

# Raw wastewater irrigation for urban agriculture in three African cities increases the abundance of transferable antibiotic resistance genes in soil, including those encoding extended spectrum $\beta$ -lactamases (ESBLs)

Bougnom, Blaise; Thiele-Bruhn, Sören; Ricci, Vito; Zongo, Cheikna; Piddock, Laura

DOI:

[10.1016/j.scitotenv.2019.134201](https://doi.org/10.1016/j.scitotenv.2019.134201)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Bougnom, B, Thiele-Bruhn, S, Ricci, V, Zongo, C & Piddock, L 2020, 'Raw wastewater irrigation for urban agriculture in three African cities increases the abundance of transferable antibiotic resistance genes in soil, including those encoding extended spectrum  $\beta$ -lactamases (ESBLs)', *Science of the Total Environment*, vol. 698, 134201. <https://doi.org/10.1016/j.scitotenv.2019.134201>

[Link to publication on Research at Birmingham portal](#)

## General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

Download date: 23. Apr. 2024

1 **Raw wastewater irrigation for urban agriculture in three African cities increases the**  
2 **abundance of transferable antibiotic resistance genes in soil, including those encoding**  
3 **Extended Spectrum  $\beta$ -Lactamases (ESBLs)**

4 B. P. Bougnom <sup>a,b</sup>; S. Thiele-Bruhn <sup>c</sup>; V. Ricci <sup>a</sup>; C. Zongo <sup>d</sup>; L.J.V Piddock <sup>a\*</sup>

5 <sup>a</sup> Institute of Microbiology and Infection, University of Birmingham, B15 2TT, UK

6 <sup>b</sup> Department of Microbiology, Faculty of Science, University of Yaounde 1, P.O. Box 812 Yaounde, Cameroon

7 <sup>c</sup> Department of Soil Science, University of Trier, D-54286 Trier, Germany

8 <sup>d</sup> Department of Biochemistry and Microbiology, University Ouaga I Pr Joseph KI-ZERBO, 03 BP 7021

9 Ouagadougou 03, Burkina Faso

10 \*Corresponding author. Institute of Microbiology and Infection, University of Birmingham, B15 2TT, UK,

11 E-mail address: l.j.v.piddock@bham.ac.uk

12 **Abstract**

13 A study was conducted to investigate the impact of raw wastewater use for irrigation on dissemination  
14 of bacterial resistance in urban agriculture in African cities. The pollution of agricultural fields by selected  
15 antibiotic residues was assessed. The structure and functions of the soil microbial communities, presence of  
16 antibiotic resistance genes of human clinical importance and *Enterobacteriaceae* plasmid replicons were analysed  
17 using high throughput metagenomic sequencing. In irrigated fields, the richness of *Bacteroidetes* and *Firmicutes*  
18 phyla increased by 65% and 15.7%, respectively; functions allocated to microbial communities' adaptation and  
19 development increased by 3%. Abundance of antibiotic resistance genes of medical interest was 27% greater in  
20 irrigated fields. Extended spectrum  $\beta$ -lactamase genes identified in irrigated fields included *bla*<sub>CARB-3</sub>, *bla*<sub>OXA-347</sub>,  
21 *bla*<sub>OXA-5</sub> and *bla*<sub>Rm3</sub>. The presence of ARGs encoding resistance to amphenicols,  $\beta$ -lactams, and tetracyclines were  
22 associated with the higher concentrations of ciprofloxacin, enrofloxacin and sulfamethoxazole in irrigated fields.  
23 Ten *Enterobacteriaceae* plasmid amplicon groups involved in the wide distribution of ARGs were identified in  
24 the fields. IncQ2, ColE, IncFIC, IncQ1, and IncFII were found in both farming systems; IncW and IncP1 in  
25 irrigated fields; and IncY, IncFIB and IncFIA in non-irrigated fields. In conclusion, raw wastewater irrigated soils  
26 in African cities could represent a vector for the spread of antibiotic resistance, thus threatening human and animal  
27 health. Consumers of products from these farms and farmers could be at risk of acquiring infections due to drug-  
28 resistant bacteria.

29 **Key words:** Wastewater irrigation; agricultural fields; bacterial resistance; metagenomics; ESBLs; Africa.

30           **1. Introduction**

31   Antibiotics are known for their properties to either stop bacteria from growing or to kill them. Bacterial drug  
32   resistance emergence and dissemination has compromised healthcare systems and public health policy in many  
33   countries. This is a source of concern in low- and middle-income countries (LMICs) where antibiotics of last  
34   resort are not available to most people (WHO, 2014). Furthermore, in LMICs, a high infectious disease burden  
35   commonly co-exists so antibiotic resistance compromises the treatment of many infections that were, until  
36   recently, treatable (Laxminarayan et al. 2016). Drug-resistant bacteria are a critical public health concern even in  
37   developed countries (Piddock, 2012). In 2011, an epidemic of *Escherichia coli* infections caused by contaminated  
38   bean sprouts affected up to 5,000 people in Europe, with over 48 deaths (Buchholz et al. 2011). To efficiently  
39   tackle the increasing bacterial resistance, environmental, agricultural, and medical aspects need to be handled at  
40   a global scale (Wellington et al. 2013).

41           Arable lands reported to be irrigated with wastewaters worldwide cover approximately 20 million  
42   hectares; which equates to 10% of the total global irrigated land (Mateo-Sagasta et al. 2011). Wastewaters  
43   originating from slaughterhouses, private use and applications in hospitals can contain high concentrations of  
44   antibiotics along with many drug-resistant pathogenic bacteria. Their application on agricultural fields can  
45   influence the structure of the soil microbiota (Dickin et al. 2016). Whereas in the past several research teams have  
46   addressed questions related to the application of manure, which is contaminated with antibiotics used in animal  
47   husbandry, much less is known about the effect of raw wastewater irrigation on the development of antibiotic  
48   resistant bacteria (ARB) in irrigated fields. This is despite the fact that raw wastewater irrigation is often applied  
49   in LMICs as a cheap alternative during water scarcity and to avoid expensive commercial fertilizers. In many  
50   cities in LMICs, wastewater irrigation has been a common practice for decades (Adegoke et al. 2018).  
51   Wastewaters can contain high concentrations of antibiotics from private use and applications in hospitals, along  
52   with pathogenic or drug-resistant bacteria or both (Igbinosa et al. 2011).

