Microbiological quality of irrigation water on highly diverse fresh produce smallholder farms: elucidating environmental routes of contamination

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Abstract

Aim: To evaluate the microbiological safety, potential multidrug resistant bacterial presence and genetic relatedness (DNA fingerprints) of *Escherichia coli* isolated from the water-soil-plant nexus on highly diverse fresh produce smallholder farms.

Methods and Results: Irrigation water (n=44), soil (n=85), and fresh produce (n=95) samples, from six smallholder farms with different production systems, were analysed for hygiene indicator bacterial counts and the presence of shigatoxigenic *E. coli* and *Salmonella* spp., using standard microbiological methods. Identities of isolates were confirmed using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and the genetic relatedness of the *E. coli* isolates determined using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-

PCR) analysis. Irrigation water *E. coli* levels ranged between 0 – 3.45 log MPN.100mL^{-1} with five farms having acceptable levels according to the World Health Organization limit (3 log MPN.100mL^{-1}). Fresh produce samples on four farms (n=65) harboured *E. coli* at low levels (<1 log CFU.g^{-1}) except for one sample from kale, spring onion, green pepper, onion and two tomato samples, which exceeded international acceptable limits (100 CFU.g^{-1}). Only one baby carrot fresh produce sample tested positive for *Salmonella* spp. Of the 224 samples, *E. coli* isolates were identified in 40% (n=90) of all water, soil and fresh produce types after enrichment. Additionally, the DNA fingerprints of *E. coli* isolates from the water-soil-plant nexus of each respective farm clustered together at high similarity values (>90%), with all phenotypically characterised as multidrug resistant.

Conclusions: The clustering of *E. coli* isolated throughout the water-soil-plant nexus, implicated irrigation water in fresh produce contamination. Highlighting the importance of complying with irrigation water microbiological quality guidelines to limit the spread of potential foodborne pathogens throughout the fresh produce supply chain.

Significance and impact of Study: Identifying contamination hotspots allows for the introduction of proper intervention strategies necessary for ensuring microbiological quality and furthermore, the potential spread of multidrug resistant pathogens in food-producing environments.

Keywords: *E. coli*, food safety, antimicrobial resistance, microbial contamination, irrigation water

Introduction

In peri-urban and rural areas of South Africa, subsistence farming has given rise to a high diversity of smallholder farmers, with different production systems, who not only grow fresh produce for their own use, but for retail to their local communities (Mdluli *et al.* 2013, Mugambiwa and Tirivangasi 2017). This trend is supported by growing consumer interest in fresh, healthy, and unprocessed foods, which has led to an increase in demand for whole and/or minimally processed fresh produce (Castro-Ibanez *et al.* 2017, Machado-Moreira *et al.* 2019). Typically, smallholder farmers have limited resources and lack access to water filtering and irrigation systems, thus rely mainly on often contaminated, easily-available water sources for crop production (van Koppen *et al.* 2017, Nephawe

et al. 2021). In South Africa, the majority (59%) of available water sources are used for irrigation purposes (Bonthuys 2018), which includes surface, borehole (groundwater), municipal, or rainwater (Iwu and Okoh 2019). However, the microbiological quality of water sources, in particular surface water, is frequently compromised due to municipal wastewater discharge, sewage from informal settlements, wastes from animal husbandry, industrial companies, hospital effluents, and the mining sector (Adesifoye and Okoh 2017, Verlicchi and Grillini 2020).

Irrigation water is increasingly being recognized as a major risk factor for microbiological contamination of fresh produce (Gurtler and Gibson 2022). Standards and guidelines are important to provide a framework for safe and quality water used for agricultural irrigation purposes and for promoting sustainable agricultural practices as well as protecting human health (Rock *et al.* 2021). Generally, international standards state that the microbial quality of water being used for irrigation purposes must be determined using a quantitative method, with an acceptable limit of <1000 *Escherichia coli*.100ml^{-1} (DWAF 1996, WHO 2006, Rock *et al.* 2021). Additionally, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have reported that relying solely on assessing *E. coli* levels in irrigation water to ensure food safety is not a suitable approach (FAO and WHO 2019). The report emphasized that *E. coli* levels do not adequately represent the variety of potential pathogens that could be present at a given time (FAO and WHO 2019). Other testing measures, such as physicochemical analyses, provide additional information and parameters important for measuring water quality (Iwu *et al.* 2020, Alegbeleye and Sant'Ana 2023). These measures typically include five crucial parameters: pH, temperature, total dissolved oxygen, conductivity, and turbidity levels (Bwire *et al.* 2020). Using contaminated water sources for irrigation can also lead to the potential transfer of pathogenic microorganisms like shiga-toxin producing *E. coli* (STEC) and *Salmonella* spp., onto crops, especially those that are consumed raw and undergo minimal post-harvest processing (Self *et al.* 2019, CDC 2020). Many smallholder farmers in South Africa do not have access to water sources that meet acceptable standards and regulations, thus rely on sources that are often contaminated and exceed the set-out guidelines (Iwu *et al.* 2021, Nephawe *et al.* 2021). Moreover, researchers have reported the need for fit-for-purpose standards for irrigation water used in the informal sector considering these unique challenges present, particularly in water

sources that generally do not meet the current microbiological quality standards (du Plessis *et al.* 2021).

Although most fresh produce contains harmless microorganisms, human pathogenic bacteria such as *E. coli* and *Salmonella* spp. may be introduced during the production and processing stages through the use of manure-amended soil, contaminated irrigation water, and unhygienic handling practices (Tope *et al.* 2016, Alegbeleye *et al.* 2018). Indeed, fresh fruits and vegetables are a common pathway for transmission of foodborne pathogens and are a primary cause of foodborne disease outbreaks (Murray *et al.* 2017). Produce types, including leafy greens, tomatoes, and bell peppers, among others have been found to be more frequently implicated in fresh produce-associated disease outbreaks (Gambushe *et al.* 2022, Rocha *et al.* 2022). Shiga-toxin producing *E. coli* (STEC) are responsible for causing diseases in humans worldwide (Rubab and Oh 2020). While there are over 100 serotypes that are highly pathogenic to humans, serotype O157:H7 is the main cause of food- and water-borne infections (Gambushe *et al.* 2022).

The most recent USA multistate *E. coli* O157:H7 outbreak connected to romaine lettuce was reported by the Center for Disease Control (CDC) in 2020 (CDC 2020). Another *E. coli* O157:H7-associated outbreak that led to 250 cases, with 96 hospitalizations and five deaths, also reported contaminated romaine lettuce as the source (CDC 2018). Additionally, *Salmonella* spp. have been linked to foodborne outbreaks, with reported cases of salmonellosis connected to onions in 2023, resulting in 73 confirmed cases and 15 hospitalisations (CDC 2023). Moreover, another outbreak caused by *Salmonella* spp. was traced back to the consumption of leafy vegetables, infecting a total of 31 individuals with four hospitalisations in 2021 (CDC 2021). Machado – Moreira *et al.* (2019) reported that contaminated leafy greens were associated with just over half of the reported outbreak cases in low- and middle-income countries (LMICs) worldwide, involving both *E. coli* and *Salmonella* spp. Through enterobacterial repetitive intergenic consensus (ERIC) PCRs, enteric microbes can be linked to isolates from a particular host to identify the possible sources of microbial contamination in environmental samples such as water, soil, and food (Abakpa *et al.* 2017, Alsultan *et al.* 2022). Previous literature has shown the link between *E. coli* from animal species and their corresponding food products as well as *E. coli* from contaminated irrigation water and associated fresh produce

(Richter *et al.* 2021, Alsultan *et al.* 2022). Moreover, multidrug resistant *Escherichia coli* has been linked from farm to retail in commercial fresh produce production cross-sectional studies (Richter et al. 2022; Ratshilingano et al. 2022).

Antimicrobial resistance (AMR) is considered one of the major challenges to global health, ranking among the top ten threats to public health (WHO 2020). This led to the WHO recently publishing a protocol on integrated surveillance systems globally, particularly focusing on extended-spectrum beta-lactamase (ESBL) -producing *E. coli* as an indicator organism (WHO 2021). The preharvest environment on farms plays a significant role in the emergence and spread of multidrug-resistant (MDR) pathogenic bacteria (Iwu *et al.* 2022). Previous studies have reported on the presence of MDR potential pathogenic bacteria in the water-plant-food nexus (Holzel *et al*. 2018, Vital *et al*. 2018, le Terrier *et al*. 2020, Richter *et al*. 2021). However, studies specifically focussing on the occurrence of antimicrobial resistant bacteria isolated from the water-plant-food nexus in the informal fresh produce production environments remain limited (Richter *et al*. 2023). Surveillance of potential pathogenic foodborne pathogens, including microbial source tracking is a critical aspect of disease outbreak assessment and food safety assurance systems (WHO 2021, Richter *et al.* 2023). Unfortunately in South Africa, foodborne illnesses are often not reported or under-reported and inadequately investigated (Bisholo *et al.* 2018, Richter *et al.* 2023). Preventing foodborne illnesses associated with produce requires safe production practices, including use of uncontaminated water for irrigation, washing, or processing (Gerdes *et al.* 2022, Alegbeleye and Sant'Ana 2023). This study aimed to determine the microbiological quality and presence of potential human pathogenic *E. coli* (STEC O157:H7 and non-O157 STEC) as well as *Salmonella* spp. in irrigation water, soil, and associated fresh produce from six highly diverse smallholder farms, with different farming practices, in three South African provinces. Additionally, the phenotypic (antibiotic resistance profiles) and genotypic (ERIC-PCR analysis) characteristics of the isolates were determined, to evaluate the potential link between isolates from water, soil, and fresh produce samples.

Materials and Methods

Sampling study areas

Samples were collected from six different smallholder farms from three provinces (Gauteng, North West and Limpopo) in South Africa. The farms (Farms A-F) differed in terms of the water sources and irrigation methods used, the produce farmed, and the production practices (i.e., aquaculture, integrated farming, organic or conventional) (Table 1). Farm A practiced integrated farming by producing both fresh produce and fish, through an aquaculture/aquaponics system (where production was for both household income and consumption). Borehole water was used as the irrigation source, and subsequently pumped through the aquaculture system. Irrigation areas included an aquaponics system and a net-covered field (Table 1). Farm B practiced integrated farming by producing both fresh produce and raising livestock (pigs), where production was for both household consumption and income. The water source on the farm was from a borehole, which was used for irrigation, with pig manure added directly to the fields as a soil amendment (Table 1). Farm C practiced organic farming and produced a variety of fresh produce (spinach, radishes, rocket, and lettuce) using borehole water via overhead sprinklers (Table 1). Organic compost piles were used as a fertiliser, while the farmed produce was for both household consumption and income. Farm D practiced conventional farming and produced fresh produce (baby carrots, kale, rocket, spring onions and leeks) using river water via overhead irrigation. The farm supplied fresh produce to various local formal retailers, as well as the local community. Farm E practiced integrated farming by producing fresh produce (tomatoes, bell peppers, spring onions, and maize) as well as raising livestock (goats and sheep). The main irrigation water source was supplied by the Rust de Winter dam where water was stored, until pumped onto the fields. The fresh produce on the farm was mostly irrigated via overhead irrigation, except for the green pepper fields which were irrigated via a drip irrigation system (Table 1). Farm F practiced integrated farming by producing fresh produce (spinach, bell peppers, cabbages and onions) as well as raising chickens. The main water supply was municipal water, used to irrigate the fresh produce on the farm via an extensive drip irrigation system. The soil in the fields were amended with chicken manure and liquid fertiliser through the irrigation system (Table 1).

Table 1: Summary of the cultivation and production practices of the six smallholder farms assessed in the current study.

