applied microbiology

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Characterization of *Escherichia coli* from Water and Food Sold on the Streets of Maputo: Molecular Typing, Virulence Genes, and Antibiotic Resistance

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Abstract: The aim of this study was to investigate the pathogenic potential and antibiotic resistance of 59 *Escherichia coli* isolates from ready-to-eat (RTE) street food (n = 31) and drinking water (n = 28) sold in the city of Maputo, Mozambique. The isolates were characterized by XbaI subtyping analysis via pulsed field gel electrophoresis. Multiplex PCRs were performed targeting five virulence genes (stx, lt, st, astA, and eae) and three groups of antibiotic-resistant genes, namely ß-lactamases (*extended-spectrum ß-lactamase* and *AmpC*), tetracycline (*tetA*, *tetB*, and *tetM*) and sulfamethoxazole/trimethoprim (*sul1*, *sul2*, and *sul3*). The stx virulence gene, encoding the *Shiga/Vero* (VT) toxin produced by the verotoxin-producing *E. coli* (VTEC), was identified with similar frequency in isolates from food (5/31) and water (6/28). The highest percentages of resistant isolates from food and water were found for ß-lactams imipenem (35.5 and 39.3%, respectively) and ampicillin (39.3 and 46.4%, respectively). Multidrug resistance was observed in 31.3% of the isolates, being higher in *E. coli* isolates from water (45.5%) compared to RTE street food isolates (19.2%). Virulence genes were detected in 73% of the multidrug-resistant isolates. Concerning antibiotic-resistant genes, ESBL was the most frequent (57.7%) among β-lactamases while *tetA* was the most frequent (50%) among non-β-lactamases.

Keywords: Mozambique; street food; drinking water; *E. coli*; PFGE; multidrug resistance; antibiotic resistance genes; virulence genes

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Citation: Salamandane, A.; Alves, S.; Chambel, L.; Malfeito-Ferreira, M.; Brito, L. Characterization of *Escherichia coli* from Water and Food Sold on the Streets of Maputo: Molecular Typing, Virulence Genes, and Antibiotic Resistance. *Appl. Microbiol.* **2022**, *2*, 133–147. https://doi.org/10.3390/applmicrobiol2010008

Academic Editor: Teresa Aymerich

Received: 5 November 2021
Accepted: 21 January 2022
Published: 25 January 2022

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1. Introduction

In developing countries, enteric infections and diarrheal diseases represent some of the main causes of hospitalization and a serious health problem [1,2]. Their occurrence is closely associated with deficiencies in the water supply and sanitation systems and poor personal hygiene conditions, which are common in developing countries. These diarrheal diseases can be caused by bacteria, parasites, and viruses [3,4]. The exposure to these pathogens in sub-Saharan Africa is favored by the climate and is mainly related to the lack of adequate hygiene and sanitation conditions and the consumption of contaminated ready-to-eat (RTE) foods and vegetables [1,2,5].

The bacterial species *E. coli* represents one of the main hazards in food and water sources [3,6,7]. However, not all *E. coli* cause diarrheal diseases [8]. Its association with the occurrence of diarrhea is based on the presence of genes that encode different virulence factors such as *stx*, *eae*, and *ehxA* in the *Shiga* toxin-producing *E. coli* or enterohemorrhagic *E. coli* (STEC/EHEC), *bfp* (bundle-forming pilus), and *eae* (intimin) [8,9]. The presence and/or association of these virulence factors determine the *E. coli* pathotypes that cause

high morbidity and mortality worldwide and are collectively known as diarrheagenic *E. coli* (DEC) [6,9,10].

In developing countries, the growing use of antibiotics in veterinary medicine, mainly by small poultry farmers, has been associated with increased levels of antimicrobial resistance in *E. coli* strains [11–13]. In fact, meat and meat products are frequently linked to the prevalence of antimicrobial resistance genes [13]. Since bacteria are highly prone to exchanging genetic material, these resistance genes can be transferred to other bacteria, particularly Enterobacteriaceae [14].

The sale of RTE street food, despite representing an important source of income for many unemployed families, has often been associated with outbreaks of diarrheal diseases [15]. In Maputo, Mozambique, these foods are often exposed to dust and sold without temperature control and minimum hygiene conditions. This increases the risk of diarrheal diseases, which are responsible for 35% of the total mortality [1,2]. The incidence of diarrheal diseases has led to the overuse of antibiotics. Often, these antibiotics can be purchased at informal markets without a prescription [11,12]. This non-medically assisted antibiotic intake is also frequently associated with incomplete antibiotic therapy, contributing to the increase in antimicrobial resistance [11,12].

In Mozambique, despite the declining rates in recent years, diarrhea remains one of the main causes of morbidity and mortality in children under 5 years old [16]. The most common diarrheal pathogens isolated from patients attending healthcare facilities in Mozambique include DEC, *Salmonella*, *Shigella* spp., *Campylobacter jejuni*, *Entamoeba histolytica*, *Rotavirus*, and *Giardia lamblia* [2,17]. Despite the small number of studies, DEC are the most frequently isolated and most studied pathogens in Mozambique. Furthermore, the few studies that report the occurrence of DEC in Mozambique are focused on clinical strains [16–19], which makes it difficult to fully study its epidemiology, especially the link with the consumption of contaminated food and water.

Within this framework, the main objective of this study was to investigate the pathogenic potential and antibiotic resistance of *E. coli* isolated from RTE food and drinking water sold in the streets of Maputo, Mozambique.

2. Material and Methods

Overall, 59 *E. coli* isolates from ready-to-eat street food and water were investigated. Of these, 31 food isolates were recovered from 83 street food samples and isolated according to the ISO 16649-2 [20], as described in Salamandane et al. [1]. The other 28 *E. coli* isolates were recovered according to ISO 9308-1 [21], from a total of 118 water samples consumed in the city of Maputo, including home-bottled street water, tap water, and water from supply wells, as described previously [22]. The evaluation of the microbiological quality of the samples indicated that in general, the samples were unsatisfactory for consumption. From each plate that presented well isolated colonies and characteristics of *E. coli*, a CFU was selected.

2.1. E. coli Isolates

Biochemical tests were carried out to characterize these presumptive *E. coli* isolates from chromogenic medium, namely Gram staining, catalase, and oxidase tests [1,22]. Subsequently, molecular identification was performed, targeting the 16S rRNA gene (401 bp) with the AB035924 forward primer (5′ CCC CCT GGA CGA AGA CTG A-3′) and the AB035924 reverse primer (5′-ACC GCT GGC AAC AAA GGA T-3′), as previously reported [1,22]. Subsequently, the 59 isolates were characterized by antibiotic resistance profiling, PFGE subtyping, and the detection of virulence genes and antibiotic resistance genes as described below.