53           The release of pharmaceuticals in the environment selects for drug-resistant bacteria (Andersson and  
54   Hughes, 2010). To combat antibiotics in ecosystems, bacteria have evolved a plethora of different antibiotic  
55   resistance genes (ARGs) of which many are mobile and can easily spread between species including human and  
56   animal pathogens. Environmental drug-resistant bacteria can transfer ARGs to pathogenic bacteria by horizontal  
57   gene transfer (Forsberg et al. 2012). Owing to its complex intrinsic and acquired antibiotic resistome, some studies  
58   have highlighted the importance of soil as the potentially largest reservoir of genes coding for antibiotic resistance  
59   (Wang et al. 2014; Nesme and Simonet, 2015). There is a crucial need to identify the principal reservoirs of ARGs

60 for humans, animals and the environment, since there is insufficient information about the conditions and factors  
61 that lead to the mobilization, selection and movement of resistant drug bacteria into and between environment,  
62 human and animal populations (Wellington et al. 2013).

63 Next generation sequencing has been successfully used to track drug-resistant bacteria and ARGs which  
64 may spread quickly across the soil microbial community following selection pressure due to antibiotic application  
65 (Fahrenfeld et al. 2014; Guo et al. 2018). Metagenomics provides an understanding of the factors driving transfer  
66 of ARGs and the prevalence of different antibiotic resistance mechanisms (Amos et al. 2014).

67 In this study, we assessed the influence of raw wastewater commonly used in urban agriculture in LMICs  
68 on the structure and functions of soil microorganisms, and presence of drug-resistant bacteria and genes. It is  
69 postulated that in agricultural fields irrigated with raw wastewater, bacteria have adapted to survive antibiotic  
70 exposure by vertical and horizontal gene transfer producing high numbers of bacteria containing clinically relevant  
71 ARGs.

72

## 73 **2. Material and methods**

### 74 **2.1. Experimental design and soil sampling**

75 The experiment was conducted in three cities, in two African countries, namely Ouagadougou (12°23' N, 1°29')  
76 in Burkina Faso, Ngaoundere (7°19' N, 13°35') and Yaounde (3°52' N, 11°31') in Cameroon. Their respective  
77 annual mean of temperature and precipitations are for: Ouagadougou (30°C; 867 mm); Ngaoundere (22°C;  
78 1497mm) and Yaounde (24°C; 1628 mm), respectively. At each city two blocks were investigated, comprising  
79 three agricultural fields that were irrigated (IRI) with raw wastewater, and as control soils, 500 m away, three  
80 non-irrigated agricultural fields (NIR), with comparable soil properties. Wastewater was coming from  
81 dwellings, hospitals, agriculture, markets and slaughterhouses (Bougnom et al. (2019a; 2019b). Tomato and  
82 salad were regularly cultivated in the fields. We had Ouagadougou (IRI1 and NIR1); Ngaoundere (IRI2 and  
83 NIR2), and Yaounde (IRI3 and NIR3). The agricultural fields were approximately 0.2 ha each and watered  
84 manually twice per day with watering cans. In each field, 100 g of soil was randomly sampled at 10 different  
85 places from 0-20 cm depth, using soil cores. Replicate samples were pooled together, receiving 1 kg-composite  
86 samples. The collected soil samples were kept on ice during transport and stored at -80°C before analysis.

### 87 **2.2. Soil physical and chemical analysis**

88 Soil pH was measured in a 1:2.5 (soil: demineralised water) ratio using a glass electrode. Total C and N  
89 were analysed using a TOC-V<sub>CPN</sub>-analyzer (Shimadzu, Duisburg, Germany).

90 Soil antibiotic residues were extracted using solid phase extraction (SPE) according to Blackwell et al.  
91 (2004). The detailed description is reported in supplementary text S1. SPE was conducted from 4 g of air-dried  
92 soil, using SAX and HLB SPE cartridges (Thermofisher, Massachusetts, USA) set up in tandem. Prior to SPE,  
93 the cartridges were conditioned with 5mL methanol then conditioned with 5mL buffer. After SPE, the SAX  
94 cartridges were removed and the HLB cartridges were washed with 5ml conditioning buffer. Thereafter, the  
95 HLB cartridges were air dried for 10 min and antibiotic residues were eluted with  $2 \times 1$  mL of methanol. Liquid  
96 chromatography-mass spectrometry (LC-MS) was used to quantify antibiotic residue concentrations (Michelini  
97 et al. (2012). Ciprofloxacin, enrofloxacin, chlortetracycline, sulfadimidine, sulfadiazine, sulfamethoxazole,  
98 oxytetracycline, tetracycline, trimethoprim and tylosin were the analyzed pharmaceuticals. These  
99 pharmaceuticals were analysed because of their molecular structure and physicochemical properties which allow  
100 them to resist transformation or degradation in soils (Cycon et al. 2019; Kumar et al. 2019).

## 101 **2.3. Microbiological analysis**

### 102 **2.3.1. Soil Biomass Purification**

103 Soil biomass purification was conducted to collect mainly the bacterial cells from the different soils  
104 (Sentchilo et al. 2013). Briefly, 15g soil samples were homogenized by magnetic stirring for 15min, in ice-cold  
105 poly (beta-amino) esters (PBAE) buffer (PBAE buffer is 10mM Na-phosphate, 10mM ascorbate, 5mM EDTA,  
106 pH 7.0), at 10 mL g<sup>-1</sup> of soil. Low speed centrifugation in 50-mL conical tubes at 160 g for 6 min was used to  
107 remove bigger particles (>10 µm) such as coarse particles, large eukaryotic cells and bacterial flocs. Microbial  
108 biomass was collected after centrifugation of the supernatants at 10,000 g/5 min.

### 109 **2.3.2. DNA extraction and quantification, and sequencing**

110 DNeasy PowerSoil Kit (Qiagen, Germany) was used to extract soil DNA according to the manufacturer's  
111 instructions. Fluorometry was used to determine DNA concentration, using the Qubit™ 3.0 Fluorometer (Qubit,  
112 Life Technologies, USA). The three DNA samples extracted from each block were pooled together in equal  
113 nanogram quantities. Six DNA samples representative of the three cities were sent to Edinburgh Genomics for  
114 high-throughput sequencing. Sequencing was conducted using Illumina Hiseq4000 (Illumina, Inc, USA),  
115 TruSeq DNA. Nano gel free library (350 bp insert) was used to prepare the libraries. Raw data consisted of  
116 190.5 Gb sequences. The sequenced reads raw data have been deposited in MG-RAST server (project IDs:  
117 [mgm4815682.3](#) ; [mgm4815683.3](#); [mgm4815684.3](#); [mgm4815685.3](#); [mgm4815686.3](#); and [mgm4815687.3](#)).

## 118 **2.4. Bioinformatic analyses**

### 119 **2.4.1. Taxonomic and functional annotations**

120 The raw sequence reads were uploaded to the metagenomics analysis server (MG- RAST) version 4.0.3  
121 (Glass et al. 2010). Soil microbial communities' profile and metabolic functions was determined with the use of  
122 both SEED database and SEED subsystems database, respectively. The SEED subsystems is a collection of  
123 protein families sharing the same functions. Both microbial and metabolic profiles were generated using a  
124 minimum identity  $\geq 80\%$  and minimum alignment length  $\geq 20$  amino acids, at E-value  $10^{-5}$  (Glass et al. 2010).

#### 125 **2.4.2. Identification and quantification of antibiotic resistance genes.**

126 Short Better Representative Extract Dataset (ShortBRED) was used to identify and quantify antibiotic  
127 resistance genes (ARGs) from the metagenome (Kaminski et al. 2015). ShortBRED profiles protein family  
128 abundance in metagenomes in two-steps: (i) *ShortBRED-Identify* isolates representative peptide sequences  
129 (markers) for the protein families, and (ii) *ShortBRED-Quantify* maps metagenomic reads against these markers  
130 to determine the relative abundance of their corresponding families based on reads per kilobase million  
131 (RPKM). Minimum identity of 95% and minimum fragment length of 30 amino acids were considered positive.  
132 ARG markers were generated from the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et  
133 al. 2013) was used to generate, using UniRef50 as a reference protein database. Antibiotic resistance ontology  
134 (ARO) numbers in CARD was used to aggregate, annotate and associate the ARGs to the corresponding  
135 resistance family.

#### 136 **2.4.3. Identification of plasmid amplicons of clinical relevance**

137 *Enterobacteriaceae* plasmid replicon sequences were downloaded from the PlasmidFinder database 1.3  
138 (<https://cge.cbs.dtu.dk/services/PlasmidFinder>). The nucleotide sequences were aligned against the  
139 metagenomic reads using BLAST-like alignment tool (BLAT). The parameters were BLAT hit with a sequence  
140 identity  $\geq 80\%$  and E-value cut-off of  $10^{-5}$  (Carattoli et al., 2014).

#### 141 **2.5. Data Analysis**

142 The differences in relative abundance of the different bacterial phyla and families of interest, functional  
143 categories present in the metagenomic reads, ARGs, and *Enterobacteriaceae* plasmid replicon groups detected  
144 in the agricultural fields were analysed using the Student's *t*-test, at  $P < 0.05$ . Heatmaps were generated to  
145 visualize tabular abundance of ARGs in the fields, using hclust2 (Asnicar et al. 2015).

146

### 147 **3. Results and Discussion**

#### 148 **3.1. Antibiotic residues in soil**

149 Enrofloxacin oxytetracycline and sulfamethoxazole concentrations were greater in irrigated fields. The  
150 concentration of enrofloxacin was greater (1.10 ng. g<sup>-1</sup>) in irrigated fields while that of sulfadimidine was greater  
151 (0.81 ng. g<sup>-1</sup>) in non-irrigated fields. In irrigated fields, antibiotic concentrations ranged from 0.09 to 0.92; in non-  
152 irrigated fields, they ranged from 0.04 to 0.44 ng g<sup>-1</sup> (Table 1). Sulfadiazine, chlortetracycline, tetracycline and  
153 tylosin residues were not detected in any soil from either farming systems. In irrigated fields, the concentrations  
154 of sulfadimidine and oxytetracycline was greater than in the non-irrigated fields by 10 %, ciprofloxacin by 52%,  
155 enrofloxacin by 63.6%, and sulfamethoxazole by 77.8%.

156 The 20 years irrigation of soils with raw wastewater (Cisse et al. 2002; Kengne et al. 2002) containing  
157 substantial amounts of organic matter led to higher pH, organic carbon and nitrogen. This could be consequent to  
158 the increase in the soil organic matter (SOM) pool (Bougnom et al. 2009). The levels of antibiotic residues  
159 concomitantly increased in irrigated soils. This confirms the studies reporting the accumulation of antibiotic  
160 compounds in agricultural fields following irrigation with treated or raw wastewater (Calderon-Preciado et al.  
161 2011; Grossberger et al. 2014; Wang et al. 2014). Soil pH, organic matter content and soil texture are reported to  
162 be among the factors impacting the fate of pharmaceuticals in soil (Thiele-Bruhn et al. 2004; Du and Liu, 2012).  
163 Antibiotic residues are less bioavailable, and thus less biodegradable in soils with high SOM and clay content,  
164 owing to stronger sorption to SOM and the formation of non-extractable residues (Luo et al. 2011; Müller et al.  
165 2013; Cheng et al. 2016). Both higher SOM content and higher contents of enrofloxacin, oxytetracycline and  
166 sulfamethoxazole residues were found in irrigated fields. The prevalence of fluoroquinolones and  
167 sulfamethoxazole may be due to the input or related to the stronger retardation of these antibiotics in acidic and  
168 iron oxide-rich tropical soils (Essington et al. 2010). Antibiotic residues were also determined in non-irrigated  
169 farms but at lower contents. This contamination could be due to previous (not reported to us) use of animal manure  
170 as fertilizer, deposition of wastewater aerosol and soil dust, respectively, derived from the nearest irrigated sites  
171 by wind erosion, and human transport of agricultural materials between fields (Dalkmann et al. 2012).

### 172 **3.2. Effects of urban agriculture and wastewater irrigation on soil microorganisms**

#### 173 **3.2.1. Microbial diversity and functionality**

174 The taxonomic analysis of the microbial communities at kingdom level in irrigated and non-irrigated fields  
175 showed that the highest proportion of metagenomic reads mapped to Bacteria (99.1% and 99.2%), followed by  
176 Archaea (0.48% and 0.42%), Eukaryota (0.26% and 0.34%), and unassigned (0.05% and 0.06%). Metagenomic  
177 reads allocated to viruses represented 0.01% in samples from both farming systems. Ten and nine bacterial phyla  
178 with a relative prevalence  $\geq 0.5\%$  of the reads were identified in irrigated and non-irrigated fields, respectively

179 (Figure 1a). The dominant bacteria phyla in both farming systems were *Proteobacteria*, *Actinobacteria* and  
180 *Bacteroidetes* ( $\geq 89.6\%$  of all bacterial phyla). In irrigated fields, the relative abundance of *Bacteroidetes* and  
181 *Firmicutes* were 65% and 15.7% greater, respectively. The phylum *Gemmatinodetes* was not among the most  
182 prevalent phyla in irrigated fields. Figure 1b shows the top 22 bacterial families found in the different farming  
183 systems. The relative abundance and order of bacterial families differed in the farming systems. In irrigated  
184 agricultural fields, the 10 most prevalent bacterial families were 1. *Xanthomonadaceae*, 2. *Caulobacteraceae*, 3.  
185 *Comamonadaceae*, 4. *Sphingomonadaceae*, 5. *Flavobacteriaceae*, 6. *Mycobacteriaceae*, 7. *Pseudomonadaceae*,  
186 8. *Planctomycetaceae*, 9. *Bradyrhizobiaceae*, and 10. *Nocardioideaceae*. The top 10 in non-irrigated fields were  
187 (deviating numbers from irrigated fields are added in brackets) 1. (2) *Caulobacteraceae*, 2. (1)  
188 *Xanthomonadaceae*, 3. *Comamonadaceae*, 4. (7) *Pseudomonadaceae*, 5. *Flavobacteriaceae*, 6. (-)  
189 *Conexibacteraceae*, 7. (6) *Mycobacteriaceae*, 8. (9) *Bradyrhizobiaceae*, 9. (8) *Planctomycetaceae* and 10.  
190 *Nocardioideaceae*.

191 Soil pH, texture, nutrients, carbon content, pollutants, and agricultural management influence soil  
192 microbial structure and functions in soil (Jangid et al. 2011; Kuramae et al. 2011). In this context, it is noted that  
193 irrigation wastewater contains nutrient elements, chemical, physical and biological pollutants, as well as  
194 degradable organic matter from anthropogenic activities (Deblonde et al. 2011). Since many pollutants can have  
195 a stimulating or inhibitory effect on microbial cells, changes in both soil microbial structure and functions  
196 following irrigation with wastewater were as expected. The identified bacterial phyla in both irrigated and non-  
197 irrigated fields are generally encountered in soil (Fierer et al. 2012; Nacke et al. 2014). Previous field and  
198 mesocosm-scale studies have indicated the transfer of *Bacteroidetes* phyla members into the soil following  
199 wastewater irrigation (Broszat et al. 2014; Frenk et al. 2018). The increased number of this phyla is explained by  
200 its copiotrophic activity and high grow rate in the presence of nutrients, moisture and labile organic C found in  
201 wastewater (Broszat et al. 2014). This elucidates their greater prevalence in irrigated fields. *Firmicutes* can form  
202 spores in unfavourable environmental conditions and will then sporulate and give rise to bacterial growth in  
203 response to the additional nutrients (Onyenwoke et al. 2004). This could explain their increase in irrigated fields,  
204 which contain nutritious organic matter. Tropical soils are depleted of nutrients because of the high mineralisation  
205 rate. *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia* and *Planctomycetes* have been reported to follow oligotrophic  
206 strategies with limited growth rates and thriving capacity in nutrient-poor ecosystems (Lauber et al. 2013; Kielak  
207 et al. 2016). *Caulobacteraceae* family members are primarily known as oligotrophic microorganisms (Poindexter  
208 et al. 1981). This could explain the prevalence of these bacterial phyla and families in non-irrigated fields. The



209 greater abundance of *Xanthomonadaceae* family members following wastewater irrigation could present a health  
210 issue. The family *Xanthomonadaceae* contains plant-pathogenic genera including *Xanthomonas* and *Xylella* (Hajri  
211 et al. 2009). Therefore, plants grown in irrigated fields are more exposed to bacterial infections affecting crop  
212 productivity. Outbreaks of *Xanthomonas* wilt disease of banana are frequently reported in the region (Ocimati et  
213 al. 2019).

214 Functional metabolic diversity analysis of the six metagenome reads from the soil samples using the  
215 SEED database revealed that 14 subsystems were most frequent in the soil microbial communities (Table 2). The  
216 most prevalent functional categories were in both farming systems “Carbohydrates”, “Clustering-based  
217 subsystems” and “Amino acids and derivatives”. Comparative analysis using the Student’s *t*-test showed that  
218 sequence reads coding for functional subsystems “Clustering-based subsystems”, “DNA metabolism”,  
219 “Nucleosides and nucleotides” and “Stress response” were significantly higher in irrigated fields ( $P < 0.05$ ).

220 The analysis of metagenome reads using SEED provided insights into the functional metagenomic  
221 profiling of microorganisms living in the investigated fields. Considering changes in the microbial diversity  
222 structure observed between the two farming systems, some differences in the metabolic potential of the soil  
223 microbiota were expected. The functions ‘clustering-based subsystems’ (functional coupling evidence but  
224 unknown function), ‘DNA metabolism’ (DNA repair, bacterial), ‘nucleosides and nucleotides and stress response’  
225 translate a higher bacterial and enzymatic activity in irrigated fields consequent to the introduction of nutrients,  
226 organic matter and several pollutants. The soil microbiota must develop functional redundancy and adopt  
227 mechanisms to adapt, survive and grow. Therefore, wastewater irrigation affects microbial community structure  
228 and functions.

### 229 3.2.2. Antibiotic resistance genes and *Enterobacteriaceae* plasmid replicons

230 Transferable ARGs abundance was 27% greater in irrigated soils. ARGs commonly associated with mobile  
231 genetic elements accounted for 33 and 26 out of the 45 and 39 detected ARGs in irrigated and non-irrigated fields  
232 sequence reads, respectively (Figure 2). The transferable ARGs confer resistance to trimethoprim (2) and nine  
233 major classes of antibiotics that encode resistance to aminoglycosides (10),  $\beta$ -lactams (7), amphenicols (6),  
234 tetracyclines (5), sulphonamides (3), macrolides (2), quinolones (1), phosphonic antibiotics (1) and nucleoside  
235 antibiotics (1). Twenty-one types were common to both farming systems, 12 (*aac(6’)-Ib7*, *ant(9)-Ia*, *catIII*, *catQ*,  
236 *bla<sub>CARB-3</sub>*, *bla<sub>OXA-347</sub>*, *bla<sub>OXA-5</sub>*, *bla<sub>Rm3</sub>*, *fosB*, *sul3*, *tetC* and *tetX*), and five (*mphG*, *bla<sub>LCR-1</sub>*, *ereA2*, *qnrVC1*, and  
237 *tetB(P)*) were found in irrigated and non-irrigated fields, respectively. The relative abundance of ARGs common  
238 to both farming systems did not show any significant difference ( $P < 0.05$ ). The heat map reporting the relative

239 abundance (RPKM) of ARGs showed that agricultural fields clustered per origin. The only exception was in block  
240 2, where the ARG imprint following wastewater irrigation was more pronounced. Bivariate correlation analysis  
241 between prevalence of both ARGs and antibiotic residues showed positive correlations between the concentration  
242 of sulfamethoxazole, ciprofloxacin, enrofloxacin, trimethoprim and some ARGs (Table 3). The concentration of  
243 sulfamethoxazole and ciprofloxacin had the greatest number of positive relationships (nine), followed by  
244 enrofloxacin (eight), and trimethoprim (one). Trimethoprim was positively correlated to *dfrA1*. There was a  
245 positive correlation between sulfamethoxazole, ciprofloxacin and enrofloxacin, and the presence of *catIII*, *floR*,  
246 *bla<sub>OXA-347</sub>*, *bla<sub>OXA-5</sub>*, *bla<sub>CARB-3</sub>*, *bla<sub>tm3</sub>*, *sul3*, *tetC*, and *tetX*; except *floR*.

247 Because of the presence of an abundant and diverse community of antibiotic producers, soil is the potentially  
248 largest reservoir of drug-resistant bacteria and genes (Nesme and Simonet, 2015). The presence and abundance  
249 of transferable ARGs in agricultural fields in three African cities is in accordance with previous studies from  
250 China, the US and Lithuania (Zhu et al. 2013; McKinney et al. 2018; Armalytė et al. 2019). Furthermore, an  
251 additional effect of wastewater irrigation on the soil resistome was found. Studies of Chen et al. (2016) ; Broszat  
252 et al. (2014) ; Dungan et al. (2018) in China, Mexico, and US, have reported an increase of ARGs in soils following  
253 wastewater irrigation. Some pathways likely to select for bacterial resistance in soil include, influx of antibiotic  
254 residues which can induce a selective pressure; transfer and survival of ARB; and influx of transferable plasmids  
255 harbouring ARGs (Von Wintersdorff et al. 2016).

256 In previous studies, the wastewaters used to irrigate the investigated fields were reported as strong vectors  
257 for bacterial resistance dissemination (Bougnom et al. 2019a; 2019b). Thus, a more diverse ARB community and  
258 ARGs in irrigated fields was anticipated. All the ARGs present in the fields had been found in the wastewaters.  
259 Nevertheless, the abundance of ARGs reported in the soil was lesser. This is most likely a consequence of the  
260 death of many ARB when transferred from wastewater to soil where the bacteria must develop mechanisms to  
261 adapt, survive and grow. Among the transferable ARGs found solely in irrigated fields, four among the 12 were  
262 genes encoding ESBLs. Gram-negative bacteria are primarily carriers of ESBL genes, and application of animal  
263 manure to soil sustains the survival and growth of pathogens, ARB and ARGs (Rawat and Nair, 2010; Sharma  
264 and Reynnells, 2016). *Enterobacteriaceae* producing ESBLs can survive for a long time in irrigated fields and  
265 contaminate humans and animals via direct contact or the food chain. ESBL producers pose critical issues in  
266 clinical settings since they are able to inactivate  $\beta$ -lactams, thus requiring the administration of more expensive  
267 antibiotics. Direct contact with urban farm workers and consumption of crops from these fields could pose a  
268 serious health risk. Changes in the distribution of the mechanisms of antibiotic resistance in the irrigated field

269 was consequent to the modification of the soil resistome following wastewater irrigation. Fertilisation or irrigation  
270 of soil with material rich in organic material could introduce ARB and ARGs (Binh et al. 2008; Negreanu et al.  
271 2012; Nesme and Simonet, 2015). This could explain the greater abundance of transferable ARGs in soils from  
272 irrigated fields. Two non-irrigated farms from Yaounde and Ngaoundere used to be fertilised with cow manure,  
273 possibly explaining the greater prevalence of enteric bacteria in these fields.

274         There was a positive correlation between the concentrations of sulfamethoxazole and trimethoprim and  
275 relative prevalence of *sul3* and *dfrA1*, respectively, suggesting that pollution by these antibiotics influences the  
276 selection of these genes. Our data add to growing evidence that antibiotic residues in the environment exert a  
277 selective pressure on the acquisition of ARGs coding resistance against them (Cheng et al. 2016; Pan and Chu  
278 2018). We found that sulfamethoxazole, ciprofloxacin and enrofloxacin were associated with multiple and cross  
279 resistance. Thus, presence of these antibiotics could foster the maintenance of drug-resistance genes for antibiotics  
280 of other classes and hence multidrug resistant plasmids (Levy, 2002; Blanco et al. 2016). Botts et al. (2017) in the  
281 US have reported multidrug resistant plasmids encoding resistance genes for amphenicols, aminoglycosides,  $\beta$ -  
282 lactams, fluoroquinolones, tetracyclines, and sulfonamides, in coastal wetland impacted by wastewater. Therefore,  
283 abundance of multidrug resistant plasmids could be observed in the case of selective pressure exerted by one of  
284 these antibiotics. This shows the complex nature of factors selecting ARB and ARGs in the environment.

285         *Enterobacteriaceae* plasmids are well known to be the main carriers of clinically relevant ARGs  
286 (Carattoli, 2009). Ten *Enterobacteriaceae* plasmid amplicon incompatibility (Inc) groups were identified in the  
287 agricultural fields (Figure 3). Five (IncQ2, ColE, IncFIC, IncQ1, and IncFII) were common to both, while two  
288 (IncW and IncP1) and three (IncY, IncFIB, and IncFIA) were found in irrigated and non-irrigated fields,  
289 respectively.

290         Considering the wide and diverse ARGs in the wastewaters used to irrigate these fields (Bougnom et al.  
291 2019a; 2019b), the presence of *Enterobacteriaceae* plasmid replicons was not surprising. However, the different  
292 amplicon groups found in the fields were less diverse than those found in the wastewater. This suggests that  
293 following transfer of some ARBs from wastewater to soil, there is loss of some ARGs. IncFIB has been reported  
294 to be predominant in cattle faeces in Nigeria (Inwezerua et al. 2014); thus, fertilisation with animal manure could  
295 explain the presence of plasmid replicons in non-irrigated fields. Considering the epidemiological aspects,  
296 *Enterobacteriaceae* plasmid replicons found in irrigated farms represent greater public health issues. All Gram-  
297 negative bacteria are potential hosts of IncP and IncW plasmids (Dröge et al., 2000), while IncF and incY have a  
298 “narrow” host range (Hawkey, P. 2008; Mshana et al. 2009).

299

#### 300 **4. Conclusions**

301 In conclusion, LC-MS and high throughput sequencing allowed the assessment of bacterial resistance  
302 dissemination in urban agriculture in African cities. Following wastewater irrigation, distribution and metabolic  
303 functions of soil microbial community were influenced. Sulfamethoxazole, ciprofloxacin and enrofloxacin  
304 antibiotic residues may select for ARGs not oriented against their action. Wastewater irrigation increases the  
305 abundance of ARGs of clinical importance in soil, including *Enterobacteriaceae* plasmid replicons. The collected  
306 information suggests that raw wastewater irrigated soils in African cities could represent a vector for the spread  
307 of antibiotic resistance, thus threatening human and animal health. Consumers of products from these farms and  
308 farmers could be at risk of acquiring infections due to drug-resistant bacteria.

309

#### 310 **Conflict of Interest**

311 The authors declare no conflict of interest.

312

#### 313 **Acknowledgments**

314 We thank the European Commission; this project has received funding from the European Union's Horizon 2020  
315 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 655398.

316 We are grateful to Petra Ziegler and Elvira Sieberger, University of Trier, for assistance in the physical and  
317 chemical analyses, and members of the Antimicrobials Research Group, University of Birmingham, for helpful  
318 and constructive discussions.

319

#### 320 **References**

- 321 Adegoke, A.A., Isaac D. Amoah, I.D., Thor A. Stenström, T.A., Verbyla, M.E., Mihelcic, J.R., 2018.  
322 Epidemiological evidence and health risks associated with agricultural reuse of partially treated and untreated  
323 wastewater: A review. *Front Public Health*. 6, 337.
- 324 Alves, L.D.F., Westmann, C.A., Lovate, G.L., de Siqueira, G.M.V., Borelli, T.C., Guazzaroni, M., 2018.  
325 Metagenomic approaches for understanding new concepts in microbial science. *Int. J. Genomics*. 2312987.
- 326 Amos, G.C.A., Hawkey, P.M., Gaze, W.H., Wellington, E.M., 2014. Wastewater effluent contributes to the  
327 dissemination of CTX-M-15 in the natural environment. *J. Antimicrob. Chemoth.* 69, 1785-1791.

328 Andersson, D.I., Hughes, D., 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat.  
329 Rev. Microbiol.8, 260-271.

330 Armalytė, J., Skerniškytė, J., Bakienė, E., Krasauskas, R., Šiugždinienė, R., Kareivienė, V., Kerzienė, S.,  
331 Klimienė, I., Sužiedėlienė, E., Ružauskas, M., 2019. Microbial diversity and antimicrobial resistance profile in  
332 microbiota from soils of conventional and organic farming systems. Front. Microbiol.  
333 <https://doi.org/10.3389/fmicb.2019.00892>

334 Asnicar, F., Weingart, G., Tickle, T.L., Huttenhower, C., Segata, N., 2015. Compact graphical representation of  
335 phylogenetic data and metadata with GraPhlAn. Peerj 3.

336 Blackwell, P.A., Holten Lutzhoft, H.C., Ma, H.P., Halling-Sørensen, B., Boxall, A.B.A., Kay, P., 2004.  
337 Ultrasonic extraction of veterinary antibiotics from soils and pig slurry with SPE clean-up and LC-UV and  
338 fluorescence detection. Talanta 64,1058-64.

339 Blanco, P., Hernando-Amado, S., Reales-Calderon, J., Corona, F., Lira, F., Alcalde-Rico, M., Bernardini, A.,  
340 Sanchez, M.B., Martinez, J.L., 2016. Bacterial multidrug efflux pumps: much more than antibiotic resistance  
341 determinants. Microorganisms 4:E14.

342 Binh, C.T.T., Heuer, H., Kaupenjohann, M., Smalla, K., 2008. Pig-gery manure used for soil fertilization is a  
343 reservoir for transferable antibiotic resistance plasmids. FEMS Microbiol. Ecol. 66:25-37.

344 Botts, R.T., Apffel, B.A., Walters, C.J., Davidson, K.E., Echols, R.S., Geiger, M.R., Guzman, V.L., Haase,  
345 V.S., Montana, M.A., La Chat, C.A., Mielke, J.A., Mullen, K.L., Virtue, C.C, Brown, C.J., Top, E.M.,  
346 Cummings, D.E., 2017. Characterization of four multidrug resistance plasmids captured from the sediments of  
347 an urban coastal wetland. Front. Microbiol. 8:1922.

348 Bougnom, B. P., Zongo C., McNally A., Ricci V., Etoa F.X., Thiele-Bruhn S., Piddock L.J.V., 2019a.  
349 Wastewater used for urban agriculture in West Africa as a reservoir for antibacterial resistance dissemination.  
350 Environ Res 168, 14-24.

351 Bougnom, B.P., McNally, A., Etoa F.X., Piddock, L.J.V., 2019b. Urban population density impacts on the  
352 abundance of antibiotic resistance genes, except those encoding ESBL in raw sewage used for urban agriculture  
353 in Africa. Environ Pollut 251, 146-154.

354 Broszat, M., Nacke, H., Blasi, R., Siebe, C., Huebner, J., Daniel, R., Grohmann, E., 2014. Wastewater irrigation  
355 increases abundance of potentially harmful Gammaproteobacteria in soils from Mezquital Valley, Mexico.  
356 Appl. Environ. Microbiol. 80, 5282-5291.

357 Buchholz, U., Bernard, H., Werber, D., Böhmer, M.M., Remschmidt, C., Wilking, H., Deleré, Y., an der Heiden,  
358 M., Adlhoch, C., Dreesman, J., Ehlers, J., Ethelberg, S., Faber, M., Frank, C., Fricke, G., Greiner, M., Höhle,  
359 M., Ivarsson, S., Jark, U., Kirchner, M., Koch, J., Krause, G., Lubert, P., Rosner, B., Stark, K., Kühne, M. 2011.  
360 German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N. Engl. J. Med. 365, 1763-1770.  
361 Calderón-Preciado, D., Jiménez-Cartagena, C., Matamoros, V., Bayona, J.M., 2011. Screening of 47 organic  
362 microcontaminants in agricultural irrigation waters and their soil loading. Water Res. 45, 221-231.  
363 Carattoli, A., 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. 53,  
364 2227-2238.  
365 Carattoli, A., Zankari, E., Garcia-Fernandez, A., Larsen, M.V., Lund, O., Villa, L., Aarestrup, F.M. and  
366 Hasman, H., 2014. In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus  
367 Sequence Typing. Antimicrob Agents Ch 58, 3895-3903.  
368 Cheng, W.X., Li, J.N., Wu, Y., LK, Xu, Su, C., Qian, Y.Y., et al., 2016. Behavior of antibiotics and antibiotic  
369 resistance genes in eco-agricultural system: a case study. J. Hazard. Mater. 304, 18-25.  
370 Cisse, G., Kientga, M., Ouedraogo, B., Tanner, M., 2002. Developpement du maraichage autour des eaux de  
371 barrage a Ouagadougou: quels sont les risques sanitaires a prendre en compte? Cah. Agric. 11, 31–38.  
372 Cycon, M., Mrozik, A., Piotrowska-Seget, Z., 2019. Antibiotics in the soil environment degradation and their  
373 impact on microbial activity and diversity. Front. Microbiol. 10, 338.  
374 Dalkmann, P., Broszat, M., Siebe, C., Willaschek, E., Sakinc, T., Huebner, J., Amelung, W., Grohmann, E.,  
375 Siemens, J., 2012. Accumulation of pharmaceuticals, *enterococcus*, and resistance genes in soils irrigated with  
376 wastewater for zero to 100 years in central Mexico. PLoS One 7 (9), e45397.  
377 Deblonde, T., Cossu-Leguille, C., Hartemann, P., 2011. Emerging pollutants in wastewater: a review of the  
378 literature. Int. J. Hyg. Environ. Heal. 214, 442-448.  
379 Dickin, S.K., Schuster-Wallace, C.J., Qadir, M., Pizzacalla, K., 2016. A Review of Health Risks and Pathways  
380 for Exposure to Wastewater Use in Agriculture. Environ. Health. Persp. 124, 900-909.  
381 Droge, M., Puhler, A., Selbitschka, W., 2000. Phenotypic and molecular characterization of conjugative  
382 antibiotic resistance plasmids isolated from bacterial communities of activated sludge. Mol. Gen. Genet. 263,  
383 471-82.  
384 Du, L., Liu, W., 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agron  
385 Sustain Dev 32, 309-327.

386 Dungan, R.S., McKinney, C.W., Leytem, A.B., 2018. Tracking antibiotic resistance genes in soil irrigated with  
387 dairy wastewater. *Sci. Total Environ.* 635, 1477-1483.

388 Essington, M.E., Lee, J., Seo, Y., 2010. Adsorption of antibiotics by montmorillonite and kaolinite. *Soil Sci.*  
389 *Soc. Am. J.* 74, 1557-1588.

390 Fahrenfeld, N., Knowlton, K., Krometis, L.A., Hession, W.C., Xia, K., Lipscomb, E., Libuit, K., Green, B.L.,  
391 Pruden, A., 2014. Effect of manure application on abundance of antibiotic resistance genes and their attenuation  
392 rates in soil: field-scale mass balance approach. *Environ. Sci. Technol.* 48, 2643–2650.

393 Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall,  
394 D.H., Caporaso, J.G., 2012. Cross-biome metagenomic analyses of soil microbial communities and their  
395 functional attributes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 21390-21395.

396 Forsberg, K.J., Reyes, A., Bin, W., Selleck, E.M., Sommer, M.O.A., Dantas, G., 2012. The Shared Antibiotic  
397 Resistome of Soil Bacteria and Human Pathogens. *Science* 337, 1107-1111.

398 Glass E.M., Wilkening J., Wilke, A., Antonopoulos, D., Meyer, F., 2010. Using the metagenomics RAST server  
399 (MG-RAST) for analyzing shotgun metagenomes. *Cold Spring Harb Protoc: prot* 5368.

400 Grossberger, A., Hadar, Y., Borch, T., Chefetz, B., 2014. Biodegradability of pharmaceutical compounds in  
401 agricultural soils irrigated with treated wastewater. *Environ. Pollut.* 185 :168-177.

402 Guo, T., Lou, C., Zhai, W., Tang, X., Hashmi, M.Z., M, R., Liu, X., Xu, J., 2018. Increased occur-rence of  
403 heavy metals, antibiotics and resistance genes in surface soil after long-term application of manure. *Sci. Total*  
404 *Environ.* 635, 995–1003.

405 Hajri A, Brin C, Hunault G, Lardeux F, Lemaire C, Manceau C, Boureau T, Poussier S (2009) A “repertoire for  
406 repertoire” hypothesis: repertoires of type three effectors are candidate determinants of host specificity in  
407 *Xanthomonas*. *PLoS One* 4:e6632

408 Hawkey, P. 2008. Molecular epidemiology of clinically significant antibiotic resistance genes. *Br. J.*  
409 *Pharmacol.* 153: S406-13.

410 Heuer H., Schmitt H., Smalla K. 2011. Antibiotic resistance gene spread due to manure application on  
411 agricultural fields. *Curr. Opin. Microbiol.* 14, 236-24310.

412 Igbinsosa, E.O., Obi, L.C., Tom, M., Okoh, A.I., 2011. Detection of potential risk of wastewater effluents for  
413 transmission of antibiotic resistance from *Vibrio* species as a reservoir in a peri-urban community in South  
414 Africa. *International Journal of Environmental Health Research* 21, 402-414.

415 Inwezerua, C., Mendonc, N., Calhau, V., Domingues, S., Adeleke, O.E., Da Silva, G.J. 2014. Occurrence of  
416 extended-spectrum beta-lactamases in human and bovine isolates of *Escherichia coli* from Oyo state, Nigeria, J.  
417 Infect Dev Countr, 8, 774-779.

418 Jangid, K., Williams, M.A., Franzluebbbers, A.J., Sanderlin, J.S., Reeves, J.H., Jenkins, M.B., Endale, D.M.,  
419 Coleman, D.C., Whitman, W.B., 2008. Relative impacts of land-use, management intensity and fertilization  
420 upon soil microbial community structure in agricultural systems. Soil Biology and Biochemistry 40, 2843-2853.

421 Kaminski, J., Gibson, M.K., Franzosa, E.A., Segata, N., Dantas, G., Huttenhower, C., 2015. High-Specificity  
422 Targeted Functional Profiling in Microbial Communities with ShortBRED. Plos Comput Biol 11(12).

423 Kengne, I.M., Endamana, D., Soh, L., Nya, J., 2002. Réutilisation des eaux usées pour l'agriculture urbaine à  
424 Yaoundé. Actes de l'atelier international CTA/ETC-RVAF/CREPA sur la réutilisation des eaux usées en  
425 agriculture urbaine. Ouagadougou, 116-123.

426 Kielak, A., Barreto, C., Kowalchuk, G.A., van Veen J.A., Kuramae, E.E., 2016. The ecology of Acidobacteria:  
427 moving beyond genes and genomes. Front Microbiol 7, 00744.

428 Kumar, M., Jaiswal, S., Sodhi, K.K., Shree, P., Singh, D.K., Agrawal, P.K., Shukla, P., 2019.  
429 Antibiotics bioremediation: perspectives on its ecotoxicity and resistance. Environ.  
430 Int. 124, 448-461.

431 Kuramae, E.E., Yergeau E., Wong, L.C., Pijl, A.S., van Veen, J.A., Kowalchuk, G.A., 2011. Soil characteristics  
432 more strongly influence soil bacterial communities than land-use type. FEMS Microbiol. Ecol. 79, 12-24.

433 Laxminarayan R., Sridhar D., Blaser M., Wang M., Woolhouse M., 2016. Achieving global targets for  
434 antimicrobial resistance. Science, 353(6302),874e5.

435 Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in soil microbial  
436 communities across land-use types. ISME J 7, 1641-1650.

437 Levy, S.B. 2002. Factors impacting on the problem of antibiotic resistance. Journal of Antimicrobial  
438 Chemotherapy 49, 25-30.

439 Luo, Y., Xu, L., Rysz, M., Wang, Y.Q., Zhang, H., Alvarez, P.J.J., 2011. Occurrence and transport of  
440 tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River basin, China. Environ. Sci.  
441 Technol. 45, 1827-1833.

442 Mateo-Sagasta, J., Medlicott, K., Qadir, M., Rashid-Sally, L., Dreschel, P., Liebe, J., 2013. Proceedings of the  
443 UN-Water project on the Safe Use of Wastewater in Agriculture. UNW-DPC Proceedings Series, August 2013.  
444 [http://collections.unu.edu/eserv/UNU:2661/proceedings-no-11\\_WEB.pdf](http://collections.unu.edu/eserv/UNU:2661/proceedings-no-11_WEB.pdf).



445 McArthur, A.G., Waglechner, N., Nizam, F., Yan, A., Azad, M.A., Baylay, A.J., Bhullar, K., Canova, M.J., De  
446 Pascale, G., Ejim, L., Kalan, L., King, A.M., Koteva, K., Morar, M., Mulvey, M.R., O'Brien, J.S., Pawlowski,  
447 A.C., Piddock, L.J.V., Spanogiannopoulos, P., Sutherland, A.D., Tang, I., Taylor, P.L., Thaker, M., Wang,  
448 W.L., Yan, M., Yu, T. and Wright, G.D., 2013. The Comprehensive Antibiotic Resistance Database. *Antimicrob*  
449 *Agents Ch* 57, 3348-3357.

450 Michelini L., Reichel R., Werner W., Ghisi R., Thiele-Bruhn S. (2012) Sulfadiazine uptake and effects on *Salix*  
451 *fragilis* L. and *Zea maize* L. plants. *Water, