *Bacillus* spp. | ERY, AUG, GEN, TET, CHL, COT, CXC | GEN, AUG, CHL, COT CXC | 5 |
| CD[7]V | *Corynebacterium Diphtheriae* | ERY, GEN, AUG, STR, TET, CHL, COT, CXC | ERY, GEN, AUG, STR, CXC | 5 |
| EN[8]V | *Enterococci* spp. | ERY, GEN, STR, CHL, COT, CXC | CXC | 1 |
| SA[9]V | *S. aureus* | ERY, GEN, STR, TET, CHL, COT, CXC | GEN, STR, TET, CHL COT, CXC | 6 |
| EN[10]V | *Enterococci* spp. | AUG, STR, TET, CHL, COT, CXC | GEN, STR, TET, CHL | 4 |
| BA[11]V | *Bacillus* spp. | ERY, AUG, STR, TET, COT CXC, CHL | GEN, STR, TET, CHL COT, CXC | 6 |
| BA[12]V | Bacillus spp. | ERY, AUG, STR, CHL, COT, CXC | GEN, STR, TET, CHL, COT, CXC | 6 |
| EN[13]S | *Enterococcus* spp. | ERY, GEN, AUG, TET, CHL COT, CXC | ERY, GEN, STR, TET, CHL, CXC | 6 |
| CD[14]V | *Corynebacterium Diphtheriae* | ERY, GEN, AUG, TET, CHL, COT, CXC | ERY, GEN, AUG, STR, TET, COT, CXC | 7 |
| SA[15]V | *S. aureus* | ERY, GEN, AUG, TET, CHL, COT, CXC | ERY, GEN, AUG, TET, CHL, COT, CXC | 7 |
| EN[16]V | *Enterococcus* spp. | ERY, GEN, STR, TET, CHL, COT, CXC | GEN | 1 |
| EN[17]S | *Enterococcus* spp. | ERY, GEN, AUG, STR, TET, CHL, COT, CXC | ERY, GEN, STR, TET CHL, COT, AUG | 7 |
| EN[18]S | *Enterococcus* spp. | ERY, GEN, AUG, STR, TET, CHL COT, CXC | GEN, AUG, STR, TET CHL, COT | 6 |
| EN[19]S | *Enterococcus* spp. | ERY, AUG, STR, TET, CHL COT, CXC | ERY, AUG, STR | 3 |
| BA[20]V | *Bacillus* spp. | ERY, AUG, STR, TET, COT, CXC | ERY, AUG, STR, TET, CHL, COT | 6 |
| SA[21]S | *S. aureus* | ERY, STR, TET, COT, CXC | GEN, AUG, STR, TET, CHL, COT | 6 |

**Table 5.** Transfer of resistance in Gram-negative isolates

| Code of isolates | Isolates | Resistant profile Of the donor | Resistant profile of transconjugants | Number of transfer resistance |
|---|---|---|---|---|
| $KP^{22}V$ | *Klebsiella Pneumoniae* | CRY, GEN, CXM, OFL, AUG, NIT, CPR, CAZ | CAZ, CRX, GEN, CFR, CXM, OFL, AUG, NIT | 8 |
| $EC^{23}V$ | *E. coli* | CRX, GEN, OFL, AUG, NIT, CPR, CAZ | CRX, GEN, AUG, NIT, CPR | 5 |
| $KP^{24}V$ | *Klebsiella Pneumoniae* | CRX, GEN, CXM, AUG, NIT, CAZ | GEN | 1 |
| $EC^{25}V$ | *E. coli* | CRX, GEN, CXM, OFL, AUG, NIT, CAZ | CRZ, GEN, CXM, AUG, CPR | 5 |
| $KP^{26}S$ | *Klebsiella Pneumoniae* | CRX, CXM, OFL, AUG, CPR, CAZ | CAZ, GEN, OFL, AUG, CPR | 5 |
| $SM^{27}S$ | *Salmonella typhi* | CRX, GEN, CXM, AUG, NIT CPR, CAZ | - | 0 |
| $KP^{28}V$ | *Klebsiella Pneumoniae* | CRX, CXM, OFL, AUG, CPR, CAZ | CRX, GEN, CXM, AUG | 4 |

## DISCUSSION

Poultry manure is a rich mixture of nutrients containing excreted substances like pathogens, heavy metals, and antibiotics. It is also used in vegetable farming. Some studies have shown that animal manure application promotes the diversity and abundance of antimicrobial-resistant bacteria in the soil (Bamidele *et al.*, 2022; Van *et al.,* 2021; Geta et al., 2021). The bacteria observed in this study (Fig. 1) not only colonised the soil but also had the ability to colonize the vegetables planted. Thus, soil amended with poultry manure may serve as a reservoir for resistance genes, with food safety issues with potential risk to human health (Zhang *et al.,* 2017; Fang *et al.*, 2015; Campos *et al.*, 2013). The most prevalent Gram-negative isolates were *E. coli* and *K. pneumoniae,* while *Bacillus* spp., *S. aureus.* and *Enterococcus* spp. were the most common Gram-positive isolate. These organisms are among the potential human pathogens isolated from chicken manure in many studies in Nigeria (Zurfluh *et al.,* 2015; Omojowo and Omojasola, 2013). The perturbing aspect of the organisms isolated in this study was the presence of *Bacillus* spp. and *Enterococcus* spp., two important foodborne pathogens known for their role in contaminating and colonizing fruits and vegetables (Chajęcka-Wierzchowska *et al.,* 2021; Ngbede *et al.,* 2017). Enterococci can survive high temperatures and grow at high NaCl concentrations and in a wide pH range. They can form residual organisms on vegetables and ready-to-eat foods. In like manner, *B. subtilis* can tolerate extreme environmental conditions, as it is a spore former. Several studies have reported *Bacillus* spp. in vegetables (Koilybayeva *et al.,* 2023; Hammerum, 2012; Fiedler *et al.,* 2019). *Bacillus* spp. is also associated with hospital infections and is reportedly resistant to tetracycline and erythromycin (Park *et al.,* 2018; Zhai *et al.,* 2023). Generally, in this study, all Gram-positive isolates obtained from the soil and vegetables exhibited high rates of resistance, especially to chloramphenicol and tetracycline (≥50%) (Table 1). All *B. subtills* obtained from vegetables in this study were resistant to erythromycin and 50% resistant to tetracycline. *Enterococcus* spp. obtained from the soil were 100%, 75% and 50% resistant to gentamycin, tetracycline, and chloramphenicol, respectively. A high resistance of 75% to erythromycin was observed in *Enterococcus* spp. Similarly, high resistance levels of between 50% to 66% were also observed in *Enterococcus* spp. obtained from vegetables to these antibiotics. The higher prevalence rate observed for tetracycline in the soil could be because it is one of the antibiotics that have the propensity to absorb soil particles, thus making it recalcitrant to biodegradation. They can thus bioaccumulate even in trace concentration in arable soil enriched with manure, modifying the soil environmental niche and prolonging the persistence of resistance genes (Xu and Zhang, 2023 ). A prevalence rate of 97.6% has been reported for tetracycline in broiler farms in Madagascar (Aliyu *et al.,* 2022). Tetracycline and chloramphenicol are two widely used antibiotics in the poultry industry for prophylactics, growth promotion and therapeutic purposes primarily due to their broad-spectrum activities and their relative cheapness compared to other classes of antibiotics (Gay *et al.,* 2016; Ljubojević *et al.,* 2017). Another concern with these antibiotics is their poor absorption in the guts of the

birds, as well as their contamination of poultry meat and droppings by unabsorbed and unmetabolized drugs. For instance, Gonzalez *et al.* (2017) reported a relatively higher quantity of these antibiotics in chicken farces, but more concerning is the finding of antibiotic resistance genes in the intestinal contents by these studies. The high prevalence rate observed for both tetracycline and chloramphenicol in this study could be attributed to the indiscriminate use of cheap antibiotics in Nigeria (Amri and Juma, 2016; Mahmoud and Abdel-Mohsein, 2019; Egbule, 2016; Egbule *et al.,* 2020; Egbule, 2022).

*Enterococcus* spp. is an important aetiologic agent of poultry production (Omojowo and Omojasola, 2013) and hospital-associated infections (Iweriebor *et al*., 2022). Clinical *Enterococcus* spp. with high-level resistance to gentamicin, tetracycline and erythromycin has been reported (Khan *et al.*, 2015). This underscores poor outcomes when these antibiotics are used for therapeutic clinical purpose. Omojowo and Omojasola (2013) also reported that the highest frequencies of resistance in *Enterococcus* were to erythromycin and tetracycline among isolates from poultry sources. Some authors in Nigeria have observed that in addition to tetracycline, macrolides are also an abused group in animal production in Nigeria (Anderson *et al.,* 2016; Adeyemi *et al.,* 2019; Adesokan *et al.,* 2015; Olonitola *et al*., 2015). However, contrary to the high rate of chloramphenicol resistance observed in *Enterococcus* spp. in this study, Johnston *et al.* (2004) reported 3% chloramphenicol resistance in *E. faecalis*. Abriouel *et al.* (2016) found low resistance in chloramphenicol of about 12% in *E. faecalis* isolated from vegetables and fruits in Spain. The low rates of chloramphenicol resistance in *Enterococcus* spp. reported in these European countries is due to the ban on the use of chloramphenicol in food animals from 1994.

Gram-negative isolates include K. pneumonia, E. coli, and *Salmonella* spp. obtained from the soil were between 75% to 100% resistant to the cephalosporins and augmentin. Similarly, these isolates obtained from vegetables exhibited high resistance levels to cephalosporin and augmentin, though slightly lower (Fig. 2).

The abysmal level of resistance observed in vegetables to these clinically important bacteria is akin to intrinsic resistance, though not in this study. However, the most plausible explanation is that these vegetables were collected directly from farms. Abriouel *et al.* (2008) reported that harbouring resistant bacteria is at the expense of viability. These freshly harvested vegetables are more viable than those purchased by consumers at the open market or supermarket; some time passes until the vegetables arrive at sales points. Some processing steps, such as removal of leaves or roots, cooling, and washing, were bypassed in this study, which might be additional stress factors for the colonizers. Samtiya *et al.* (2022) reported that resistance rates of bacteria isolated from farm produce were higher than those of the retail market and showed that carrying out the processing steps in the vegetables and viability was the reason for the low resistance observed in their vegetables from the retail market.

The multidrug resistance bacteria on the vegetables may have resulted from the contaminated soil. Zhu *et al.* (2010) and Guron *et al.* (2017) reported that antibiotic-resistant bacteria present in soil and manure have the capacity to colonize the roots of plants in the soil and the plant rhizosphere or colonize the leaves, possibly from particulates in the air or the root rhizosphere (Chen *et al*., 2019) to agricultural produce making it an ideal route for human acquisition of antibiotic-resistant genes through the food chain (Wang *et al*., 2019). Transmission through poor hygienic handling practices by marketers is also an important route. The multi-drug-resistant bacteria in vegetables may be a threat to public health because they may transmit these pathogens to humans and to the environments that surround them (Wang *et al*., 2022). However, further systematic studies are needed to source track contamination routes of antibiotic-resistant bacteria and antibiotic-resistant genes in poultry production and to determine if their role transcends colonization. The composition of resistant bacteria in the MDR profile of the Gram-positive isolates showed the genotypic presence of chloramphenicol, tetracycline and erythromycin, as revealed by the curing experiment, as all donor isolates transferred chloramphenicol, tetracycline, and erythromycin-resistant gene to the recipient strains at varying rates (Table 4). This ease of transfer could be attributed to the resistance being carried on plasmid (Egbule, 2016). As observed in MDR transfer of Gram-positive isolates, the transfer of resistance was equally high in Gram-negative isolates. Of important note was the high prevalence of MDR Gram-negative isolates resistant to cephalosporin and augumentin. This underscores the production of β-lactamases, which has serious health consequences. The production of β-lactamases such as Extended Spectrum Beta Lactamases are associated with severe outcomes in humans, limiting the range of treatment options (Husna *et al.,* 2023).

There are numerous studies on manure usage and its implications in cultivating vegetables. One of the major reported implications is the rapid spread of antimicrobial resistance via vegetables (Büdel *et al*., 2020) as a result of the horizontal transfer of resistance genes in the environment, thus escalating the levels of antimicrobial resistance in the community, subsequently creating the emergence of MDR bacteria (Samtiya *et al*., 2022; Moroni et al., 2020 ; Levinreisman *et al.,* 2017). Following the criteria (≥6 resistant antibiotics) for selecting MDR donor isolates in this study, it was observed that more eligible donor isolates were obtained

from vegetables. Therefore, there is a remarkably high potential for resistance transfer from soil isolates to vegetables. This study confirmed the ease of transfer in the conjugation experiment conducted using *Bacillus* spp.*, S. aureus, P. aeruginosa* and *E. coli*, a bacterium commensal to the human gastrointestinal tract as the recipient. All the isolates served as donors harboured plasmids and transferred between 1 to 8 antibiotics resistances to the recipient strain in about 99% of the cases. This may indicate that the resistance gene was carried on conjugative plasmid or other mobile genetic elements (MGE) such as transposons and integrons.

## Conclusion

The present study revealed high-risk contamination of vegetables from farms in Delta State, Nigeria, high levels of resistant bacteria, multidrug resistance, and plasmids. The conjugation experiment indicated a high rate of antibiotic transfer to recipient bacteria, which may seriously threaten food safety and public health.

## ACKNOWLEDGEMENTS

We thank the Microbiology Department, Delta State University, and Abraka laboratory staff for their support during this work.

## Conflict of interest

The authors declare that they have no conflict of interest.

## REFERENCES

1. Abriouel, H., Omar, N.B., Molinos, A.C., López, R.L., Grande, M.J., Martínez-Viedma, P, Ortega, E., Cañamero, M.M. & Galvez, A. (2008). Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples. *International Journal of Food Microbiology,* 123*,* 38–49. doi: 10.1016/j.ijfoodmicro.2007.11.067.
2. Adesokan, H.K., Akanbi, I.O, Akanbi, I.M. & Obaweda, R.A. (2015). Pattern of antimicrobial usage in livestock animals in south western Nigeria: The need for alternative plans, Onderstepoort. *Journal of Veterinary Research*, *82,* 816. doi: 10.4102/ojvr.v82i1.816
3. Aliyu, M., Halim, M., Mohamed, A.H. & Tahir, I.B.H. (2022). Adsorption tetracycline from aqueous solution using a novel polymeric adsorbent derived from the rubber waste. *Journal of Taiwan Institute of Chemical Engineers,* 136, 104333. DOI: 10.1016/j.jtice.2022.104333
4. Amri, E. & Juma, S. (2016). Evaluation of antimicrobial activity and qualitative phytochemical screening of solvent extracts of *Dalbergia melanoxylon* (Guill. *And Perr.). International Journal of Current Microbiology and Applied Sciences*, 5(7), 412–423. doi: 10.20546/ijcmas.2016.507.045. Jul
5. Anderson, A.C., Jonas, D., Huber, I., Karygianni, L., Wölber, J., Hellwig, E., Arweiler, N., Vach, K., Wittmer, A., Al-Ahmad, A. (2016). Enterococcus faecalis from Food, Clinical Specimens, & Oral Sites: Prevalence of Virulence Factors in Association with Biofilm Formation. *Frontiers in Microbiology*,11: 6:1534. doi: 10.3389/fmicb.2015.01534.
6. 
7. Bamidele, O., Amole, T.A., Oyewale, O.A., Bamidele, O.O., Yakubu, A., Ogundu, U.E., Ajayi, F.O., Hassan, WA. (2022). Antimicrobial Usage in Smallholder Poultry Production in Nigeria. *Vet Med Int.* 2022:7746144. doi: 10.1155/2022/7746144.
8. Black, Z., Balta, I., Black, L., Naughton, P.J., Dooley, J.S.G., Corcionivoschi, N. (2021). The Fate of Foodborne Pathogens in Manure Treated Soil. *Frontiers Microbiology,* 12:781357. doi: 10.3389/fmicb.2021.781357
9. Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., et al. (2015). Tackling antibiotic resistance: the environmental framework. *Nature Review Microbiology.* 13(5):310-7. doi: 10.1038/nrmicro3439.
10. 
11. Büdel, T., Kuenzli, E., Campos-Madueno, E.I., Mohammed, A.H., Hassan, N.K. & Zinsstag, J. (2020). On the island of Zanzibar people in the community are frequently colonized with the same MDR Enterobacterales found in poultry and retailed chicken meat. *Journal of Antimicrobial Chemotherapy,* 75(9),2432-2441. doi: 10.1093/jac/dkaa198.
12. Campos, J., Mourao, J., Pestana, N., Peixe, L., Novais, C. & Antunes, P. (2013). Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. *International Journal of Food Microbiology*, 166, 464–470. 10.1016/j.ijfoodmicro.2013.08.005.
13. Cappacino, J.G. & Sherman, N. Editors (2013). Microbiology: A Laboratory Manual. 10th ed. Sterling Heights, MI, USA: Pearson 13–23.
14. Chah, J.M., Nwankwo, S.C., Uddin, I.O. & Chah, K.F. (2022). Knowledge and practices regarding antibiotic use among small-scale poultry farmers in Enugu State, Nigeria. *Heliyon.* 8(4),e09342. doi: 10.1016/j.heliyon.2022.e09342.
15. Chah K.F., Ugwu I.C., Okpala A., Adamu K.Y., Andrea C., Ceballos S., Nwanta J.N. & Torres C. (2018). Detection and molecular characterisation of extended-spectrum Beta - lactamase-producing enteric bacteria from pigs and chickens in Nsukka, Nigeria. *Journal of Global Antimicrobial Resistance,* 15,36-40. doi: 10.1016/j.jgar.2018.06.002.
16. Chajęcka-Wierzchowska, W., Zarzecka, U. & Zadernowska, A. (2021). Enterococci isolated from plant-derived food - Analysis of antibiotic resistance and the occurrence of resistance genes. *LWT.* 139, 110549. https://doi.org/10.1016/j.lwt.2020.110549.
17. Chen, Q.L., Cui, H.L., Su, J.Q., Su, J., Ma, Y. & Zhu., Y.G. (2019). Antibiotic resistomes in plant microbiomes. *Trends in Plant Science.* 24: 530–541. doi: 10.1016/j.tplants.2019.02.010.
18. Clinical and Laboratory Standards Institute (2016). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard: Clinical and

Laboratory Standards Institute.M7-A6, 14th ed. Wayne, PA USA.
19. EARA (2020). Department of Agriculture, Nutrients Action Programme (NAP) 2019-2022. London: EARA.
20. Egbule, O. S. (2016). Antimicrobial Resistance and *β*-Lactamase Production among Hospital Dumpsite Isolates. *Journal of Environmental Protection,* 7 (07), 1057-1063
21. Egbule, O.S. (2022). Occurrence of extended spectrum beta-lactamases and sul1 in multi drug resistance Escherichia coli and Salmonella isolate from poultry feeds. Scientific Africa, 18; e01362. https://doi.org/10.1016/j.sciaf.2022.e01362
22. Egbule, O.S. & Yusuf, I. (2019). Multiple Antibiotic Resistances in Escherichia coli Isolated from Cattle and Poultry Faeces in Abraka, South-South Nigeria. *Tropical Agricultural Science,* 42(2): 585-594.
23. Egbule, O.S., Iweriebor, B.C. & Edward, I.O. (2020). Beta-Lactamase-Producing Escherichia coli Isolates Recovered from Pig Handlers in Retail Shops and Abattoirs in Selected Localities in Southern Nigeria: Implications for Public Health. Implication for Public Health. Antibiotics. 10(1):9. https://doi.org/10.3390/antibiotics10010009
24. Esperón, F., Albero, B., Ugarte-Ruíz, M., Domínguez, L., Carballo, M., Tadeo, J. L., et al. (2020). Assessing the benefits of composting poultry manure in reducing antimicrobial residues, pathogenic bacteria, and antimicrobial resistance genes: a field-scale study. *Environmental Science and Pollution Research International.* 27, 27738–27749. doi: 10.1007/s11356-020-09097-1
25. Fang, H., Wang, H.F., Cai, L. & Yu, Y.L. (2015). Prevalence of antibiotic resistance genes and bacterial pathogens in long-term manured greenhouse soils as revealed by metagenomic survey. *Environmental Science and Technology*, 49, 1095–1104. doi: 10.3390/ijerph16050683
26. Fatoba, D. O., Abia, A. L. K., Amoako, D. G. & Essack, S. Y. (2021). Rethinking manure application: increase in multidrug-resistant enterococcus spp. in agricultural soil following chicken litter application. Microorganisms, 9:885. doi: 10.3390/microorganisms9050885
27. Fiedler, G., Schneider, C., Igbinosa, E.O., Kabisch, J., Brinks, E., Becker, B., Stoll, D.A., Cho, G.-S., Huch, M. & Franz, C.M.A.P (2019). Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. *BMC Microbiology*, 19; 250. https://doi.org/10.1186/s12866-019-1632-2
28. Gay, N., Leclaire, A., Laval, M., Miltgen, G., Jégo, M., Stéphane, R., Jaubert, J., Belmonte, O. & Cardinale, E. (2016) Risk factors of extended-spectrum β-lactamase producing *Enterobacteriaceae* occurrence in farms in Reunion, Madagascar and Mayotte Islands 2016 2016–2017. *Veterinary Science.* 5,22. doi: 10.3390/vetsci5010022.
29. Geta K., Kibret M (2021). Knowledge, attitudes and practices of animal farm owners/workers on antibiotic use and resistance in Amhara region, north western Ethiopia. *Scientific Reports.* 11(1),1–13. doi: 10.1038/s41598-021-00617-8.
30. Gonzalez, R., Angeles, M. & Hernandez, J.C. (2017). Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. *Food Control,* 72, 255–267. 10.1016/j.foodcont.2016.03.001.
31. Gurmessa, B., Ashworth, A. J., Yang, Y., Savin, M., Moore, P. A., Ricke, S. C., et al. (2021). Variations in bacterial community structure and antimicrobial resistance gene abundance in cattle manure and poultry litter. *Environmental Research.* 197,111011. doi: 10.1016/j.envres.2021.111011
32. Guron, G.K.P., Arango-Argoty, G., Zhang, L., Pruden, A. & Ponder, M.A. (2019). Effects of dairy manure-based amendments and soil texture on lettuce- and radish-associated microbiota and resistomes. *MSphere* 4, e239-19. doi: 10.1128/mSphere.00239-19.
33. Hammerum, A.M. (2012). Enterococci of animal origin and their significance for public health. *Clinical Microbiology and Infection.* 18(7),619-25. doi:10.1111/j.1469-0691.2012.03829. x.
34. Husna, A.; Rahman, M.M., Badruzzaman, A.T.M., Sikder, M.H., Islam, M.R., Rahman, M.T.,Alam, J. & Ashour, H.M. (2023). Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities. *Biomedicines* 11; 2937. https://doi.org/10.3390/biomedicines11112937
35. Iweriebor, B. C., Egbule, O.S., Obi, L.C. (2022). The Emergence of Colistin- and Imipenem-Associated Multidrug Resistance in Escherichia Coli Isolates from Retail Meat. *Polish Journal Microbiology* 71(4), 519–528. doi: 10.33073/pjm-2022-046.
36. Johnston, L.M., Jaykus, L.A. (2004). Antimicrobial resistance of Enterococcus species isolated from produce. *Applied Environmental Microbiology* 70, 3133–3137. doi: 10.1128/AEM.70.5.3133-3137.2004
37. Khan, H.A., Ahmad, A. & Mehboob, R. (2015). Nosocomial infections and their control strategies, *Asian Pacific Journal of Tropical Biomedicine*, 5, 509 – 514. https://doi.org/10.1016/j.apjtb.2015.05.001
38. Koilybayeva, M., Shynykul, Z., Ustenova, G., Abzaliyeva, S., Alimzhanova, M. Amirkhanova, A., Turgumbayeva, A., Mustafina, K., Yeleken, G., Raganina, K., *et al* . (2023). Molecular Characterization of Some Bacillus Species from Vegetables and Evaluation of Their Antimicrobial and Antibiotic Potency. *Molecules,* 28, 3210. https://doi.org/10.3390/molecules28073210
39. Laconi, A., Mughini-Gras, L., Tolosi, R., Grilli, G., Trocino, A., Carraro, L., *et al.* (2021). Microbial community composition and antimicrobial resistance in agricultural soils fertilized with livestock manure from conventional farming in Northern Italy. *Science of Total Environment.* 760, 143404. doi: 10.1016/j.scitotenv.2020.143404
40. Levinreisman, I., Ronin, I. & Gefen, O. (2017). Antibiotic tolerance facilitates the evolution of resistance. Science 355, 826–830. doi: 10.1126/science.aaj2191
41. Ljubojević, D., Pelić, M., Puvača, N. & Milanov, D. (2017). Resistance to tetracycline in *Escherichia coli* isolates from poultry meat: epidemiology, policy and perspective. *World's Pollution Science Journal,* 73: 409–417. 10.1017/S0043933917000216.
42. Mahmoud, M.A.M. & Abdel-Mohsein, H.S. (2019). Hysterical tetracycline in intensive poultry farms accountable for substantial gene resistance, health and ecological risk in Egypt- manure and fish. *Environmental Pollution*, 255, 113039. 10.1016/j.envpol.2019.113039.
43. Moroni, F. J., Gascon-Aldana, P. J. & Rogiers, S. Y. (2020). Characterizing the efficacy of a film-forming anti-transpirant on raspberry foliar and fruit transpira-tion. *Biology*, 9(9), 255. https://doi.org/10.3390/biology9090255

44. Ngbede, E.O., Raj, M.A., Kwanashie, C.N. & Kwaga, J.K.P. (2017). Antimicrobial resistance and virulence profile of enterococci isolated from poultry and cattle sources in Nigeria. *Tropical Animal Health and Production.* 49 (3),451-458. doi: 10.1007/s11250-016-1212-5.
45. Nsofor C.A., Olatoye I.O., Amosun E.A., Iroegbu C.U., Davis M.A., Orfe L.H. & Call D.R (2013). *Escherichia coli* from Nigeria exhibit a high prevalence of antibiotic resistance where reliance on antibiotics in poultry production is a potential contributing factor. *African Journal Microbiological Research.* 7, 4646–4654.
46. Nwiyi P., Chah K.F., Shoyinka S.V.O. (2018). Detection of some resistance genes in Salmonella isolated from poultry farms in Abia and Imo states, Southeastern Nigeria. Niger. *Veterinary Journal*. 39:124–132.
47. Adeyemi O.A, Fejukui, B. M. & Adeyemi, O.O. (2019). "Microbial contamination of fresh vegetable salads from food vendors in oyo metropolis," *Nigerian Journal of Pure and Applied Science*. 32; no. 1.
48. Olonitola, O.S., Fahrenfeld, N. & Pruden, A. (2015). Antibiotic resistance profile among mesophilic aerobic bacteria in Nigerian chicken litter and associated antibiotic resistance genes. *Poultry Science*, 94, 867 – 874. doi: 10.3382/ps/pev069.
49. Omojowo, F. & Omojasola, P.F. (2013). Antibiotic Resistance Pattern of Bacterial Pathogens Isolated from Poultry Manure Used to Fertilize Fish Ponds in New Bussa, Nigeria. *Albanian Journal of Agricultural Science*, 12 (1), 81-85
50. Park, K.M., Jeong, M., Park, K.J. & Koo, M. (2018). Prevalence, Enterotoxin Genes, and Antibiotic Resistance of Bacillus Cereus Isolated from Raw Vegetables in Korea. *Journal of Food Protection,* 81(10), 1590-1597. https://doi.org/10.4315/0362-028X.JFP-18-205.
51. Pezzuto, A., Belluco, S., Losasso, C., Patuzzi, I., Bordin, P., Piovesana, A., Comin, D., Mioni, R. & Ricci, A. (2016). Effectiveness of Washing Procedures in Reducing Salmonella enterica and Listeria monocytogenes on a Raw Leafy Green Vegetable (Eruca vesicaria). *Frontiers in Microbiology,* 7,1663. doi: 10.3389/fmicb.2016.01663
52. Samtiya, M., Matthews, K.R., Dhewa, T. & Puniya, A.K. (2022). Antimicrobial Resistance in the Food Chain: Trends, Mechanisms, Pathways, and Possible Regulation Strategies. *Foods.*11(19),2966. doi: 10.3390/foods11192966.
53. Sijhary, T.J., Bermann, M.L. & Enquist, L.W. (1984). Experiment with Gene Fusions 5th Edition, Cold Spring Harbor Laboratory Press, New York, USA.
54. Szott, V. & Friese, A. (2021). Emission sources of *Campylobacter* from agricultural farms, impact on environmental contamination and intervention strategies. *Current Topics in Microbiology and Immunology,* 431, 103–125. doi: 10.1007/978-3-030-65481-8_5
55. Tan, W., Wang, J., Bai, W., Qi, J. & Chen, W. (2020). Soil bacterial diversity correlates with precipitation and soil pH in long-term maize cropping systems. Scientific Report, 10:6012. doi: 10.1038/s41598-020-62919-7
56. Van T. T. H., Yidana Z., Smooker P. M. & Coloe P. J. (2020). Antibiotic use in food animals worldwide, with a focus on Africa: pluses and minuses. *Journal of Global Antimicrobial Resistance*, 20,170–177. doi: 10.1016/j.jgar.2019.07.031.
57. Wang, F., Sun, R., Hu, H., Duan, G., Meng, L. & Qiao, M. (2019). The overlap of soil and vegetable microbes drives the transfer of antibiotic resistance genes from manure-amended soil to vegetables. *Science of Total Environment,* 1, 828:154463. doi: 10.1016/j.scitotenv.2022.154463.
58. Wang, F.H., Qiao, M. & Chen, Z. (2015). Antibiotic resistance genes in manure-amended soil and vegetables at harvest. *Journal of Hazard Materials,* 299, 215–221. doi: 10.1016/j.jhazmat.2015.05.028.
59. Wang, J,, Zhang, Q., Chu, H. & Wang, Q. (2022). Distribution and co-occurrence patterns of antibiotic resistance genes in black soils in Northeast China, *Journal of Environmental Management*, 1:319:115640. doi: 10.1016/j.jenvman.2022.115640.
60. Xu, Q. & Zhang, M (2023). Effects of Combined Pollution of Tetracycline and Sulfamethazine on Tomato Growth and Antibiotic Absorption. *Agronomy,* 13, 762. https://doi.org/10.3390/agronomy13030762
61. Zhai, Z., Cui, C., Li, X., Yan, J., Sun, E., Wang, C., Guo, H. & Hao, Y (2023). Prevalence, antimicrobial susceptibility, and antibiotic resistance gene transfer of Bacillus strains isolated from pasteurized milk, *Journal of Dairy Science*, 106, 1, 75-83, https://doi.org/10.3168/jds.2022-22199.
62. Zhang, Y.J., Hu, H.W., Gou, M., Wang, J.T., Chen, D. & He. J.Z (2017). Temporal succession of soil antibiotic resistance genes following application of swine, cattle and poultry manures spiked with or without antibiotics. *Environ Pollut.* 231( 2),1621-1632. doi: 10.1016/j.envpol.2017.09.074.
63. Zhu, B., Chen, Q., Chen, S., Zhu & Y-G. (2017). Does organically produced lettuce harbor higher abundance of antibiotic resistance genes than conventionally produced? *Environ Int 98,* 152–159. doi: 10.1016/j.envint.2016.11.001.
64. Zurfluh, K., Nüesch-Inderbinen, M., Morach, M., Zihler, B.A., Hächler, H., Stephan, R. (2015). Extended-spectrum ß-lactamase-producing-Enterobacteriaceae in vegetables imported from the Dominican Republic, India, Thailand and Vietnam. *Appl Environ Microbiol*. *81,* 3115–3120. doi: 10.1128/AEM.00258-15.