2.2. Pulsed Field Gel Electrophoresis (PFGE)

The 59 *E. coli* isolates were characterized by DNA macrorestriction analysis using PFGE in accordance with PulseNet International [23]. Briefly, bacterial isolates were inoculated

onto Tryptone Soy Agar plates (Biokar Diagnostics, Beauvais, France) and incubated at 37 °C for 14 ± 2 h. From these plates, bacterial cultures were embedded in SeaKem Gold agarose (Bio-Rad Laboratories, Milan, Italy), lysed, washed, and the DNA was in situ digested with 50 U of XbaI (Thermo Fisher Scientific, Fermentas; Waltham, MA, USA) at 37 °C for 3 h. Resolution of the generated DNA fragments was obtained with 1% (*m/v*) SeaKem Gold Agarose gels in 0.5× Tris-Borate EDTA Buffer (Bio-Rad Laboratories, Milan, Italy) at 14 °C and 6 V/cm with time ramped for 6.7/35.3 s over 18 h, using a CHEF DRII System (Bio-Rad Laboratories, Milan, Italy); pattern images were acquired in the Gel Doc™ EZ System (Bio-Rad Laboratories, Milan, Italy). The TIF images were normalized by aligning the peaks of the XbaI DNA fragments of the size standard strain Salmonella enterica serovar Braenderup H9812 DNA, which was loaded onto two lanes in each gel.

2.3. Multiplex-PCR (MPCR) for Identification of Virulence and Antibiotic Resistance Genes

As PCR templates, six bacterial colonies grown on TSA plates for 18 ± 2 h at 37 °C were suspended in 300 µL of sterile ultrapure water and incubated in a boiling bath for 15 min [24]. Subsequently, the tubes were centrifuged at 16,000× *g* for 12 min, and the lysates supernatants (lysates) were stored at −20 °C. Amplification reactions were performed in a final volume of 25 µL, containing 12.5 µL of Taq DNA Polymerase NZYTaq II2× Colorless Master Mix (MZTech, Lisbon, Portugal) and 1 µL of 50-mM $MgCl_2$.

The five targeted virulence genes coding for the respective virulence factors were *stx* (*shiga/vero* toxin, VT), *lt* (heat-labile enterotoxin, LT), *st* (heat-stable enterotoxin, ST), *astA* (heat-stable enterotoxin 1, EAST1), and *eae* (intimin). The respective five primer sets (Table 1) were added at a final concentration of 0.4 µM each. The PCR was performed with an initial denaturation step at 95 °C for 5 min, 35 cycles of 30 s at 95 °C, 40 s at 50 °C, and 72 °C for 1 min, with a final cycle at 72 °C for 10 min.

Table 1. Primers targeted virulence genes.

Primer Name	Sequence	Target Gene/ (Virulence Factor)	Size (bp)	Reference
LT—1: For LT—2: Rev	ATT TAC GGC GTT ACT ATC CTC TTT TGG TCT CGG TCA GAT ATG	*lt* (LT)	280	[25]
ST—PR1: For ST—PR2: Rev	TCT GTA TTG TCT TTT TCA CC TTA ATA GCA CCC GGT ACA AGC	*st* (ST)	195	[26]
EAE 23F: For EAE 25R: Rev	ACC AGA TCG TAA CGG CTG CCT AGT TTG GGT TAT AAC GTC TTC ATT G	*eae* (Intimin)	499	
ES—151: For ES—149: Rev	GAG CGA AAT AAT TTA TAT GT CGA AAT CCC CTC TGT ATT TGC C	*stx* (VT)	323	[27]
EAST 11: For EAST 11: Rev	CCA TCA ACA CAG TAT ATC CGA GGT CGC GAG TGA CGG CTT TGT	*astA* (EAST1)	114	[28]

For the screening of antibiotic resistance genes, 16 primer sets were used in four separate MPCR reactions. For the detection of extended-spectrum ß-lactamases (ESBL) genes, five pairs of primers (*TEM* variants, *SHV* variants, bla_{OXA} variants, *CTX-M* Group 1 variants, and *CTX-M* Group 9) were selected. Five primer pairs (*ACC* variants, *FOX* variants, *MOX* variants, *CIT* variants, and *DHA* variants) were selected for the detection of *AmpC* β-lactamase genes. For the detection of tetracycline resistance genes, three primer pairs, *tetA*, *tetB*, and *tetM*, were selected. For the detection of sulfonamide resistance genes, the primers *sul1*, *sul2*, and *sul3* were selected. The used primers and the respective sizes of the PCR products, as well as the final concentrations, are listed in Table 2.

Table 2. Primers targeted antibiotic resistance genes.

Primer Name	Sequence	Target Gene	Size (bp)	Final Concentration	Ref
MultiTSO-T-For MultiTSO-T-Rev	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	*TEM* variantes (*TEM-1* and *TEM-2*)	800	0.4 µM	[29]
MultiTSO-S-For MultiTSO-S-Rev	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC	*SHV* variants (including *SHV-1*)	713	0.4 µM	
MultiTSO-O-For MultiTSO-O-Rev	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG	*OXA*-variants (*OXA-1*, *OXA-4*, *OXA-30*)	564	0.4 µM	
CTX-MGrp1—For CTX-MGrp1—Rev	TTAGGAARTGTGCCGCTGYA CGATATCGTTGGTGGTRCCAT	Multi *CTX-M*Grp1 (*CTX-M-1*, *CTX-M-3*, *CTX-M-15*)	688	0.4 µM	
CTX-MGrp9—For CTX-MGrp9—Rev	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG	*CTX-M* Group 9 (*CTX-M-9*, *CTX-M-14*)	561	0.4 µM	
MultiACC-For MultiACC-Rev	CACCTCCAGCGACTTGTTAC GTTAGCCAGCATCACGATCC	*ACC* variants (*ACC-1 and ACC-2*)	346	0.2 µM	[29]
MultiFOX-For MultiFOX-Rev	CTACAGTGCGGGTGGTTT CTATTTGCGGCCAGGTGA	*FOX* variants (*FOX-1 to FOX-5*)	162	0.5 µM	
MultiMOX_For MultiMOX_Rev	GCAACAACGACAATCCATCCT GGGATAGGCGTAACTCTCCCAA	*MOX* variants (*MOX-1*, *MOX-2*, *CMY-1*, *CMY-8 to CMY-11*, and *CMY-19*)	895	0.2 µM	
MultiCIT-For MultiCIT-Rev	CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY	*CIT* variants (*LAT-1 to 3*, *BIL-1*, *CMY-2 to 7*, *CMY-12 to -18*, and *CMY-21 -23*)	538	0.3 µM	
MultiDHA_For MultiDHA-Rev	TGATGGCACAGCAGGATATTC GCTTTGACTCTTTCGGTATTCG	*DHA* variants	997	0.5 µM	
Tetracyclines (A)-For Tetracyclines (A)-Rev	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	*tetA*	210	1 µM	[30]
Tetracyclines (B)-For Tetracyclines (B)-Rev	TTGGTTAGGGGCAAGTTTTG GTAATGGGCCAATAACACCG	*tetB*	659	0.25 µM	
Tetracyclines (M)-For Tetracyclines (M)-Rev	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	*tetM*	406	0.5 µM	
Sulphonamides 1-For Sulphonamides 1-Rev	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	*sul1*	433	0.4 µM	[30]
Sulphonamides 2-For Sulphonamides 2-Rev	GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCTCCCGT	*sul2*	293	0.4 µM	
Sulphonamides 3-For Sulphonamides 3-Rev	GAGCAAGATTTTGGAATCG CATCTGCAGCTAACCTAGGGCTTTGGA	*sul3*	790	0.4 µM	

For the detection of ESBL and *AmpC* β-lactamase genes, the reaction mixtures were subjected to the following amplification program: initial denaturation at 94 °C for 10 min, followed by 30 cycles at 94 °C for 40 s, 60 °C for 40 s, 72 °C for 1 min, and a final elongation step at 72 °C for 7 min. For tetracycline resistance genes, the reaction mixtures were subjected to the following amplification program: initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 60 s, 55 °C for 60 s, 72 °C for 1.5 min, and a final elongation step at 72 °C for 7 min. For sulfonamide resistance genes, the reaction mixtures

were subjected to the following amplification program: initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 15 s, 69 °C for 30 s, 72 °C for 1 min, and a final elongation step at 72 °C for 7 min.

All PCR reactions were run in a thermocycler GeneAmp® PCR System 9700; Applied Biosystems (Bio-Rad Laboratories, Segrate, Milan, Italy). The resulting PCR products were resolved on 2% (*m/v*) agarose gels in 1xTAE buffer in an EC330 Thermo Fisher Scientific tank (Georgia, USA) at 6 V/cm for 90 min. The gels were then stained with GelRed (Frilabo, Maia, Portugal) and analyzed using the Gel Doc™ EZ System (Bio-Rad Laboratories, Segrate, Milan, Italy). For calculating the size of the PCR products, the molecular marker 100-bp DNA Ladder (InVitrogen, Carlsbad, CA, USA) was used.

2.4. Antibiotic Susceptibility Profile

The antibiotic susceptibility profile was evaluated for 59 isolates of *E. coli*, using the disk diffusion method on Mueller-Hinton (MH) agar plates (Biokar Diagnostics, Beauvais, France) with antibiotic disks (Liofilchem, Roseto degli Abruzzi, Italy) according to the Clinical Laboratory Standards Institute (CLSI, 2021) [31]. Strain *E. coli* ATCC 25922 was used as a control. Briefly, isolated colonies grown on Trypto-Casein-Soy agar (Biokar Diagnostics) at 37 °C for 18 ± 2 h were suspended in sterile saline until the turbidity was equivalent to that of the McFarlands 0.5 standard (ca. 106 CFU/mL). The resulting bacterial suspensions were used to inoculate MH plates. After disk deposition (four per plate), plates were incubated for 18 ± 2 h at 37 °C. Fifteen antibiotics were used: amoxicillin (AMX) 10 µg, amoxicillin/clavulanic acid (AMC) 30:10 µg, ceftazidime (CAZ) 30 µg, imipenem (IPM) 10 µg, cefpirome (CPO) 30 µg, aztreonam (ATM) 30 µg, cefoxitin (FOX) 30 µg, ampicillin (AMP) 10 µg, cefotaxime (CTX) 30 µg, chloramphenicol (CHL) 30 µg, tetracycline (TET) 30 µg, gentamycin (GEN) 10 µg, trimethoprim/sulfamethoxazole (SXT) 1:19 µg, azithromycin (AZM) 15 µg, ciprofloxacin (CIP) 5 µg. The ranges of the diameters of the inhibition halos for each antibiotic used according to the CLSI 2021 [31] are listed in the Supplemental Table S1.

2.5. Data Analysis and Interpretation

The PFGE patterns were analyzed with BioNumerics® version 6.6 (Applied Maths, Belgium). The levels of similarity were based on the Dice correlation coefficient, with tolerance and optimization values of 1 and 0.5%, respectively. For cluster analysis of the patterns, the unweighted-pair group matching algorithm (UPGMA) was used. Ten isolates of *E. coli* were used to determine the experimental variation between duplicate experiments. The minimum level of repeatability of the macrorestriction conditions was calculated by running DNA samples from duplicated restrictions of the DNA of each strain. Based on the obtained results, a 95% cut-off value of similarity was established for typing identical isolates with identical outputs.

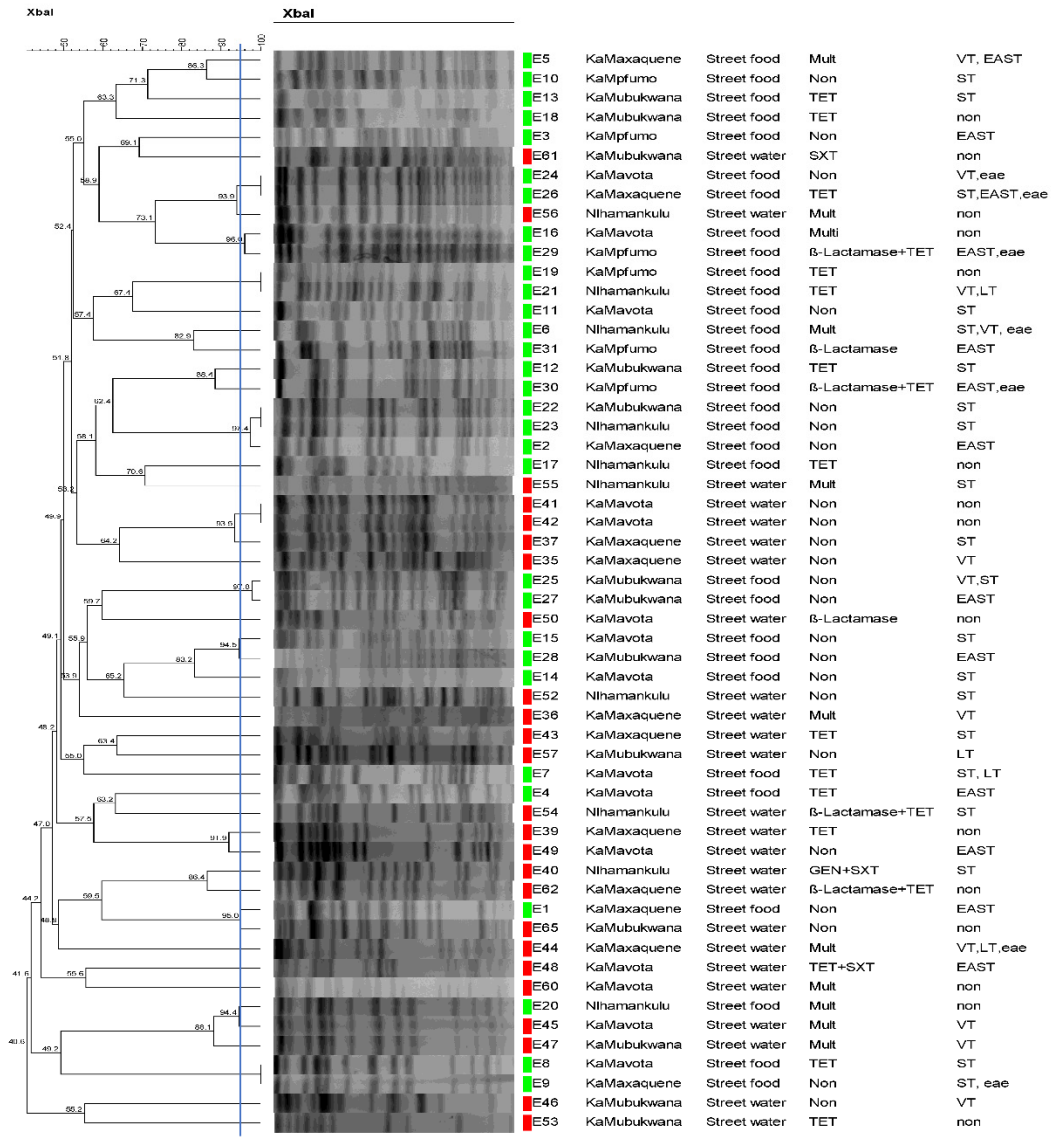
For the evaluation of antibiotic resistance, the diameters of the inhibition halos (mm) were measured and compared to those described by the CLSI, 2021 [31]. The isolates were considered non-susceptible to a certain antibiotic when they showed intermediate or full resistance to that antibiotic. Multidrug resistance was considered to be resistant to more than two unrelated antimicrobial agents.

3. Results

3.1. Pulse Typing and the Presence of Virulence Genes

The PFGE of the DNAs of the 59 isolates digested with XbaI showed 12–21 fragments, ranging from approximately 20.5 to 1135 kb in size. The relationships among *E. coli* isolates based on their XbaI PFGE profiles are shown in the dendrogram in Figure 1. Based on data obtained from the duplicate experiments, a cut-off level of 95% similarity was established for the definition of a PFGE pattern. The PFGE of the XbaI macrorestriction fragments differentiated the 59 isolates into 51 different patterns, showing a great diversity among the

isolates regardless of their food, water, or municipal district origin. These results suggest a great diversity of sources of contamination.



isolate	district	origin	antibiotic resistance	virulence gene
E5	KaMaxaquene	Street food	Mult	VT, EAST
E10	KaMpfumo	Street food	Non	ST
E13	KaMubukwana	Street food	TET	ST
E18	KaMubukwana	Street food	TET	non
E3	KaMpfumo	Street food	Non	EAST
E61	KaMubukwana	Street water	SXT	non
E24	KaMavota	Street food	Non	VT,eae
E26	KaMaxaquene	Street food	TET	ST,EAST,eae
E56	NIhamankulu	Street water	Mult	non
E16	KaMavota	Street food	Multi	non
E29	KaMpfumo	Street food	ß-Lactamase+TET	EAST,eae
E19	KaMpfumo	Street food	TET	non
E21	NIhamankulu	Street food	TET	VT,LT
E11	KaMavota	Street food	Non	ST
E6	NIhamankulu	Street food	Mult	ST,VT, eae
E31	KaMpfumo	Street food	ß-Lactamase	EAST
E12	KaMubukwana	Street food	TET	ST
E30	KaMpfumo	Street food	ß-Lactamase+TET	EAST,eae
E22	KaMubukwana	Street food	Non	ST
E23	NIhamankulu	Street food	Non	ST
E2	KaMaxaquene	Street food	Non	EAST
E17	NIhamankulu	Street food	TET	non
E55	NIhamankulu	Street water	Mult	ST
E41	KaMavota	Street water	Non	non
E42	KaMavota	Street water	Non	non
E37	KaMaxaquene	Street water	Non	ST
E35	KaMaxaquene	Street water	Non	VT
E25	KaMubukwana	Street food	Non	VT,ST
E27	KaMubukwana	Street food	Non	EAST
E50	KaMavota	Street water	ß-Lactamase	non
E15	KaMavota	Street food	Non	ST
E28	KaMubukwana	Street food	Non	EAST
E14	KaMavota	Street food	Non	ST
E52	NIhamankulu	Street water	Non	ST
E36	KaMaxaquene	Street water	Mult	VT
E43	KaMaxaquene	Street water	TET	ST
E57	KaMubukwana	Street water	Non	LT
E7	KaMavota	Street food	TET	ST, LT
E4	KaMavota	Street food	TET	EAST
E54	NIhamankulu	Street water	ß-Lactamase+TET	ST
E39	KaMaxaquene	Street water	TET	non
E49	KaMavota	Street water	Non	EAST
E40	NIhamankulu	Street water	GEN+SXT	ST
E62	KaMaxaquene	Street water	ß-Lactamase+TET	non
E1	KaMaxaquene	Street food	Non	EAST
E65	KaMubukwana	Street water	Non	non
E44	KaMaxaquene	Street water	Mult	VT,LT,eae
E48	KaMavota	Street water	TET+SXT	EAST
E60	KaMavota	Street water	Mult	non
E20	NIhamankulu	Street food	Mult	non
E45	KaMavota	Street water	Mult	VT
E47	KaMubukwana	Street water	Mult	VT
E8	KaMavota	Street food	TET	ST
E9	KaMaxaquene	Street food	Non	ST, eae
E46	KaMubukwana	Street water	Non	VT
E53	KaMubukwana	Street water	TET	non

Figure 1. Dendrogram (based on Dice correlation coefficient and UPGMA clustering) of *E. coli* XbaI PFGE patterns for 59 isolates. BioNumerics® version 6.6 was used with a band position tolerance of 1% and an optimization of 0.5%. The reference of the isolates as well as their municipal district, origin, antibiotic resistance, and presence of virulence genes are indicated. Red are isolates from drinking water; green are isolates from street food; Non = the virulence genes searched were not detected; non = susceptible for all antibiotic detected; Mult = multi drug resistance.

However, at 85% similarity, there were some clusters with a few isolates from street food from adjoining municipal districts, although the isolates in these clusters may differ regarding the presence of virulence genes and antibiotic resistance profiles.

In RTE street foods, the most predominant genes were those coding for thermo-stable (ST) toxin, *st* (33.3%), followed by *astA* (EAST1) (30.6%). The intimin (AEA) *eae* gene was present in 16.7% of the isolates, whereas 13.9% of the isolates presented *stx* coding for the verotoxine (VT) and 5.6% presented genes coding for thermo label (LT) toxin (*lt*).

The coexistence of different combinations of two or more virulence genes coding for the respective virulence factors was observed in eight food isolates: *stx* (VT) and *astA* (EAST1), *st* (ST), *stx* (VT) and *eae* (intimin), *st* (ST) and *lt* (LT), *st* (ST) and *eae* (intimin), *stx* (VT) and *lt* (LT), *stx* (VT) and *eae* (intimin), *stx* (VT) and *st* (ST), *st* (ST), *astA* (EAST1) and *eae* (intimin) (Figure 1).

In water, the most predominant genes were *stx* (35.3%), *st* (35.3%), *lt* (11.8%), *astA* (11.8%), and *eae* (5.9%). In one water isolate, the combination of *stx* (VT), *lt* (LT), and eae (intimin) was observed. The percentage of isolates that did not present any of the searched virulence genes was 16.1% in those recovered from RTE street food and 39.3% in those recovered from water (Figure 1).

3.2. Antibiotic Resistance Profiles

Fifteen antibiotics were tested against the 59 *E. coli* isolates from RTE food and water sold in the streets of Maputo, and 46 (78%) isolates showed an antibiotic resistance profile. The highest percentages of resistant isolates from street food were observed in the ß-lactams IPM (35.5%), AMP (22.6%), AMX and CAZ (19.4%), and the non-ß-lactams TET (58.1%), CHL (12.9%), and SXT (9.7%) (Table 3). No resistance profiles were observed against four antibiotics tested (CPO, ATM, FOX, and CIP) (Table 3). The isolates exhibiting antibiotic resistance profiles were 26 (83.8%). Five out of the 26 non-susceptible food isolates (19.2%) even presented multidrug resistance profiles (Figure 1 and Table 4), whereas 21 food isolates (80.8%) presented non-multidrug resistance profiles (Table 4). Five isolates (16.2%) were susceptible to the 15 tested antibiotics.

Table 3. Antibiotic resistance profiles of 59 *E. coli* isolates from RTE street food and drinking water.

Group	Antibiotic	RTE Street Food (*n* = 31)		Water (*n* = 28)	
		Susceptible	Resistant	Susceptible	Resistant
ß-lactams	AMX (10 µg)	25 (80.6%)	6 (19.4%)	18 (64.3%)	10 (35.7%)
	AMC (20/10 µg)	27 (87.1%)	4 (12.9%)	24 (85.7%)	4 (14.3%)
	CAZ (30 µg)	25(80.6%)	6 (19.4%)	25 (89.3%)	3 (10.7%)
	IPM (10 µg)	20 (64.5%)	11 (35.5%)	17 (60.7%)	11 (39.3%)
	CPO (30µg)	31(100%)	0	28 (100%)	0
	ATM (30 µg)	31(100%)	0	27 (96.4%)	1 (3.6%)
	FOX (30 µg)	31(100%)	0	23 (81.1%)	5 (17.8%)
	AMP (10 µg)	24 (77.4%)	7 (22.6%)	15 (53.6%)	13 (46.4%)
	CTX (30 µg)	29 (93.5%)	2 (6.5%)	25 (89.3%)	3 (10.7%)
Non-ß-lactams	CHL (30 µg)	27(87.1%)	4 (12.9%)	22 (78.6%)	6 (21.4%)
	TET (30 µg)	13 (41.9%)	18 (58.1%)	14 (50%)	14 (50%)
	GEN (10 µg)	29 (93.5%)	2 (6.5%)	25 (89.3%)	3 (10.7%)
	SXT (23.75/1.25 µg)	28 (90.3%)	3 (9.7%)	17 (60.7%)	11 (39.3%)
	AZM (15 µg)	30 (96.8%)	1 (3.2%)	24 (85.7%)	4 (14.3%)
	CIP (5 µg)	31 (100%)	0	28 (100%)	0

Amoxicillin (AMX); Amoxicillin/clavulanic Acid (AMC); Ceftazidime (CAZ); Imipenem (IPM); Cefpirome (CPO); Aztreonam (ATM); Cefoxitin (FOX); Ampicillin (AMP); Cefotaxime (CTX); Chloramphenicol (CHL); Tetracycline (TET), Gentamycin (GEN), Trimethoprim/Sulfamethoxazole (SXT) 1:19, Azithromycin (AZM); Ciprofloxacin (CIP).

Table 4. Prevalence of multidrug resistance in 48 non-susceptible *E. coli* isolates from RTE street food (n = 26) and drinking water (n = 22).

Type of Resistance	Group of Antibiotics	Number of Isolates	
		RTE Street Food	Drinking Water
Multi-resistant	AMX, AMC, CAZ, AMP, CHL, SXT	-	1 (4.5%)
	IPM, CHL, TET, AZM	-	1 (4.5%)
	AMX, FOX AMP, TET, SXT	-	1 (4.5%)
	IPM, GEN, SXT	-	1 (4.5%)
	TET, GEN, SXT	-	1 (4.5%)
	AMX, AMC, IPM, FOX, AMP, GEN, SXT, AMZ	-	1 (4.5%)
	AMX, AMC, CAZ, AMP, CHL, GEN, SXT	1 (3.8%)	-
	AMX, AMP, CHL, SXT	-	1 (4.5%)
	AMX, AMC, AMP, CHL, TET, SXT	1 (3.8%)	-
	AMP, TET, AZM	1 (3.8%)	-
	AMX, AMP, TET, SXT	-	1 (4.5%)
	AMX, AMP, TET, AZM	1 (3.8%)	-
	AMX, AMC, IPM, FOX, AMP, TET, GEN, SXT	-	1 (4.5%)
	AMX, CAZ, IPM, FOX, AMP, CHL, TET, AZM	-	1 (4.5%)
	AMX, AMC, AMP, CHL, SXT	1 (3.8%)	-
Total		**5 (19.2%)**	**10 (45.5%)**
Non-multi resistant	CTX, TET	-	1 (4.5%)
	IPM, AMP, CTX, TET	-	1 (4.5%)
	IPM, AMP, TET	-	1 (3.6%)
	CAZ, IPM, TET	1 (3.8%)	-
	AMX, CAZ, IMP, ATM, FOX, AMP, CTX, CHL	-	1 (4.5%)
	AMX, AMC, AMP, TET	-	1 (4.5%)
	AMX, IPM, AMP, TET	1 (3.4%)	-
	IPM, TET	2 (7.7%)	-
	IPM, CPO	1 (3.8%)	-
	TET, SXT	-	1 (4.5%)
	SXT	-	1 (4.5%)
	AMX, AMP, TET	-	1 (4.5%)
	TET	7 (27%)	-
	AMP, TET, AZM	1 (3.8%)	-
	TET, SXT	-	1 (4.5%)
	CAZ, GEN	1 (3.8%)	-
	AMP, TET	-	1 (4.5%)
	AMP, CTX	1 (3.8%)	-
	TET, GEN	1 (3.8%)	-
	CAZ	1 (3.8%)	-
	IMP	3 (11.5%)	1 (4.5%)
	AMX, IPM, AMP, TET	1 (3.8%)	-
	CHL	-	1 (4.5%)
Total		**21 (80.8%)**	**12 (54.5%)**

Amoxicillin (AMX); Amoxicillin/clavulanic Acid (AMC); Ceftazidime (CAZ); Imipenem (IPM); Cefpirome (CPO); Aztreonam (ATM); Cefoxitin (FOX); Ampicillin (AMP); Cefotaxime (CTX); Chloramphenicol (CHL); Tetracycline (TET), Gentamycin (GEN)g, Trimethoprim/Sulfamethoxazole (SXT), Azithromycin (AZM); Ciprofloxacin (CIP).

Among the isolates recovered from water, the highest percentage of resistant isolates was found for ß-lactams AMP (46.4%), followed by IPM (39.3%) and AMX (35.7%). For the non-ß-lactams, the highest percentages of resistant isolates were found for TET (50%), SXT (39.3%), and CHL (21.4%) (Table 3). No resistance profiles were observed for CPO and CIP (Table 3). Additionally, 22 (78.6%) isolates from water presented an antibiotic resistance profile, of which 10 (45.5%) were multidrug-resistant (Figure 1 and Table 4) and 12 (54.5%) were non-multi-resistant (Table 4). Six isolates (21.4%) were susceptible to the 15 tested antibiotics.

3.3. Antibiotic Resistance Genes

A total of 116 fragments genes were amplified from 16 variants of antibiotic resistance genes screened in the 48 non-susceptible *E. coli* isolates. In food isolates, 59 fragments of antibiotic resistance genes were detected, whereas in water isolates, this number was 57 (Table 5). Among the 59 genes detected in isolates from food, 38 (64.4%) were ß-lactam resistance genes and 21 (35.6%) were genes encoding non-ß-lactam resistance. From the 38 β-lactamase genes, 22 (57.9%) were ESBL genes, and 16 (42.1%) were potential *AmpC* producers. From the 21 non-β-lactamase genes, 16 (76.2%) were *tet* genes and five (23.8%) were *sul* genes. Each of the bla_{SHV} variants and the *ACC* variants accounted for 23.7% of the total ß-lactam resistance genes, making them the most frequent genes, followed by the *FOX* variants (18.4%), *MCTX-M* Group 9 variants, and bla_{TEM} variants (15.8%, respectively). The *tetA* gene was the most frequent non-ß-lactamase resistance gene (71.4%), followed by the *sul2* gene (14.3%) and *sul3* (9.5%) (Table 5).

Table 5. Prevalence of resistance genes in 48 non-susceptible *E. coli* isolates from RTE street food and drinking water.

Group	Subgroup	Antibiotic Resistance Gene Variants	Frequency		
			RTE Street Food	Water	Total
ß-lactams	ESBL	bla_{tem} variants	6 (15.8%)	7 (17.5%)	13 (16.7%)
		bla_{oxa} variants	0	4 (10%)	4 (5.1%)
		bla_{shv} variants	9 (23.7%)	6 (15%)	15 (33.3%)
		MCTX-M Group 1 variants	1 (2.6%)	2 (5%)	3 (3.8%)
		MCTX-M Group 9 variants	6 (15.8%)	4 (10%)	10 (12.8%)
		Total ESBL genes	**22 (57.9%)**	**23 (57.5%)**	**45 (57.7%)**
	ß-lactams ampC	*ACC* variants	9 (23.7%)	5 (12.5%)	14 (17.9%)
		FOX variants	7 (18.4%)	4 (10%)	11 (14.1%)
		MOX variants	0	0	0
		CIT variants	0	8 (20%)	8 (10.3%)
		DHA variants	0	0	**0**
		Total ampC genes	**16 (42.1%)**	**17 (42.5%)**	**33 (42.3%)**
Total ß-lactam coding genes			**38 (48.7%)**	**40 (51.3%)**	**78 (100%)**
non-ß-lactams	Tetracyclines	*tet-A*	15 (71.4%)	4 (23.5%)	19 (50%)
		tet-B	0	0	0
		tet-M	1 (4.8%)	0	1 (2.6%)
		Total-TET genes	**16 (76.2%)**	**4 (23.5%)**	**20 (52.6%)**
	Sulphonamides	*sul-1*	0	0	0
		sul-2	3 (14.3%)	9 (52.9%)	11 (28.9%)
		sul-3	2 (9.5%)	4 (23.5%)	6 (15.8%)
		Total-*sul* genes	**5 (23.8%)**	**13 (76.5%)**	**18 (47.4%)**
Total non-ß-lactams			**21 (55.3%)**	**17 (44.7%)**	**38 (100%)**

In six (19.4%) isolates from RTE food and eight (28.6%) from water, there was the simultaneous occurrence of ESBL and *AmpC* genes. In two (6.5%) isolates from RTE and in six (21.4%) from water, we found the simultaneous occurrence of ESBL, *AmpC*, and *sul* coding genes. The simultaneous presence of ESBL and *sul* coding genes was observed in one isolate from RTE street food and water, respectively. The simultaneous occurrence of *AmpC* and *tet* coding genes was found in two isolates from RTE street food.

Regarding the 57 antibiotic resistance genes detected in *E. coli* isolated from water, 70.2% (n = 40) were ß-lactam genes and 29.8% (n = 17) non-ß-lactam genes. Of the 40 ß-lactam genes, 23 were ESBL genes and 17 were ß-lactams *AmpC* (Table 5). Of the 17 non-ß-lactams genes, four were *tet* genes and 13 were *sul* genes (Table 5). The *CIT* variants represented 20% of the total ß-lactamase resistance genes, making them the most frequent genes, followed by bla_{TEM} variants (17.5%) and bla_{SHV} variants (15.8%). For non-ß-lactams

genes, *sul2* gene (52.9%) was the most frequent one, followed by *tetA* and *sul3* (23.5%, respectively) (Table 5).

Simultaneous occurrences of more than one variant of ESBL gene were found in eight isolates from RTE street food and in four isolates from water. In RTE street food, bla_{TEM} variants and bla_{MG9} variants were simultaneous founded in three isolates, variants bla_{TEM} and bla_{SHV} in three isolates, $bla_{MG\ 9}$ variants and bla-$_{SHV}$ variants in four isolates, and bla_{Mg1} variants and bla_{SHV} variants in one isolate. In water, bla_{TEM} variants and bla_{MG9} variants were simultaneous founded in two isolates from water, The variants bla_{TEM} and bla_{SHV} one from water $bla_{MG\ 9}$ variants and bla_{SHV} variants one from water. bla_{TEM} variants and bla_{MG1} variants, bla_{OXA} variants and bla_{MG1} variants in one isolate from water. Regard *AmpC* variants, *FOX* variants and *ACC* variants were found in four isolates from RTE street food and in two isolates from water. The variants *ACC* and *CIT* were found in simultaneous in three isolates from water. In nom-ß-lactamases gens, *sul2* and *sul3* were simultaneous founded in two isolates from RTE and four from water.

4. Discussion

The PFGE subtyping results show a high diversity of isolates, either among those collected from RTE street food or from street water for human consumption. It was not possible to establish a relationship between the origin of the isolates (food or water) or the municipal district where they were sold, confirming the high diversity of fecal contamination sources of food and water sold on the streets, as previously reported [1,20]. In fact, Maputo municipal districts are non-urbanized areas, with numerous sanitation problems and water supply difficulties, contributing to the spread of pathogenic microorganisms [5,15,32]. In some cases, isolates gathered in clusters with a high level of similarity (>85%), presenting different virulence genes or different antibiotic resistance profiles, which suggests that the isolates may have had the same clonal origin but evolved in different environments where different genes were acquired, depending on adaptive mutations to cope with lethal stresses [33]. In general, isolates from food had a higher prevalence of virulence genes when compared with those from water. Nevertheless, *stx* coding for the *shiga/vero* toxin (VT) produced by the verotoxin-producing *E. coli* (VTEC) was identified with similar frequencies in isolates from food (5/31) and from water (6/28). The VTECs are considered a great threat in foodborne diseases. For example, *E. coli* O157:H7 became the first of several VTECs referred to as enterohaemorrhagic *E. coli* (EHEC), which can produce one or more *shiga* toxins (VTs). The main reservoirs for VTECs are ruminants, which continually shed bacteria into the environment contaminating food and water [34,35]. These strains can survive in fresh ground beef and on fresh leafy green vegetables [34]. Currently, *E. coli* is the most widespread indicator of fecal contamination in food handling processes, and its presence usually correlates with inadequate hygiene or cross contamination [36,37]. The isolates analyzed here were from street food and water with high fecal contamination (food *E. coli* counts above 3.5 log CFU/g [1], and between 1.6–2.8 log CFU/100 mL in water samples [22]). The occurrence of potential DECs among the isolates strongly supports the hypothesis of fecal contamination of food and water [38]. In fact, reports of lack of toilets and poor water supply at points of sale have already been published [1,2,5].

Among the street food isolates, 17 of the 26 antibiotic-resistant isolates carried one or more resistance gene. Of these, three had the gene encoding VT and were, simultaneously, multidrug-resistant. Nine had genes encoding *EAST* and showed high resistance to beta-lactams. All isolates with the *eae* gene were resistant to at least one antibiotic tested.

Among the water isolates, 14 out of 22 antibiotic-resistant isolates had virulence genes. Four of these isolates were multidrug-resistant with genes encoding VT, Lt and ST. All water isolates carrying the gene encoding *EAST* were resistant to antibiotics. The increased use of antimicrobials for prophylaxis and therapy against pathogens and for growth promotion in livestock, aquaculture and agricultural production may contribute to the increase of antibiotic resistance strains in the environment [39,40]. Multidrug-resistant Gram-negative bacteria represent significant global public health threats [41].

Information on antibiotic resistance in southern Africa is scarce, and the little existing data were obtained from clinical isolates, with a global prevalence between 25 and 50% [42]. In the present study, the highest percentages of resistant isolates from food and water were found for the ß-lactams IPM (35.5 and 39.3%, respectively) and AMP (39.3 and 46.4%, respectively). Regarding the non ß-lactams, the highest percentages of resistant isolates were found for TET (58.1% from food and 50% from water). Multidrug resistance was higher in *E. coli* isolates from water (45.5%) compared to RTE street food isolates (19.2%). Overall, three out of five and eight out of 10 multidrug-resistant isolates, from food and water, respectively, had virulence genes. The prevalence of the multidrug resistance reported here is lower than those observed for hospital isolates [15,18,43,44] but similar to those reported in South Africa in isolates from food and water [45,46]. In isolates from beef and water, Jaja et al. [45] reported a prevalence of resistance to β-lactams between 20 and 45% and a prevalence of 24% to the non-β-lactam TET. Malema et al. [46] found 50 to 75% of resistance to AMP, AUG, and TET in *E. coli* recovered in water, and multidrug resistance was 52% of the isolates [46]. In Tanzania, studies revealed an increasing trend toward the emergence of multidrug-resistant and pathogenic *E. coli* found in food animals [39].

The search for antibiotic resistance genes showed that ESBLs were the most frequent β-lactam genes, with 57.9% in isolates from RTE street food and 57.5% in water sources. The bla_{SHV} variants (23.7%), CTX-M Group 9 variants (15.8%), and ba_{TEM}-variants (15.8%) were the most predominant ESBL genes. Similar results were obtained by Faife et al. [47], who reported bla_{SHV} (85.0%), followed by the Bla CTX-M group (62.9%) and bla_{TEM} (44.4%), among the *E. coli* isolated from imported and locally produced chicken in Mozambique. However, according to Faife et al. [47], it is difficult to understand the contribution of local isolates to resistance genes, since some of the chickens were imported. Among *E. coli* isolates from children with enteric diarrhea, at the Maputo Central Hospital, Mozambique, Berendes et al. [15] found 68% of the isolates with the bla_{SHV} variants and 20% with the prevalence of the CTX-M Group 9. Estaleva et al. [43] also performed studies with isolates from clinical cases and found 77% with the CTX-M Group and 52% with the bla_{SHV} variants. The coexistence of several ESBL and *AmpC* genes were found in this study. A similar study reported a large number of clinical isolates that co-expressed bla_{CTM-1}, bla_{CTM-15}, bla_{CMY-2}, bla_{NDM-1}, bla_{NDM-5} and some other ß-lactamases [41]. ESBL-producing strains represent a major health problem worldwide [40,41]. The most common ESBL variants include blaTEM, blaSHV, and bla_{CTX-M}, which are considered the main causes of hospital and community infections [41].

In this study, *AmpC* ACC variants and FOX variants were found in RTE street food (23.7 and 18.4%, respectively) and in water isolates (12.5 and 10%, respectively), whereas CIT variants were found only in water isolates (20%). In some isolates from the Enterobacteriaceae family, such as *E. coli* and *Shigella* spp., ampC is expressed at clinically irrelevant levels [48]. These isolates do not have an ampR gene [48,49], and *AmpC* regulation is accomplished by a weak promoter and a strong attenuator [48]. Thus, these isolates are normally sensitive to cefoxitin and are non-inducible, not altering the inhibitory effect of β-lactams unless by mutations in the promoter region [48,49]. However, these mutations must be hyper-producing of ampR, making the isolates resistant to cefoxitin and third-generation cephalosporins [50]. These mutations are sporadic and much less frequent than the resistance mechanism in *E. coli* through the acquisition of ESBL gene [50].

In this study, DHA variant and MOX variant genes were not detected in food and water isolates. In isolates from clinical cases in Maputo, Chirindze et al. [51] observed a high prevalence of DHA variants (14.4%) and MOX variants (9.6%). Regarding tetracycline resistance genes, *tetA*, with 71.4% in food and 23.5% in water isolates, was the most frequent non-ß-lactam gene. Similar results were obtained by Berendes et al. [15], who reported a prevalence of tetracycline resistance genes (*tetA* and *tetB*) above 85% in isolates from children with enteric diarrhea in Maputo. Mandomando et al. [52] also found a high prevalence (71%) of *tetA* in *Shigella* and Salmonella isolates from hospitalized children. Although there are no Mozambican studies investigating the occurrence of tetracycline

resistance genes in isolates from water or food, several studies in other African countries report a high prevalence of these genes in food-borne isolates [45,53–55]. Furthermore, 13 out of 32 tetracycline resistance isolates were negative for the three tet genes searched in the present study, which leads us to infer that the enzymes involved in the phenotypic resistance to the antibiotic tetracycline are coded by genes different from those investigated here, making it necessary to search for other, less common genes.

Microbiological criteria define the acceptability of foods based on the number of microorganisms and their pathogenic potential [56]. The occurrence of potentially pathogenic *E. coli* isolates reported in the aforementioned studies [1,22] represents an increased risk to public health. The occurrence, for example, of potential VTEC in food (5/31) and water (6/28) may represent an increase in clinical cases in local hospitals. These cases are of particular concern as some of these potentially pathogenic isolates (3/5 and 5/6, from food and water, respectively) were multi-resistant isolates. The consumption of untreated water represents the main cause of occurrence of diarrheal diseases in developing countries, especially in children under five years old [57]. In low-income countries, these diarrheal diseases transmitted by water and food account for more than 35% of hospital admissions of these children [58].

5. Conclusions

A previous assessment of the microbiological quality of water and food sold on the streets of Maputo indicated that they were generally unsatisfactory for consumption. In the present work, although it was not possible to determine a relationship between food contamination and water contamination, a higher percentage of antibiotic resistance was found among the water isolates. This raises concerns about the possibility of contamination of water sources by antibiotics used in medical and veterinary treatments. Multidrug resistance was observed, some of these isolates also had DEC virulence genes. Other genes associated with other pathotypes, such as urinary tract infection or meningitis, have not been evaluated here. Furthermore, the antibiotic resistance profile was designed based on the most locally used antibiotics. The results obtained in this work suggest the evaluation of new generation antibiotics for diseases associated with DECs in Mozambique.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol2010008/s1, Table S1. List and evaluation intervals of the antibiotics used, according to CLSI, 2021 criteria.

Author Contributions: Conceptualization, A.S., M.M.-F. and L.B.; methodology, A.S., S.A., L.C. and L.B.; software, L.C.; validation, L.C., M.M.-F. and L.B.; formal analysis, A.S., L.C. and L.B.; investigation, A.S. and L.B.; writing—original draft preparation, A.S., S.A., M.M.-F. and L.B.; writing—review and editing, A.S., M.M.-F., L.C. and L.B.; supervision, M.M-F. and L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by national funds through FCT—Foundation for Science and Technology, I.P., under the scholarship grants which reference PRT/BD/151521/2021, and project UID/AGR/04129/2020 (LEAF) and UIDB/04046/2020 and UIDP/04046/2020 Centre grants from FCT, Portugal (to BioISI).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting the findings of this study are available within the article and its supplementary materials.

Acknowledgments: The authors thank Ana Carla Silva for the support with the work performed in Laboratory of Microbiology (ISA/ULisboa) and the authors thank project PORBIOTA—Portuguese E-Infrastructure for Information and Research on Biodiversity (POCI-01-0145-FEDER-022127), Operational Thematic Program for Competitiveness and Internationalization (POCI), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (FEDER).

Conflicts of Interest: The authors declare no conflict of interest.

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