Site	Province	Scenario	Water source/s	Produce sampled	Irrigation method	Additional observations and information
Farm A	Gauteng	Aquaculture	Borehole water Aquaculture system used for nutrient enrichment before irrigation	Kale, spinach and lettuce	Aquaponics and overhead irrigation (hosepipe)	Kale was grown in rocks Tilapia was produced as well
Farm B	Gauteng	Integrated	Borehole water	Kale, spinach and rape	Overhead irrigation (sprinklers)	Pig and chicken manure added directly to fields as fertiliser
Farm C	Gauteng	Organic	Borehole water	Lettuce, radishes and rocket	Overhead irrigation	Soil was amended with compost
Farm D	North West	Conventional river	River - canal system	Baby carrots, leeks, spring onions and rocket	Overhead irrigation	Supplied retailers and exported selected produce GLOBAL'GAP certified
Farm E	Limpopo	Conventional dam	Dam - canal system	Tomatoes, green peppers and spring onions	Overhead irrigation (Tomatoes/spring onions) Drip irrigation (green peppers)	Goats and sheep were also raised
Farm F	Gauteng	Conventional municipal	Municipal water	Spinach, green peppers and onions	Drip irrigation	Chicken manure was used to amend the fields Liquid fertiliser was used in irrigation system

Sample collection

A total of 235 samples were collected at selected sampling points from Farms A to F, depending on the production system and availability of fresh produce types. With 224 samples designated for microbiological analysis and the remaining 11 samples for physicochemical analysis. A total of 55 water samples were collected from each farm at each point in the irrigation system, i.e., either borehole water samples (n = 31), river water samples (n = 12) or municipal water samples (n = 12). The samples consisted of 100 mL, 1 L and 100 L samples collected from each respective point of the irrigation system on each farm, i.e., the water source, storage dams and irrigation points where available. The 100 mL and 1 L water samples were collected in ethanol-sterilized, air-dried plastic bottles at each respective site. The 100 L water samples were collected by filtering water at each water source site through a kidney dialysis filter (FDA 2021). Soil samples (n=85), of approximately 50g each, were collected where the fresh produce was harvested on each farm. Based on availability, at least three different types of fresh produce samples were collected at harvest at each respective farm (n = 95). Of each leafy fresh produce type (lettuce, spinach, rape, kale and rocket), five replicates were collected in an unbiased random manner across the fields. Additionally, whole produce types, consisting of a composite of at least three tomatoes/baby carrots/radishes/green peppers/onions were collected using a 70 % ethanol sterilised knife. All samples were stored and transported in a cooler box at 4 ºC until analysis within 24 hours.

Sample processing, enumeration for hygiene indicator bacteria (coliforms/*Escherichia coli*) and physicochemical characterisation of irrigation water

Water. Each of the 100 mL water samples was used for the enumeration of coliforms and *E. coli* using the MPN-based Colilert-18 system according to the manufacturer's instructions (IDEXX Laboratories Incorporated, Westbrook, ME, USA). The coliform and *E. coli* counts were recorded as log MPN.100mL^{-1}. One litre water samples were delivered within 24 hours to the Council for Scientific and Industrial Research (CSIR), and/or WaterLab (Pty) Ltd for measurement of the biological oxygen demand (BOD), chemical oxygen demand (COD), pH, turbidity, total dissolved solids (TDS), and total suspended solids (TSS) levels. Additionally, all tests were performed

according to the ISO 17025 standard in SANAS accredited (T0391) laboratories for physicochemical analyses.

Soil. A 25 g soil sample collected from each fresh produce sample type was weighed and added to 225 mL of buffered peptone water (BPW) (Merck, Johannesburg). To enumerate coliforms and *E. coli* in soil, a tenfold serial dilution of each BPW sample mixture was plated in duplicate onto *E.coli*/coliform count plates and incubated for 24 hours at 37 ºC according to the manufacturer's instructions (3M Petrifilm, 3M, St. Paul, Minnesota, USA, ISO method 4832). The remaining soil/BPW mixtures were incubated at 37 ºC for 24 hours for detection of potential human pathogens.

Fresh produce. A 50 g composite sample of each respective leafy vegetable bunch was aseptically cut and subsequently placed into a pre-labelled sterile polyethylene strainer stomacher bag containing 200 mL of BPW, in a 1:4 weight to volume ratio (3M, Johannesburg) (Richter *et al.* 2019). For other vegetable sample types (tomatoes, baby carrots, onions, and green peppers) 150 g of each was placed into pre-labelled sterile polyethylene strainer stomacher bags containing 150 mL BPW, in a 1:1 weight-to-volume ratio (Richter *et al.* 2019). To enumerate coliforms and *E. coli* in fresh produce, a tenfold serial dilution of each BPW sample mixture (obtained from the stomacher bag) was plated in duplicate as described for the soil samples. The remaining fresh produce/BPW mixtures were incubated at 37 ºC for 24 hours, for detection of foodborne pathogens as described previously.

Detection and isolation of potential pathogenic bacteria

The 1 L water samples were filtered through nitrocellulose membranes (0.45µm pore size, Sartorius, Gottingen, Germany). The membrane was subsequently placed into 50 mL BPW and incubated for 24 hours at 37°C for further selective enrichment of *E. coli* (non-O157 and O157) and *Salmonella* spp. as described below. The kidney dialysis filter membranes, used to filter the 100 L water samples, were back-flushed with 2.5 L Tween-80 dH20 and the subsequent back-flushed liquid was filtered through 0.45µm nitrocellulose membranes similar to the 1 L water samples.

***Escherichia coli* isolation.** Following incubation, each of the enriched water, soil and fresh produce samples were plated onto RAPID'E. coli two agar plates (Bio-Rad, Johannesburg), using the streak-plate method, and incubated at 37 ºC for 24 hours for isolation of typical *E. coli* colonies. Additionally, 1 mL of each sample was incubated in 9 mL STEC selective enrichment broth (SEB)

(Bio-Rad) for detection of shiga- toxin *Escherichia coli* O157:H7 and non-O157:H7 *E. coli* and incubated at 37 ºC for 24 hours. Following enrichment, the samples were streaked onto chromID O157:H7 agar for detection of typical *E. coli* O157:H7 colonies using the streak plate method.

***Salmonella* spp. isolation.** Of each BPW enriched sample, 1 mL was further incubated in 9 mL Rappaport-Vassiliadis (RV) Salmonella enrichment broth (Merck, Johannesburg) at 42 ºC for 24 hours. Subsequently, the presence of *Salmonella* spp. was assessed using the iQ-Check Salmonella II kit (BioRad) according to the manufacturer's instructions. Positive results obtained were then plated onto Xylose lysine deoxycholate (XLD) agar plates (Biolabs, Johannesburg) and subsequently incubated for 24 hours at 37 ºC. The presence of typical black colonies was then visualised, and the identity was confirmed using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS).

Isolate identification using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry.

All presumptive positive *E. coli* and *Salmonella* spp. colonies were isolated and purified, after which the isolates were identified using MALDI-TOF MS (Bruker, Bremen, Germany). MALDI-TOF MS identifies isolates to the species level (Standing *et al*. 2013) (AOAC-OMA#2017.09) as described by the manufacturer. Briefly, the purified cultures were aseptically transferred onto a MALDI-TOF target plate, then overlaid with α-cyano-4-hydroxycinnamic acid matrix (Bruker) and allowed to air dry. The target plate was then analysed using MicroFlex LT MALDI-TOF (Bruker) in conjunction with the Biotyper automation software and library (Bruker), following calibration with a bacterial standard according to the manufacturer's instructions (Bruker). An organism's identity is considered reliable for identification at the species level with a score value ranging between 2.30-3.00, whilst the best organism match value between 2.00 - 2.29 was considered reliable at the genus level. All isolates with confirmed identities were preserved in 65 % glycerol at -20 ºC for future use.

Antimicrobial susceptibility testing

A total number of 90 *E. coli* isolated from the water, soil, and fresh produce samples, were phenotypically characterised using the Kirby-Bauer disk diffusion technique as to determine the resistance patterns of the isolates (Clinical Laboratory Standard Institute [CLSI] 2020). The isolates

were cultured in 9 mL Tryptone Soy Broth (TSB) (Merck) and incubated for 24 h at 37 ºC. From each TSB sample, 100 µl was subsequently cultured in 9 mL of Brain Heart Infusion (BHI) broth (Merck) and incubated at 37 ºC for 24 h. A 120 µl aliquot was then plated, in duplicate, onto Mueller-Hinton (MH) agar plates (ThermoFisher Scientific) and incubated at 37 ºC for 24 h, after which each respective zone diameters were measured. The zone diameters were then compared to the CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria to determine if each respective isolate is resistant, intermediately resistant, or susceptible to each antibiotic (CLSI 2020 and EUCAST 2024). Additionally, the results were interpreted using the BioNumerics software program (Applied Mathematics, South Africa), with isolates resistant to three or more different antibiotic classes defined as multidrug-resistant (MDR). The antibiotics that were tested for resistance or susceptibility of each respective isolate included gentamicin (10 µg), streptomycin (10 µg), aztreonam (30 µg), chloramphenicol (30 µg), ampicillin (10 µg), amoxicillin (10 µg), imipenem (10 µg), meropenem (10 µg), norfloxacin (10 µg), ciprofloxacin (10 µg), tigecycline (15 µg), azithromycin (15 µg), erythromycin (15 µg), nalidixic acid (30 µg), trimethoprim-sulfamethoxazole/cotrimoxazole (1,25 µg/ 23,75 µg), tetracycline (30 µg), cefoxitin (30 µg), cefpodoxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg) and cefepime (30 µg) (Mast Diagnostics, Bootle, UK, supplied by Davies Diagnostics, Midrand, SA).

Molecular screening of *Escherichia coli* O157:H7 and non-O157:H7 isolates.

The presence of enterohaemorrhagic *E. coli* (EHEC) (*eaeA*, *stx1* and *stx2* genes) virulence genes were analysed by PCR, with the *mdh* gene used as an internal control in all reactions (Omar and Barnard 2010a). Control strains for the PCR reactions included *E. coli* O157:H7 ATCC 43890 (EHEC) as the positive control; and *E. coli* ATCC 25922 as the negative control. Single colonies of each *E. coli* isolate were cultured in 9ml TSB (Merck) for 24 hours at 37 ºC. The cells were pelleted by centrifugation (12 500g for 3 min), the DNA extracted using the Quick-gDNA mini-prep kit according to manufacturer's instructions (Zymo Research, Irvine, USA) and the concentration determined using the Qubit dsDNA Broad Range Assay and a Qubit 2.0 fluorometer (Life Technologies, Johannesburg). PCR was performed using a 2x DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Johannesburg), with specific primers, and thermocycling conditions for

each of the genes (Supplementary table S1). All reactions were run on a Bio-Rad T100 thermal cycler (Bio-Rad) and the amplicons visualised on a 2 % agarose gel stained with Conda®-Safe (Carl Roth GmbH & Co, Germany), and analysed using a Bio-Rad GelDoc XR in conjunction with the Image LabTM software (BioRad).

Genomic fingerprinting of *Escherichia coli* isolates by repetitive PCR.

The same *E. coli* isolates analysed for antimicrobial susceptibility and virulence genes were used to conduct repetitive PCR through the generation of enterobacterial repetitive intergenic consensus (ERIC)-PCR fingerprints for each of the isolates from the individual smallholder farms. The primer sequences and thermocycling conditions used to generate the ERIC-PCR fingerprints are summarised in Supplementary table S1. Following visualisation of the PCR amplicons on a 2 % agarose gel the band patterns were analysed digitally using BioNumerics 7.6 (Applied Maths, Saint-Marten-Latem, Belgium).

Statistical analysis

The coliform and *E. coli* counts data was analysed using the SAS version 9.3 statistical software (SAS/STAT User's Guide 1999), to determine significant differences between counts in different sample types. The Shapiro-Wilk test was performed on the standardised residuals to test for deviations from normality (Shapiro and Wilk 1965). Student's protected t-LSD (Least significant difference) was calculated at a 5 % significance level to compare means of significant source effects (Snedecor and Cochran 1980). The boxplots, representing the coliform and *E. coli* counts, in the microbiological quality analysis section below, were generated using GraphPad Prism version 9.0.0 (GraphPad Software, California, USA).

Results

Physicochemical analyses of water samples

The overall physicochemical values for the source, storage and irrigation water samples collected along the irrigation system for each Farm A-F are shown in Table 2, as well as the Department of Water Affairs and Forestry (DWAF) recommended guidelines for each test. The physicochemical values displayed are measures of the biological oxygen demand (BOD), chemical oxygen demand

Table 2: Physicochemical analyses of water samples from various smallholder farms around South Africa as well as the guidelines for irrigation water recommended by the Department of Water Affairs and Forestry (DWAF)

Reference – recommended guidelines			BOD ($mg.L^{-1}$)	COD ($mg.L^{-1}$)	pH	TDS ($mg.L^{-1}$)	TSS ($mg.L^{-1}$)	Turbidity (NTU)
Department of Water Affairs and Forestry (DWAF), 1996			<30	<250	6-9	<450	<37.4	<1 (not >5)
Farm		**Sample type**	**BOD ($mg.L^{-1}$)**	**COD ($mg.L^{-1}$)**	**pH**	**TDS ($mg.L^{-1}$)**	**TSS ($mg.L^{-1}$)**	**Turbidity (NTU)**
Scenario	**Number**							
Aquaculture	A	Aquaculture	14	125	7.21	535	15	6.6
Integrated	B	Borehole source	10	10	8.33	220	0.3	0.25
Organic	C	Borehole source	10	16	6.76	37	0.3	0.5
Conventional/river	D	River source	144	206	7.2	178	24	11
		Holding dam	10	10	8.1	176	9.3	15
		Overhead irrigation point	10	10	7.9	186	185	38
Conventional/dam	E	Holding dam	10	20	9.7	46	3.3	2
		Overhead irrigation point	10	12	8.3	70	5.3	17
Conventional/municipal	F	Municipal source	10	12	7.8	196	1	0.3
		Storage tank	10	16	7.8	168	1	0.3
		Drip irrigation point	10	12	7.8	168	1	0.3

*BOD - Biological oxygen demand, *COD - Chemical oxygen demand, *TDS - Total dissolved solids, *TSS - Total suspended solids

(COD), pH, total dissolved solids (TDS), total saturated solids (TSS) and turbidity levels in each respective sample (Table 2).

Biological oxygen demand levels. The BOD level for the aquaculture water sample (Farm A) was found to be 14 $mg.L^{-1}$, while the borehole water, collected from the integrated (Farm B) and organic (Farm C) farms were 10 $mg.L^{-1}$ (Table 2). The river source water sample (Farm D) had a level of 144 $mg.L^{-1}$, whereas the holding dam and overhead irrigation water samples had levels of 10 $mg.L^{-1}$ (Table 2). The river water exceeded the recommended standard, as suggested by DWAF for irrigation water (Table 2) (DWAF 1996) and was the highest level amongst all the water samples collected. The holding dam and irrigation point water samples from Farm E both had the same level of 10 $mg.L^{-1}$. Similarly, the municipal source, storage tank, and drip irrigation water samples from Farm F all had a level of 10 $mg.L^{-1}$ (Table 2).

Chemical oxygen demand levels. The aquaculture water sample had a COD level of 125 $mg.L^{-1}$, in contrast to the borehole water source samples' from Farm B (integrated) and Farm C (organic) at 10 and 16 $mg.L^{-1}$, respectively (Table 2). The river source water sample (Farm D) had a level of 206 $mg.L^{-1}$, whereas the holding dam and overhead irrigation water samples had a level of 10 $mg.L^{-1}$ (Table 2). Similar to the BOD levels, the COD level for the river water was the highest amongst the rest of the water samples from all the farms in the study. The holding dam and irrigation point water samples collected from Farm E both had levels of 20 and 12 $mg.L^{-1}$ respectively (Table 2). Lastly, from Farm F, the municipal source and drip irrigation water samples both had a level of 12 $mg.L^{-1}$, whereas the storage tank levels was 16 $mg.L^{-1}$ (Table 2). None of the water samples, from Farms A-F, exceeded the recommended guidelines (Table 2) (DWAF 1996).

pH values. The pH for the aquaculture water sample was 7.21, whereas the borehole source water from Farm B and C were 8.33 and 6.76, respectively (Table 2). On Farm D, the river source water sample had a pH of 7.2, in contrast to the holding dam and overhead irrigation water samples at 8.1 and 7.9, respectively (Table 2). The holding dam and irrigation point water samples collected from Farm E had values of 9.7 and 8.3, respectively, with the holding dam water having the highest pH level as well as exceeding DWAF regulations (pH = 6-9) (Table 2) (DWAF 1996). Interestingly, the

municipal water source, storage tank and drip irrigation samples of Farm F all had a neutral pH of 7.8 (Table 2).

Total dissolved solids levels. The TDS levels for the aquaculture water sample was 535 $mg.L^{-1}$, in contrast to the borehole water source samples' from Farm B and C, at 220 and 37 $mg.L^{-1}$, respectively (Table 2). The aquaculture water sample exceeded the recommend guidelines (Table 2) (DWAF 1996), and was the highest level compared to the water samples from the other farms. The river source water sample had a level of 178 $mg.L^{-1}$, whereas the holding dam water and overhead irrigation water samples had levels of 176 and 186 $mg.L^{-1}$, respectively (Table 2). The holding dam and irrigation point water samples of Farm E had levels of 46 and 70 $mg.L^{-1}$, respectively. Lastly, the municipal source, storage tank and drip irrigation water samples from Farm F had levels at 196, 168, and 168 $mg.L^{-1}$ respectively (Table 2).

Total suspended solids levels. The aquaculture water sample's TSS levels was found to be 15 $mg.L^{-1}$, in contrast to the borehole water source samples (Farm B and C) at 0.3 mg/L (Table 2). The river source water sample (Farm D) had a level of 24 $mg.L^{-1}$, whereas the holding dam water had a level of 9.3 $mg.L^{-1}$ and the overhead irrigation water sample a level of 185 $mg.L^{-1}$ (Table 2). The irrigation point sample was the only water sample to exceed the recommended standards (Table 2) (DWAF 1996) and was the highest TSS level amongst all the water samples. The holding dam and irrigation point water samples had levels of 3.3 and 53 $mg.L^{-1}$ respectively for Farm E. Lastly the municipal source, storage tank and drip irrigation water samples from Farm F all had a level of 1 $mg.L^{-1}$ (Table 2).

Turbidity levels. The turbidity for the aquaculture water was found to be 6.6 NTU, in contrast to both borehole water samples, from Farm B (0.25 NTU) and Farm C (0.5 NTU) (Table 2). Additionally, the aquaculture water was above the recommended standards (Table 2) (DWAF 1996). On Farm D, the turbidity increased as the river source water (11 NTU) flowed throughout the irrigation system, at the holding dam (15 NTU) and overhead irrigation point (38 NTU) (Table 2). Moreover, the turbidity levels of the entire irrigation system on Farm D exceeded the recommended standards (Table 2) (DWAF 1996), with the irrigation water point samples having the highest level

compared to the other water samples. The holding dam and irrigation point water samples of Farm E had levels of 2 and 17 NTU, respectively, with the later turbidity level above the standards (Table 2). Interestingly the municipal source, storage tank, and drip irrigation water samples of Farm F all had a turbidity level of 0.3 NTU (Table 2).

Microbiological quality analyses

The coliform and *E. coli* counts for the water, soil, and fresh produce samples for Farms A-F are shown in Figures 1 and 2. Additionally, the fluctuations of counts within each respective farm and results of statistical analysis are shown in Tables 3 to 5.

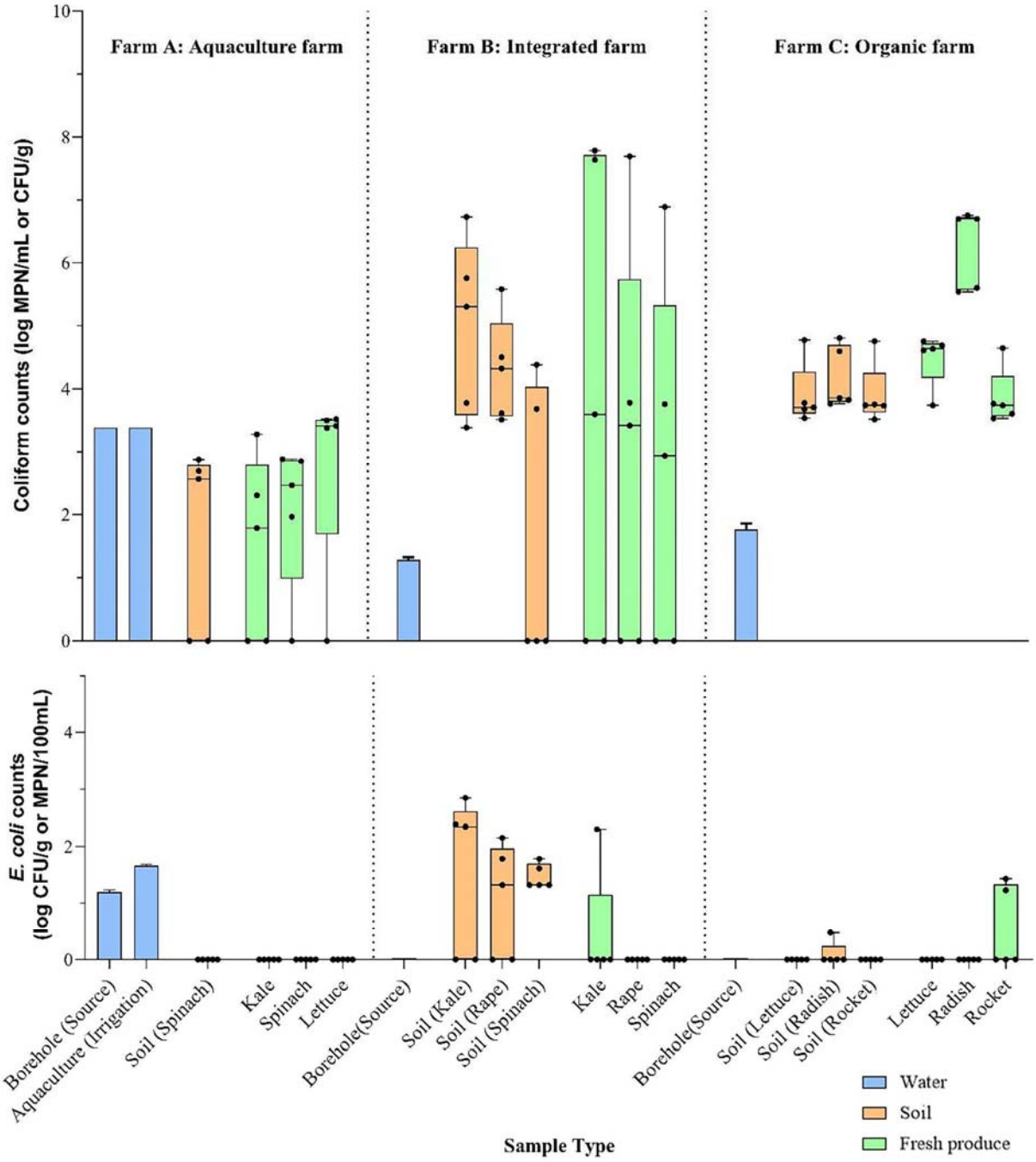

Figure 1: Indicator bacteria levels for water, soil and fresh produce samples collected from three smallholder farms (aquaculture, integrated and organic farms), who used borehole water as an irrigation source, as well as manure or compost as a fertiliser for fresh produce production. Water counts were measured as log MPN.100mL^{-1}, with soil and fresh produce counts as log CFU.g^{-1}. Water samples are represented in blue, soil samples in orange and fresh produce in green. CFU – colony forming unit; MPN – most probable number.

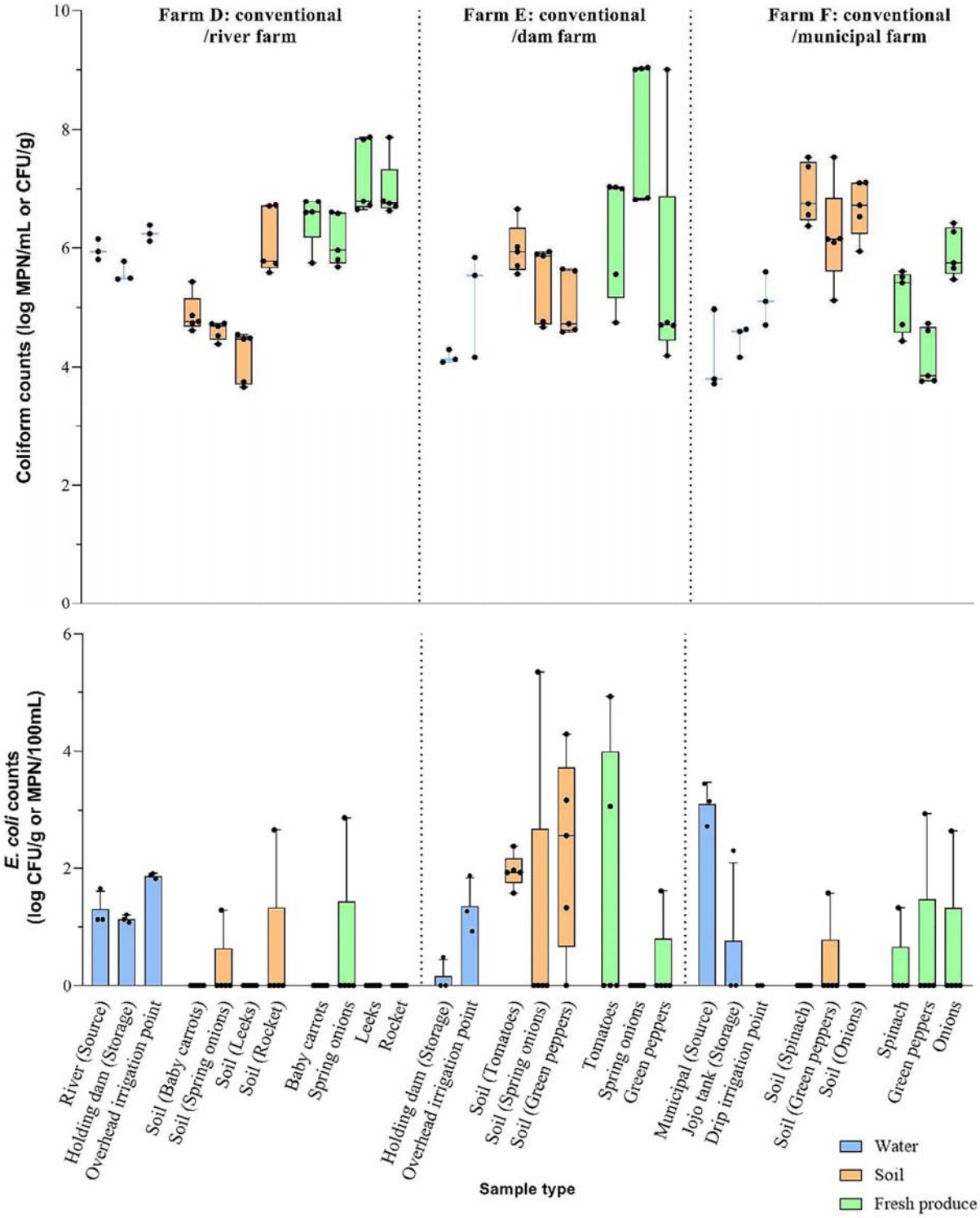

Figure 2: Indicator bacteria levels for water, soil and fresh produce samples collected from three smallholder farms (all conventional) who used either river (D), dam (E) or municipal (F) water as an irrigation source for fresh produce production. Water counts were measured as log MPN.100mL^{-1}, with soil and fresh produce counts as log CFU.g^{-1}. Water samples are represented in blue, soil samples in orange and fresh produce in green. CFU – colony forming unit; MPN – most probable number.

Table 3 Total Coliform and *Escherichia coli* loads present in water samples from smallholder farms in South Africa, at a 95% lower and upper confidence limit

Farm	Number of samples (% harbouring coliforms)	Total coliforms (log MPN.100mL^{-1})				Number of samples (% harbouring *E. coli*)	*E. coli* (log MPN.100mL^{-1})			
		Range	Lower	Upper	Mean		Range	Lower	Upper	Mean
Farm A (Aquaculture/aquaponics)										
Borehole (Source)	3 (100)	> 3.38	N/A	Infinite	3.38	3 (100)	1.16 - 1.24	0.95	1.41	1.2
Irrigation (Aquaponics)	3 (100)	> 3.38	N/A	Infinite	3.38	3 (100)	1.65 - 1.69	1.50	1.81	1.66
Farm B (Mixed farming)										
Borehole (Source)	3 (100)	1.24 - 1.31	1.06	1.49	1.28	3 (0)	0	0	0.63	0
Farm C (Organic farming)										
Borehole (Source)	3 (100)	1.67 - 1.86	1.61	1.9	1.76	3 (0)	0	0	0.63	0
Farm D (Conventional/river)										
River (Source)	3 (100)	5.81 - 6.15	5.83	6.11	5.96	3 (100)	1.13 - 1.65	1.08	1.5	1.30
Holding dam (Storage)	3 (100)	5.47 - 5.77	5.48	5.67	5.58	3 (100)	1.08 - 1.20	0.88	1.42	1.14
Irrigation (Overhead)	3 (100)	6.11 - 6.38	6.07	6.46	6.24	3 (100)	1.82 - 1.91	1.72	2	1.87
Farm E (Conventional/Dam-canal)										
Holding dam (Storage)	3 (100)	4.08 - 4.29	0.92	1.39	4.17	1 (33)	0.49	0.15	0.95	0.16
Irrigation (Overhead)	3 (100)	4.16 - 5.83	1.99	2.35	5.18	3 (100)	0.93 - 1.87	1.12	1.55	1.36
Farm F (Conventional/Municipal)										
Municipal (Source)	3 (100)	3.72 - 4.97	3.88	4.44	4.16	3 (100)	2.72 - 3.45	2.8	3.34	3.12
Jojo tanks (Storage)	3 (100)	4.16 - 4.64	4.27	4.64	4.47	2 (66)	0.94 - 2.23	0.65	2.85	1.06
Irrigation point (Drip)	3 (100)	4.7 - 5.60	4.98	5.28	5.14	3 (0)	0	0	0.63	0

Table 4 Total coliform and *Escherichia coli* loads present in soil samples, collected during fresh produce harvesting, from smallholder farms in South Africa

Farm	Number of samples (% harbouring coliforms)	Total coliforms (log CFU.g^{-1})		Number of samples (% harbouring *E. coli*)	*E. coli* (log CFU.g^{-1})	
		Range	Mean ± SE		Range	Mean ± SE
Farm A (Aquaculture/aquaponics)						
Soil (Spinach)	5 (60)	0 - 2.88	2.71 ± 0.06	5 (0)	0	0
Total	5 (60)			5 (0)		
Farm B (Mixed farming)						
Soil (Kale)	5 (100)	3.38 - 6.73	4.31 ± 0.46	5 (60)	0 - 2.85	2.52 ± 0.10
Soil (Rape)	5 (100)	3.51 - 5.58	4.50 ± 0.36	5 (60)	0 - 2.15	0.60 ± 0.38
Soil (Spinach)	5 (40)	0 - 4.38	2.69 ± 0.86	5 (100)	1.32 - 1.79	1.57 ± 0.09
Total	15 (80)			15 (73)		
Farm C (Organic farming)						
Soil (Lettuce)	5 (100)	3.53 - 4.77	3.72 ± 0.02	5 (0)	0	0
Soil (Radishes)	5 (100)	3.76 - 4.80	4.09 ± 0.16	5 (20)	0 - 0.48	0
Soil (Rocket)	5 (100)	3.51 - 4.75	3.9 ± 0.19	5 (40)	0 - 1.43	0.89 ± 0.29
Total	15 (100)			15 (20)		
Farm D (Conventional/river)						
Soil (Baby carrots)	5 (100)	4.74 - 5.43	4.88 ± 0.13	5 (0)	0	0
Soil (Spring onions)	5 (100)	4.39 - 4.73	4.61 ± 0.06	5 (0)	0	0
Soil (Leeks)	5 (100)	3.66 - 4.55	4.19 ± 0.18	5 (0)	0	0
Soil (Rocket)	5 (100)	5.58 - 6.72	6.11 ± 0.22	5 (20)	0 - 2.66	0.89 ± 0.56
Total	20 (100)			15 (5)		
Farm E (Conventional/Dam-canal)						
Soil (Tomatoes)	5 (100)	5.56 - 6.65	5.97 ± 0.17	5 (100)	1.57 - 2.38	1.95 ± 0.15
Soil (Spring onions)	5 (100)	4.67 - 5.94	5.43 ± 0.26	5 (20)	0 - 5.35	1.78 ± 1.13
Soil (Green pepper)	5 (100)	4.59 - 5.61	5.04 ± 0.22	5 (80)	0 - 4.29	2.27 ± 0.54
Total	15 (100)			15 (67)		
Farm F (Conventional/Municipal)						
Soil (Spinach)	5 (100)	6.37 - 7.53	6.92 ± 0.20	5 (0)	0	0
Soil (Green pepper)	5 (100)	5.11 - 7.54	6.21 ± 0.35	5 (20)	0 - 1.57	0.52 ± 0.33
Soil (Onions)	5 (100)	5.94 - 7.11	6.68 ± 0.19	5 (0)	0	0
Total	15 (100)			15 (7)		

Table 5 Total coliform and *Escherichia coli* loads present in fresh produce samples from smallholder farms in South Africa

Farm	Number of samples (% harbouring coliforms)	Total coliforms (log CFU.g^{-1})		Number of samples (% harbouring *E. coli*)	*E. coli* (log CFU.g^{-1})	
		Range	Mean ± SE		Range	Mean ± SE
Farm A (Aquaculture/aquaponics)						
Kale	5 (100)	0 - 3.28	1.47 ± 0.38	5 (0)	0	0
Spinach	5 (100)	0 - 2.88	2.03 ± 0.57	5 (0)	0	0
Lettuce	5 (100)	0 - 3.52	2.76 ± 0.73	5 (0)	0	0
Total	15 (100)			15 (0)		
Farm B (Mixed farming)						
Kale	5 (100)	0 - 7.79	2.60 ± 1.64	5 (20)	0 - 2.3	0
Rape	5 (100)	0 - 7.70	3.82 ± 1.41	5 (0)	0	0
Spinach	5 (100)	0 - 6.89	2.30 ± 1.45	5 (0)	0	0
Total	15 (100)			15 (7)		
Farm C (Organic farming)						
Lettuce	5 (100)	3.74 - 4.75	4.39 ± 0.21	5 (0)	0	0
Radishes	5 (100)	5.54 - 6.76	5.95 ± 0.24	5 (0)	0	0
Rocket	5 (100)	3.53 - 4.64	3.62 ± 0.04	5 (0)	0	0
Total	15 (100)			15 (0)		
Farm D (Conventional/river)						
Baby carrots	5 (100)	5.75 - 6.79	6.50 ± 0.17	5 (0)	0	0
Spring onions	5 (100)	5.68 - 6.60	6.13 ± 0.18	5 (20)	0 - 2.86	0.95 ± 0.6
Leeks	5 (100)	6.65 - 7.87	7.17 ± 0.25	5 (0)	0	0
Rocket	5 (100)	6.63 - 7.87	6.95 ± 0.21	5 (0)	0	0
Total	20 (100)			15 (0)		
Farm E (Conventional/Dam-canal)						
Tomatoes	5 (100)	4.75 - 7.04	6.27 ± 0.43	5 (40)	0 - 4.93	1.02 ± 0.65
Spring onions	5 (100)	6.82 - 9.04	8.15 ± 0.48	5 (0)	0	0
Green pepper	5 (100)	4.19 - 9.00	5.47 ± 0.80	5 (20)	0 - 1.61	0.54 ± 0.34
Total	15 (100)			15 (20)		
Farm F (Conventional/Municipal)						
Spinach	5 (100)	4.44 - 5.60	5.14 ± 0.21	5 (20)	0 - 1.32	0.44 ± 0.28
Green pepper	5 (100)	3.76 - 4.73	4.15 ± 0.19	5 (20)	0 - 2.94	0.98 ± 0.62
Onions	5 (100)	5.47 - 6.41	5.91 ± 0.16	5 (20)	0 - 2.64	0.88 ± 0.56
Total	15 (100)			15 (20)		

No significant difference (p=0.1702) was seen between the coliform counts for the water, soil and fresh produce samples on Farm A (Figure 1). Interestingly, all the coliform counts for the water samples were the same at 3.38 log MPN.100mL^{-1}. Coliform counts for soil samples ranged from <limit of detection (LOD) - 2.88 log CFU.g^{-1}, and for fresh produce samples from <LOD – 3.52 log CFU.g^{-1} (Figure 1). *Escherichia coli* counts for the water samples on Farm A ranged between 1.16 and 1.69 log MPN.100mL^{-1} (Figure 1), with the aquaculture water significantly higher than the borehole source water (p<0.001). *Escherichia coli* was not detected from any of the soil or fresh produce samples (Figure 1). The coliform counts for the borehole water from Farm B ranged from 1.24 – 1.31 log MPN.100mL^{-1} (Figure 1). The leafy vegetable coliform counts ranged from <LOD - 7.90 log CFU.g^{-1}, with the soil counts between <LOD and 6.76 log CFU.g^{-1}, respectively (Figure 1). No significant differences (p=0.2945) were observed in any of the coliform counts between all sample types (water, soil, leafy vegetables) on Farm B, with no *E. coli* detected from the borehole water on Farm B (Figure 1). The *E. coli* counts for the soil samples ranged from <LOD – 2.85 log CFU.g^{-1}, with soil collected during kale harvesting (±2.52 log CFU.g^{-1}) significantly higher (p=0.0097) than the other soil samples that ranged from 0.6 and 1.57 log CFU.g^{-1} (Figure 1). This can be attributed to the uneven spread of untreated animal manure that was observed on the fields. Additionally, *E. coli* was only detected from one fresh produce sample, namely a kale sample at 2.3 log CFU.g^{-1} (Figure 1). The coliform counts for the borehole water from Farm C ranged between 1.67 and 1.86 log MPN.100mL^{-1} (Figure 1). Coliform counts for the soil samples, collected during lettuce and rocket harvesting, ranged from 3.51 – 4.8 log CFU.g^{-1}, with significant differences (p<0.0001) observed for the soil collected during radish harvesting ranging from 3.76 - 4.8 log CFU.g^{-1} (Figure 1). Moreover, coliform counts were significantly higher (p<0.0001) for the radish samples (5.54 – 6.76 log CFU.g^{-1}) when compared to the other leafy fresh produce (3.53 – 4.75 log CFU.g^{-1}) (Figure 1). *Escherichia coli* were only detected from rocket and the associated soil samples on Farm C (<LOD - 1.43 log CFU.g^{-1}) (Figure 1). The higher detection of coliforms in fresh produce samples compared to soil can be attributed to the higher natural occurrence of Enterobacterales on leafy fresh produce (Blaak *et al* 2014, Richter *et al* 2021).

The coliform counts for the water samples on Farm D ranged from 5.47 – 6.38 log MPN.100mL^{-1}, with the irrigation point samples (±6.24 log MPN.100mL^{-1}), significantly higher ($p<0.0001$) than the rest of the irrigation system samples (±5.8 log MPN.100mL^{-1}) (Figure 2). The coliform counts for the soil samples ranged from 4.19 - 6.11 log CFU.g^{-1} (Figure 2), with fresh produce counts ranging between 5.68 and 7.87 log CFU.g^{-1} (Figure 2). *Escherichia coli* counts for the water samples ranged from 1.08 – 1.91 log MPN.100mL^{-1}, with the irrigation point samples (±1.9 log MPN.100mL^{-1}), significantly higher ($p=0.0007$) than the rest of the water samples (±1.2 log MPN.100mL^{-1}). *Escherichia coli* was detected from soil samples with levels from <LOD – 2.86 log CFU.g^{-1}, with counts only observed for spring onion samples between <LOD and 2.86 log CFU.g^{-1} (Figure 2). Farm E coliform levels from 4.08 – 5.83 log MPN.100mL^{-1} were detected for the water samples with the irrigation point levels (±5.2 log MPN.100mL^{-1}), significantly higher ($p<0.0001$) than the holding dam water levels (±4.2 log MPN.100mL^{-1}) (Figure 2). The coliform levels for the soil samples ranged from 4.59 – 6.65 log CFU.g^{-1}, with the fresh produce levels from 4.19 – 9.04 log CFU.g^{-1}. Coliforms detected from spring onions (±8.2 log CFU.g^{-1}), were significantly higher ($p<0.0001$) than those from tomatoes and green peppers (±5.9 log CFU.g^{-1}) (Figure 2). *Escherichia coli* counts for the irrigation water (0.93 – 1.87 log MPN.100mL^{-1}), on Farm E, were significantly higher ($p<0.0001$), compared to the holding dam water samples between <LOD and 0.49 log MPN.100mL^{-1} (Figure 2). The *E. coli* counts for the soil samples ranged from <LOD – 5.35 log CFU.g^{-1}, with fresh produce levels from <LOD – 4.93 log CFU.g^{-1}. *Escherichia coli* levels from tomatoes (±1.02 log CFU.g^{-1}), were significantly higher ($p<0.0001$), than those from spring onions and green peppers (±0.3 log CFU.g^{-1}) (Figure 2). Coliform counts for the irrigation water samples (4.7 – 5.6 log MPN.100mL^{-1}) on Farm F, were significantly higher ($p<0.0001$), compared to the rest of the water coliform counts between 3.72 and 4.97 log MPN.100 mL^{-1} (Figure 2). Coliform counts for the soil samples ranged from 5.11 – 7.54 log CFU.g^{-1}, with counts for fresh produce from 3.76 – 6.41 log CFU.g^{-1} (Figure 2). *Escherichia coli* counts were significantly higher ($p=0.0009$) for the municipal water samples (2.72 – 3.45 log MPN.100mL^{-1}), compared to the storage and irrigation point counts between <LOD and 2.23 log MPN.100mL^{-1}, respectively (Figure 2). *Escherichia coli* were also detected for soil samples, ranging from <LOD – 1.57 log CFU.g^{-1}, and fresh produce samples from <LOD – 2.94 log CFU.g^{-1}

(Figure 2). *Escherichia coli* detected from green pepper samples (±0.98 log CFU.g^{-1}) were significantly higher (p=0.0009) compared to the onion and spinach samples (±0.9 log CFU.g^{-1}) (Figure 2).

Detection of potential foodborne pathogens

In total, 90/224 samples (40.18%) harboured *E. coli* after enrichment, with the most isolates (35/90) obtained from soil, followed by water (29/90) and then fresh produce (25/90) samples. The occurrence of *E. coli* from the different water source types, was the highest in river water (nine isolates), followed by municipal (four isolates) and borehole (two isolates). A subtotal of 42 *E. coli* isolates was identified as presumptive shiga-toxin producing *E. coli* (STEC) after enrichment. None were confirmed to harbour any of the STEC virulence genes, *stx1*, *stx2* and *eaeA*, after an end-point PCR was performed. Out of all the samples (224) only one *Salmonella* spp. isolate was obtained from a baby carrot sample from Farm D.

Genomic fingerprinting and phenotypic characterisation of *Escherichia coli* isolates

ERIC-PCR cluster analysis for the aquaculture farm (Farm A) showed two distinct clusters at a 70% similarity cut-off (Figure 3, A and B). Cluster A had isolates recovered from the borehole water and soil, with a >90% similarity among the isolates. Cluster B, with a 98% similarity, had two isolates, one from the soil and the other from a hydroponically grown lettuce sample (Figure 3, A and B). Similarly, ERIC-PCR cluster analysis for *E. coli* isolates recovered from the integrated farm (Farm B) showed two distinct clusters at a 70% similarity cut-off. Cluster A had *E. coli* isolates at 80% similarity from soil, spinach and kale samples, while Cluster B had *E. coli* isolates from soil and rape samples at a near 100% similarity (Figure 3, A and B). Genomic fingerprinting of all six *E. coli* isolates from the organic farm (Farm C), displayed a cluster at 75% similarity and included isolates recovered from soil and fresh produce samples (Figure 3, A).

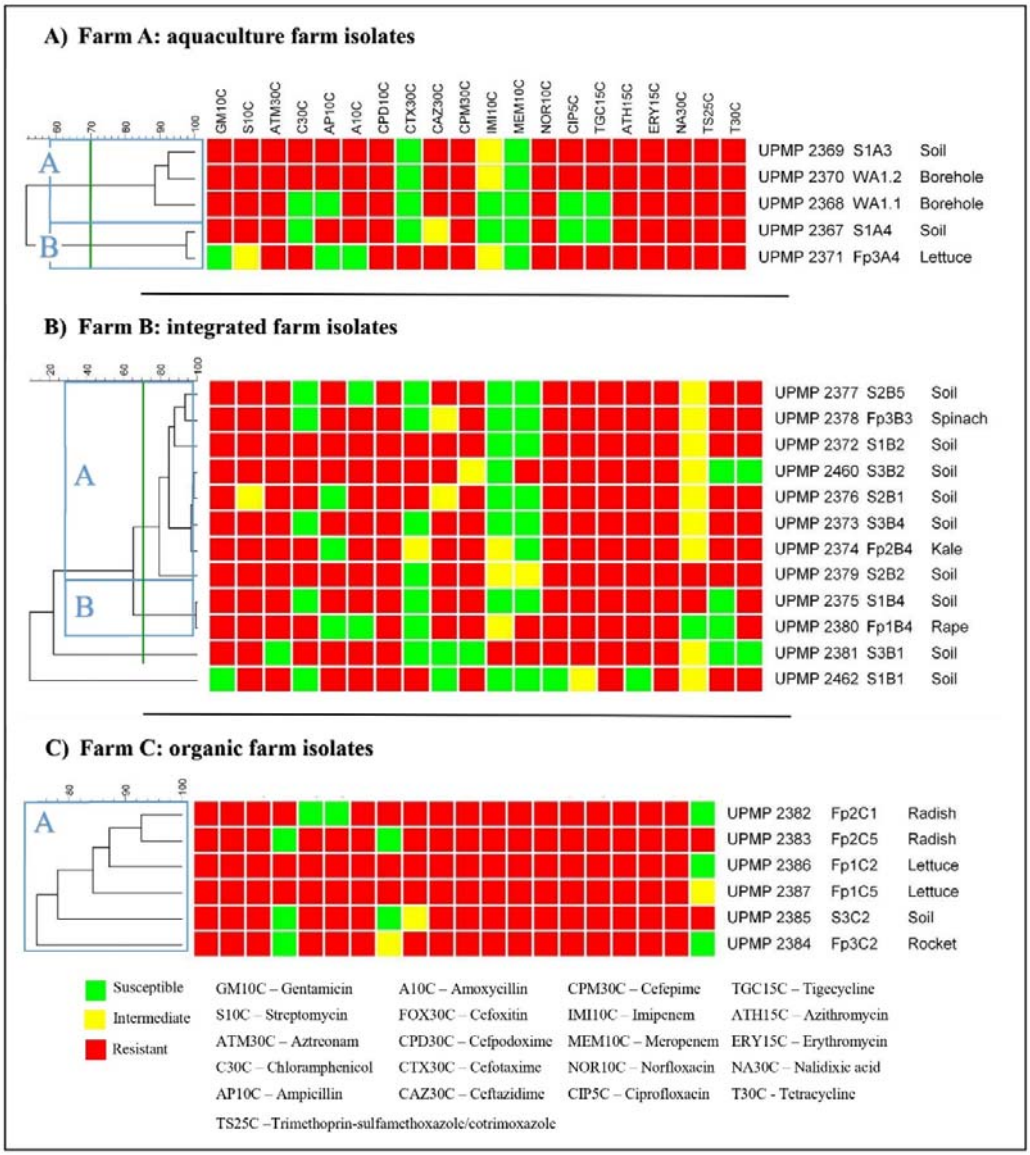

Figure 3: Genetic relatedness and antimicrobial resistance profiles between *Escherichia coli* isolates recovered from the water-soil-plant nexus from (A) the aquaculture farm, (B) the integrated farm and (C) the organic farm. A 70% similarity cut-off was used to determine the clusters of isolates, as represented by the green vertical line.

At a 70% similarity cut-off, DNA fingerprinting generated four distinct clusters for the isolates recovered from Farm D (Figure 4, A-D). Cluster A included isolates (n = 5) from the river, irrigation point and leek samples at >95% similarity. Cluster B had three isolates from the holding dam and soil at >65% similarity (Figure 4, A-B). The largest cluster (Cluster C) had 13 isolates recovered from the entire water-soil-plant nexus with a >80% similarity among all isolates. Lastly, Cluster D (n = 5) had isolates recovered from the water system on the farm (Figure 4, D).

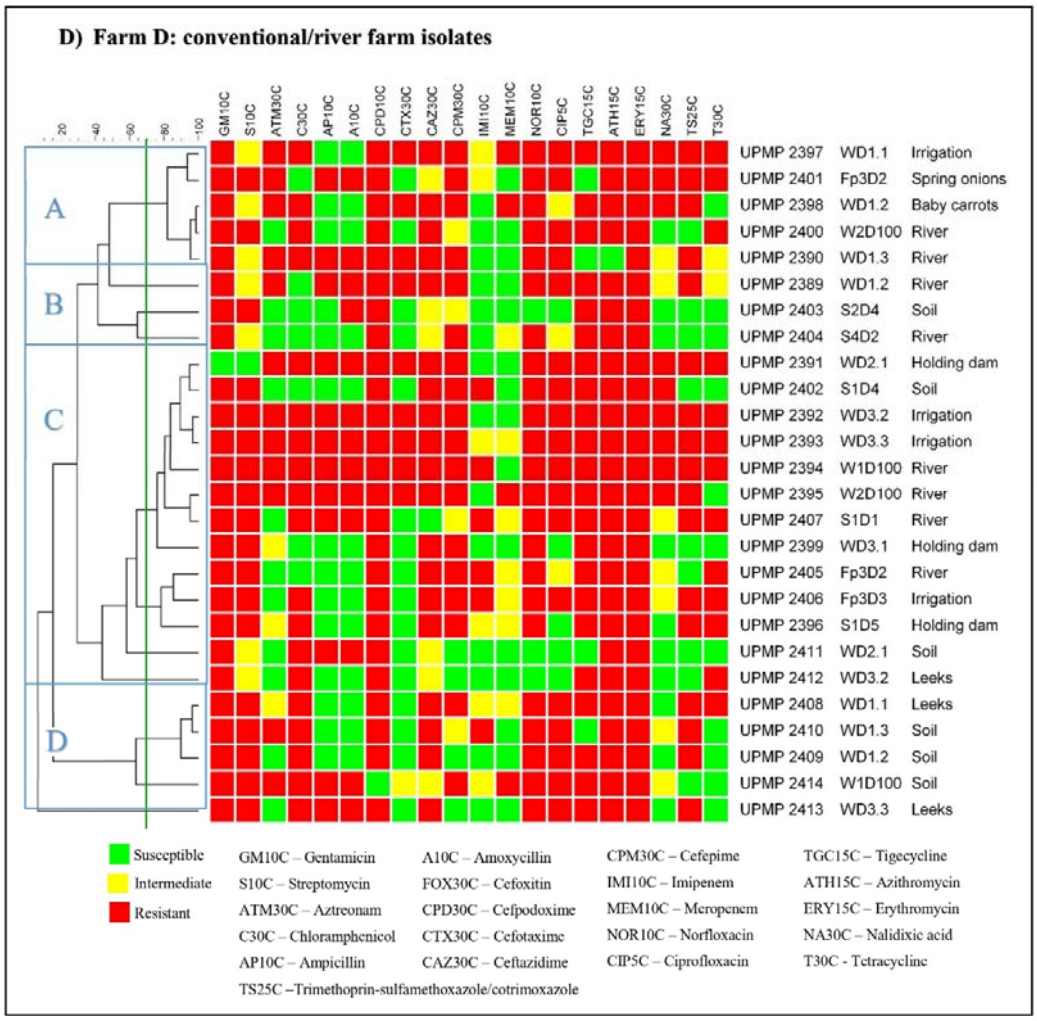

Figure 4: Genetic relatedness and antimicrobial resistance profiles between *Escherichia coli* isolates recovered from the water-soil-plant nexus from (D) the conventional/river farm (farm D). A 70% similarity cut-off was used to determine the clusters of isolates, as represented by the green vertical line. Green blocks represent susceptible isolates, while yellow and red blocks represent intermediate and resistant isolates, respectively.

The genetic fingerprinting of the *E. coli* isolates from Farm E had two distinct clusters at a 70% similarity cut-off (Figure 5, A and B). Cluster A, the largest (n = 16), had isolates recovered from the water-soil-plant nexus with close clustering (>90% similarity) between water, soil, and fresh produce samples. On the other hand, Cluster B (n = 5), had isolates from soil and tomato samples with high similarities of >80% (Figure 5, A and B). The ERIC-PCR analysis of the *E. coli* isolates recovered from Farm F showed four distinct clusters at a 70% similarity cut-off (Figure 5, A-D). The largest cluster (Cluster A) comprised of seven isolates recovered from the tank, soil, onion and spinach samples at >80% similarity. Similarly, Cluster B (n = 5), had isolates from the water system and one

spinach sample, in contrast to Cluster C and D with isolates from municipal, soil, and spinach samples at >70% similarity (Figure 5, A-D).

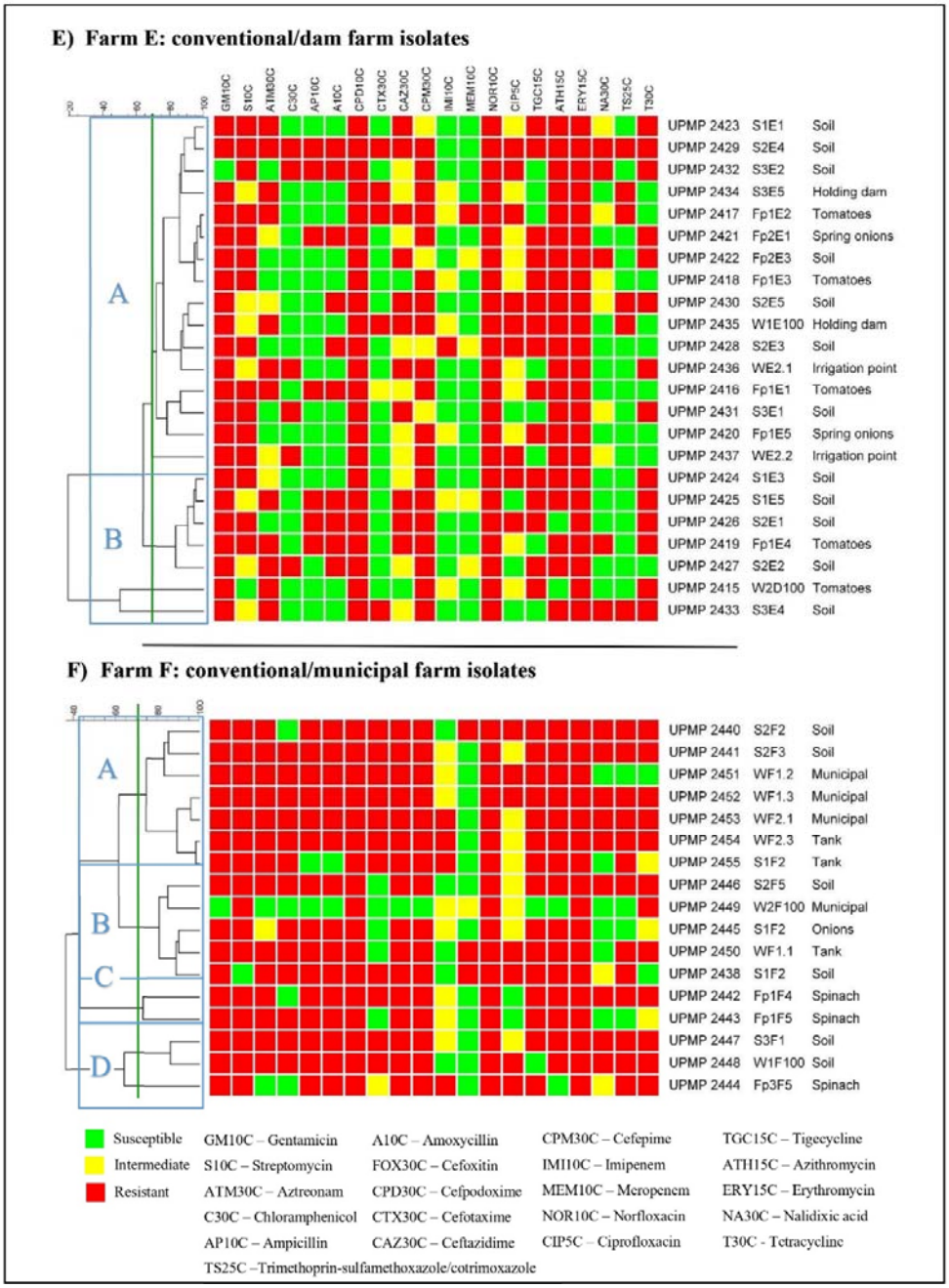

Figure 5: Genetic relatedness and antimicrobial resistance profiles between *Escherichia coli* isolates recovered from the water-soil-plant nexus from (E) the conventional/dam farm and (F) the conventional/municipal farm. A 70% similarity cut-off was used to determine the clusters of isolates, as represented by the green vertical line.

Table 6: *Escherichia coli* resistance percentages against the 21 antibiotics, and 13 antibiotic classes, tested in the study (n = 90 isolates)

Antibiotic class	Antibiotic name	Antibiotic code	Number (%) of resistant isolates from:						
			Farm A resistant isolates (n = 5)	**Farm B resistant isolates (n = 11)**	**Farm C resistant isolates (n = 6)**	**Farm D resistant isolates (n = 27)**	**Farm E resistant isolates (n = 23)**	**Farm F resistant isolates (n = 18)**	**Total resistant isolates (n = 90)**
Aminoglycosides	Gentamicin (10µg)	GM10C	4 (80)	11 (100)	6 (100)	26 (96)	22 (96)	18 (100)	87 (97)
	Streptomycin (10µg)	S10C	5 (100)	11 (100)	6 (100)	26 (96)	22 (96)	18 (100)	88 (98)
	Aztreonam (30µg)	ATM30C	5 (100)	10 (90)	6 (100)	23 (85)	23 (100)	15 (83)	82 (91)
Amphenicols	Chloramphenicol (30µg)	C30C	3 (60)	7 (64)	4 (67)	20 (74)	7 (30)	11 (61)	52 (58)
Beta-lactams: Penicillins	Ampicillin (10µg)	AP10C	4 (80)	10 (90)	5 (83)	13 (48)	9 (39)	16 (89)	57 (63)
	Amoxycillin (10µg)	A10C	4 (80)	10 (90)	5 (83)	14 (52)	13 (57)	16 (89)	62 (69)
Beta-lactams: Carbapenems	Imipenem (10µg)	IMI10C	3 (60)	5 (45)	6 (100)	15 (56)	11 (48)	14 (78)	54 (60)
	Meropenem (10µg)	MEM10C	0 (0)	4 (36)	6 (100)	17 (62)	7 (30)	7 (39)	41 (46)
Beta-lactams: Cephalosporins II	Cefoxitin (30µg)	FOX30C	2 (40)	9 (82)	3 (50)	22 (81)	17 (74)	9 (50)	62 (69)
Beta-lactams: Cephalosporins III	Cefpodoxime (10µg)	CPD10C	5 (100)	11 (100)	6 (100)	26 (96)	23 (100)	18 (100)	89 (99)
	Cefotaxime (30µg)	CTX30C	1 (20)	5 (45)	5 (83)	11 (41)	6 (26)	14 (78)	42 (47)
	Ceftazidime (30µg)	CAZ30C	5 (100)	10 (90)	6 (100)	26 (96)	22 (96)	17 (94)	86 (96)
Beta-lactams: Cephalosporins IV	Cefepime (30µg)	CPM30C	5 (100)	10 (90)	6 (100)	25 (93)	23 (100)	17 (94)	86 (96)
Fluoroquinolones	Norfloxacin (10µg)	NOR10C	3 (60)	11 (100)	6 (100)	25 (93)	23 (100)	18 (100)	25 (29)
	Ciprofloxacin (10µg)	CIP5C	4 (80)	11 (100)	6 (100)	24 (89)	20 (87)	16 (89)	23 (29)
Glycylcyclines	Tigecycline (15µg)	TGC15C	5 (100)	11 (100)	6 (100)	24 (89)	17 (74)	16 (89)	24 (31)
Macrolides	Azithromycin (15µg)	ATH15C	5 (100)	11 (100)	6 (100)	26 (96)	22 (96)	17 (94)	87 (97)
	Erythromycin (15µg)	ERY15C	5 (100)	11 (100)	6 (100)	27 (100)	23 (100)	18 (100)	90 (100)
Quinolones	Nalidixic acid (30µg)	NA30C	5 (100)	10 (90)	6 (100)	18 (67)	15 (65)	12 (67)	66 (73)
Sulfonamides	Trimethoprin-sulfamethoxazole/ cotrimoxazole (1.25µg/23.75µg)	TS25C	5 (100)	7 (64)	6 (100)	18 (67)	6 (26)	14 (78)	56 (62)
Tetracyclines	Tetracycline (30µg)	T30C	5 (100)	7 (64)	3 (50)	16 (59)	14 (61)	16 (89)	63 (70)

The AMR profiles of each isolate as well as the overall resistance percentages can be seen in Figures 3 to 5, Table 6, and Supplementary table S2. All of the 90 *E. coli* isolates obtained, were categorised as MDR with resistance to three or more antibiotic classes. Within the beta-lactam class of antibiotics, the *E. coli* isolates showed the highest resistance to 1st generation cephalosporins (99%) followed by 3rd and 4th generation cephalosporins (96%), penicillins (69%) and carbapenems (54%) (Table 6). The *E. coli* isolates from the soil samples showed higher resistance against 19/21 antimicrobials (Table 6). In contrast, isolates recovered from the water and fresh produce samples were resistant to fewer antimicrobials (2/21) than those for the soil (Table 6). The MDR patterns of all the *E. coli* isolates predominantly included resistance against β-lactams combined with aminoglycosides and macrolides (100% of the isolates), followed by β-lactams combined with glycylcyclines and fluoroquinolones (86 and 96% respectively) (Supplementary table S2).

Discussion

The results from the current study expands on the limited knowledge base of the microbiological quality and antimicrobial resistance in highly diverse smallholder farms for future water and food safety assurance (du Plessis *et al.* 2021). Notably, this study highlights the link of *E. coli* isolated throughout the water-soil-plant nexus, with contaminated irrigation water implicated in fresh produce contamination. This finding emphasizes the importance of monitoring irrigation water and enhancing water quality control methods for smallholder farmers, who depend on this limited resource.

Irrigation water is a known major risk factor for bacterial contamination of fresh produce (Gurtler and Gibson 2022). The typical irrigation water sources used by smallholder farmers in the current study were municipal, borehole, and river water, which is similar to what Beharielal *et al.* (2018) reported for water used by smallholder farmers in KwaZulu-Natal, South Africa. From the current study, only one water source had unsatisfactory *E. coli* levels according to the DWAF and WHO guidelines, namely, the municipal water source at 3.12 log MPN.100mL^{-1} (DWAF 1996, WHO 2006). This is concerning as municipal water is supplied as potable water for communities, and thus the municipal water sampled at the specific time point in the current study potentially poses a contamination risk in the supply chain. The water quality results for the municipal water samples in the current study, however, differed from previous reports where satisfactory levels were reported for

municipal water samples, in the Marianhill Agri-hub in KwaZulu Natal and Vhembe District in Limpopo, respectively (Beharielal *et al.* 2018, Edokpayi *et al.* 2018). Low-levels (1.16 – 1.24 log MPN.100mL^{-1}) of *E. coli* were enumerated from the borehole water used on the aquaculture farm (Farm A), which is similar to other South African studies where low levels of *E. coli* were enumerated from borehole water used for irrigation purposes (Iwu *et al*. 2020, Richter *et al*. 2021). Currently, the DWAF guidelines stipulate that water used for vegetable and crop irrigation should have coliform levels <1000 CFU.100mL^{-1} and that there is a likelihood of contamination of vegetables and other crops eaten raw if the *E. coli* levels range between 1-1000 CFU.100mL^{-1} (DWAF, 1996). Although the enumeration of *E. coli* is globally routinely used as an indicator of fecal contamination in irrigation water used in fresh produce production, no standardized global guidelines exist. In South Africa, fit-for-purpose irrigation quality guidelines are currently being developed, specifically for smallholder farmers, in line with the FAO risk-based water quality guidelines (Drechsel *et al*. 2023).

The general pH levels for surface water in South Africa have been reported to fluctuate around neutral pH (Mekonnen *et al.* 2018). Similarly, 5/6 farms from the current study, had neutral pH levels for their irrigation water samples, with the exception of a surface water sample from Farm E (conventional/dam) which was observed to be more alkaline (pH 9.7). Moreover, reports have noted the reduced activity of nitrifying bacteria and the bioavailability of essential nutrients to crops grown at alkaline pH levels (FAO 2014, Cerozi and Fitzsimmons 2016). The BOD levels for the river water source sample (Farm D) exceeded DWAF regulations (<30 mg.L^{-1}), with a level of 144 mg.L^{-1}, indicating potential pollution of the river. Similarly, the COD levels for the same sample was observed to be at least 15 times higher than the rest of the water samples tested in the study. Since the BOD and COD levels of the river water exceeded the water quality regulations, the source may have a reduced ability to sustain aquatic life (FAO 2014, Prambudy *et al.* 2019). The turbidity levels throughout the entire irrigation system on Farm D (conventional/river) exceeded regulations of less than 5 NTU, which is similar to reports for other rivers around South Africa and can be attributed to the current pollution in water systems around the country (Bonthuys 2018, Rangeti and Dzwairo 2021, Gqomfa *et al.* 2022). These high turbidity levels have the potential to decrease the penetration

of sunlight through water, which results in decreased photosynthesis and lower survival of aquatic microorganisms (Kuutondokwa 2021, Rangeti and Dzwairo 2021).

The microbiological quality of fresh produce plays a crucial role in ensuring both food safety and market accessibility of smallholder farmers, which significantly impacts their livelihoods (FAO and WHO 2019, Beharielal *et al.* 2023). Currently, there is no universal consensus on the microbial quality standards applied to ready-to-eat (RTE) fresh produce (DWAF 1996, WHO 2006, Health Protection Agency 2009, FPSC A-NZ 2019). Fresh produce naturally carries a higher burden of indigenous microflora which results in many countries recommending the exclusion of coliform counts when establishing microbial quality standards (Health Canada 2010, CFS 2014). Nevertheless, the South African Department of Health (DoH) guidelines, which include coliform counts but are currently under revision, state that no more than 2.3 log $CFU.g^{-1}$ coliforms are acceptable on fresh produce (DoH 2000). In this study, coliforms were detected from all fresh produce samples, with counts ranging from 2.3 – 8.15 log $CFU.g^{-1}$, which exceeded the recommended guideline (DoH 2000), similar to other studies done in South Africa that reported coliform levels above 2.3 log CFU/g (Beharielal *et al.* 2018, Richter *et al.* 2021). On the contrary, *E. coli* were only detected from 8.9% of all the fresh produce samples, in accordance with previous studies in commercial and informal sold fresh produce (Richter *et al.* 2021, Baloyi *et al.* 2022). Moreover, 91.1% of the fresh produce samples had acceptable *E. coli* levels according to the DoH guidelines of zero CFU/g, currently under revision, regarding them a low risk to public health (DoH 2000).

In this study, 40.2% of generic *E. coli* were isolated (after enrichment) from the water (32%), soil (39%) and fresh produce (28%) samples, which is similar to previous reports in smallholder fresh produce farms in South Africa (Beharielal *et al.* 2018). The ERIC-PCR profiles, in this study, showed a clear link between the *E. coli* isolated from the water-soil-plant nexus. The >80% similarity observed, is similar to other studies reported in the commercial food supply chain (Richter *et al.* 2021, Ratshilingano *et al.* 2022). To further elucidate the genetic relatedness between *E. coli* isolates from different environmental samples, more detailed genetic typing methods, such as whole-genome sequencing, should be included in future studies. Interestingly, the *E. coli* isolates within each farm in the current study (regardless of the farm scenario or water source type) showed that isolates from

irrigation water clustered together with isolates from soil and fresh produce. The results further reiterate the importance of irrigation water as a contamination source during fresh produce production, in accordance with previous studies where links between *E. coli* in irrigation water and contaminated fresh produce were evident (Decol *et al.* 2017, Douti *et al.* 2021, du Plessis *et al.* 2021). Interestingly, no STEC virulence genes were detected in any *E. coli* isolates, despite the 100% MDR resistance observed. This is similar with previous studies in South Africa, where a low incidence of virulence genes in *E. coli* was reported (Baloyi *et al.* 2022, Ratshilingano *et al.* 2022). Understanding the patterns of AMR, particularly in foodborne pathogen presence throughout fresh produce farms, is crucial in reducing treatment failures in the event of an outbreak (Kim *et al.* 2019).

The multidrug resistant *E. coli* isolates from the current study, displayed >70% prevalence of resistance against 2nd generation, 3rd generation and 4th generation cephalosporins, at 99%, 96% and 96% respectively. This raises concern, as cephalosporins (2nd, 3rd, and 4th generation) are considered the last-resort medications for treating MDR *E. coli* infections (WHO 2018). In Ghana, similar results were shown, with 100% resistance against beta-lactams and tetracyclines amongst *E. coli* isolates from fresh produce (Adzitey 2018). In the current study, a higher prevalence of MDR *E. coli* was observed in soil samples (36/90), followed by water (29/90 and fresh produce samples (25/90). Moreover, the prevalence of antimicrobial resistance in *E. coli* isolates recovered from the environment has been reported by previous studies in low- and middle-income countries (Canizalez-Roman *et al.* 2019, Richter *et al.* 2021). Surveillance of foodborne pathogens and AMR is a critical aspect of disease outbreak assessment and food safety. Unfortunately, in South Africa, foodborne illnesses are often not reported or are under-reported and inadequately investigated (Bisholo *et al.* 2018, Richter *et al.* 2023). Furthermore, the importance of safe irrigation water sources in fresh produce production on smallholder farms, was highlighted. There is a need for revised guidelines, focusing on science-based quality standards, for irrigation water used in fresh produce production, as a link between *E. coli* throughout the water-soil-plant nexus on each smallholder farm was shown.

One of the key findings of the current study is the variability in the sources and quality of water used by smallholder farmers. While some water samples met the acceptable microbial and physicochemical standards, others showed signs of contamination, highlighting the importance of

inclusive water quality assessments and regulatory standards. The presence of generic *E. coli* across the water, soil and fresh produce samples indicates the potential for contamination throughout the water-soil-plant nexus. The use of DNA fingerprinting revealed the high similarity of *E. coli* isolates from different sources (regardless of the water source), showing a clear link between isolates throughout the water-soil-plant nexus within each farm. Furthermore, the strength in following a holistic, One Health, approach when investigating the quality and safety of ready-to-eat fresh produce is demonstrated. Implementation of proper farm management, routine surveillance of MDR foodborne pathogens and mitigation measures should be implemented to prevent cross-contamination from farm-to-fork.

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Conflict of interest

No conflict of interest declared.

Ethical consideration

Ethical approval has been submitted and received at the University of Pretoria's ethics committee in April 2022 (Reference number - NAS055/2022).

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Author contribution statement

Loandi Richter, Erika du Plessis and Lise Korsten contributed to the conceptualization of the study. Sheldon Viviers performed the data extraction and execution of experimental procedures. Sheldon Viviers and Loandi Richter summarized the generated data, prepared all figures and tables, and

analysed statistical results. All authors contributed to the manuscript writing, revision, reading and approval of the submitted version.

Table S1: Primer details and PCR conditions used for screening of *Escherichia coli* O157:H7, non-O157 and DNA fingerprint generation using ERIC-PCR.

Organism	Target genes	Primer sequences (5'-3')	Thermocycling conditions	Expected amplicon size (bp)	Reference
Enterohemorrhagic *E. coli* (EHEC) O157:H7	*eaeA*	F: CTG AAC GGC GAT TAC GCG AA R: GAC GAT ACG ATC CAG	95°C, 15min; 35 cycles of (94°C, 45s; 56°C, 45s; 68°C; 2min), 72ºC, 5min; hold 4ºC	917	Omar and Barnard 2010
	stx1	F: ACA CTG GAT GAT CTC AGT GG R: CTG AAT CCC CCT CCA TTA TG		614	
	stx2	F: CCA TGA CAA CGG ACA GCA GTT R: CCT GTC AAC TGA GCA CTT TG		779	
	mdh	F: GGT ATG GAT CGT TCC GAC CT R: GGC AGA ATG GTA ACA CCA GAG T		304	
Escherichia coli	ERIC	F: ATG TAA GCT CCT GGG GAT TCAC R: AAG TAA GTG ACT GGG TGA GCG	95ºC for 4min, 30 cycles (94ºC 30s, 40ºC 1min, 72ºC 8min), hold 72ºC for 15min, 4ºC hold	DNA fingerprint	Soni *et al*. 2014

Table S2: Summary of the number of antimicrobials, resistant patterns and type of antibiotic classes to which generic *Escherichia coli* isolates from different highly diversified smallholder farms were resistant.

No of isolates (n =90)	No of antimicrobials to which isolates were resistant	No of isolates per smallholder farm						No of isolates with specific resistant pattern	Resistant pattern	No of antibiotic classes to which isolates were resistant	Antibiotic classes
		A	B	C	D	E	F				
1	10						1	1	GM10C - S10C - ATM30C - CPD10C - IMI10C - MEM10C - NOR10C - CIP5C - ERY15C - T30C	6	Aminoglycosides - Cephalosporins II - Carbapenems - Fluoroquinolones - Macrolides - Tetracyclines
3	11				1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CAZ30C - NOR10C - ATH15C - ERY15C	7	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Fluoroquinolones - Macrolides
					1			1	GM10C - S10C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - TGC15C - ATH15C - ERY15C - T30C	7	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Glycylcyclines - Macrolides - Tetracyclines
					1			1	GM10C - S10C - ATM30C - A10C - CPD10C - CAZ30C - CPM30C - IMI10C - TGC15C - ATH15C - ERY15C	8	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Glycylcyclines - Macrolides
3	12					1		1	GM10C - ATM30C - FOX30C - CPD10C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - T30C	8	Aminoglycosides - Cephalosporins II - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Tetracyclines
						1		1	GM10C - S10C - ATM30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C	7	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides
					1			1	GM10C - S10C - ATM30C - FOX30C - CPD10C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C	7	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides
2	13					1		1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - NOR10C - ATH15C - ERY15C - NA30C - T30C	9	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Macrolides - Quinolones - Tetracyclines
						1		1	GM10C - S10C - ATM30C - C30C - A10C - CPD10C - CAZ30C - CPM30C - MEM10C - NOR10C - TGC15C - ATH15C - ERY15C	9	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides

		2				2	GM10C - S10C - ATM30C - AP10C - A10C - CPD10C - CAZ30C - CPM30C - NOR10C - ATH15C - ERY15C - NA30C - TS25C - T30C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1			1	GM10C - S10C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - TS25C	9	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Fluoroquinolones - Glycylcyclines - Macrolides - Sulfonamides
				1		1	GM10C - S10C - ATM30C - A10C - CPD10C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines
8	14								
				1		1	GM10C - S10C - ATM30C - A10C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides
			1	1		2	GM10C - S10C - ATM30C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C	9	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones
			1			1	GM10C - S10C - ATM30C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C	8	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides
				1		1	S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Macrolides - Quinolones - Tetracyclines
					1	1	GM10C - S10C - AP10C - A10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ERY15C - NA30C - TS25C - T30C	11	Aminoglycosides - Beta lactams - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
11	15								
				1		1	GM10C - S10C - ATM30C - C30C - AP10C - FOX30C - CPD10C - CAZ30C - CPM30C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Macrolides - Quinolones - Tetracyclines
				1		1	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - T30C	9	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Tetracyclines

		1			1	GM10C - S10C - ATM30C - C30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - T30C	10	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Tetracyclines
				1	1	GM10C - S10C - ATM30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - TS25C - T30C	9	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1		1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - T30C	10	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Tetracyclines
				1	1	GM10C - S10C - ATM30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C	9	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides
				1	1	GM10C - S10C - ATM30C - FOX30C - CPD10C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines
				1	1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C	10	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones
				1	1	GM10C - S10C - ATM30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - TS25C	9	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Macrolides - Quinolones - Sulfonamides
				1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - TGC15C - ATH15C - ERY15C - NA30C - T30C	11	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines
11	16							
		1			1	GM10C - S10C - ATM30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines

1		1	GM10C - S10C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C	10	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Carbapenems - Fluoroquinolones - Macrolides - Quinolones - Tetracyclines
	1	1	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Macrolides - Quinolones - Tetracyclines
	1	1	GM10C - S10C - ATM30C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - T30C	11	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines
	1	1	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - TGC15C - ATH15C - ERY15C - T30C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Tetracyclines
	1	1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	10	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides
	1	1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - TS25C	10	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Macrolides - Quinolones - Sulfonamides
	1	1	GM10C - S10C - ATM30C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - TS25C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Sulfonamides
	1	1	GM10C - S10C - ATM30C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - T30C	10	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines
	1	1	GM10C - S10C - ATM30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	10	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides

				1		1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ERY15C - NA30C - T30C	11	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Tetracyclines
					1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - TS25C - T30C	11	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Sulfonamides - Tetracyclines
		2		1		3	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	11	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1			1	GM10C - S10C - ATM30C - AP10C - A10C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - TS25C - T30C	11	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Macrolides - Quinolones - Sulfonamides - Tetracyclines
					1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - T30C	11	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Tetracyclines
16	17								
			1			1	ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
		1				1	S10C - ATM30C - C30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1			1	GM10C - S10C - ATM30C - C30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
					1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C	10	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides

				1	1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - TS25C - T30C	11	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Sulfonamides - Tetracyclines
			2		2	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - TS25C - T30C	11	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Sulfonamides - Tetracyclines
			1		1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	11	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides
		1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	13	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
				1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II -Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1		1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II -Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
12	18							
			1		1	GM10C - S10C - C30C - AP10C - A10C - FOX30C - CPD10C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
		1			1	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines

	1			1	GM10C - S10C - ATM30C - AP10C - A10C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II -Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides
			1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - TGC15C - ATH15C - ERY15C - TS25C - T30C	10	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Sulfonamides
			1	1	GM10C - S10C - ATM30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
1				1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C	11	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II -Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones
	1			1	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	11	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
	1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C	11	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II -Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones
	1			1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	11	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides

		1				1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	13	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			2		1		3	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
						2	2	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	11	Aminoglycosides - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
						1	1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Macrolides - Quinolones - Sulfonamides - Tetracyclines
14	19									
			1				1	GM10C - S10C - ATM30C - C30C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	13	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
				1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
				1	1		2	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
				1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides

			1			1	GM10C - S10C - ATM30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	11	Aminoglycosides - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides
				1		1	GM10C - S10C - ATM30C - C30C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	10	Aminoglycosides - Chloramphenicols - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Glycylcyclines - Macrolides - Sulfonamides - Tetracyclines
9	20		1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			2	1		3	GM10C - S10C - ATM30C - C30C - AP10C - A10C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	13	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
				1	3	4	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C - T30C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Glycylcyclines - Macrolides - Quinolones - Sulfonamides - Tetracyclines
			1			1	GM10C - S10C - ATM30C - C30C - AP10C - A10C - FOX30C - CPD10C - CTX30C - CAZ30C - CPM30C - IMI10C - MEM10C - NOR10C - CIP5C - TGC15C - ATH15C - ERY15C - NA30C - TS25C	12	Aminoglycosides - Chloramphenicols - Beta lactams - Cephalosporins II - Cephalosporins III - Cephalosporins IV - Carbapenems - Fluoroquinolones - Glycylcyclines - Macrolides - Quinolones - Sulfonamides

Antibiotics abbreviations:
GM10C – Gentamicin (10µg), S10C – Streptomycin (10µg), ATM30C – Aztreonam (30µg), C30C – Chloramphenicol (30µg), AP10C – Ampicillin (10µg), A10C – Amoxycillin (10µg), FOX30C – Cefoxitin (30µg), CPD10C – Cefpodoxime (10µg), CAZ30C – Ceftazidime (30µg), CTX30C – Cefotaxime (30µg), CPM30C – Cefepime (30µg), IMI10C – Imipenem (10µg), MEM10C – Meropenem (10µg), NOR10C – Norfloxacin (10µg), CIP5C – Ciprofloxacin (5µg), TGC15C – Tigecycline (15µg), ATH15C – Azithromycin (15µg), ERY15C – Erythromycin (15µg), NA30C – Nalidixic acid (30µg), TS25C - Trimethoprin-sulfamethoxazole/ cotrimoxazole (1.25/23.75µg), T30C – Tetracycline (30µg)

Smallholder farms:
A – Farm A, Aquaculture farm
B – Farm B, Integrated farm
C – Farm C, Organic farm
D – Farm D, Conventional/river farm
E – Farm E, Conventional/dam farm
F – Farm F, Conventional/municipal farm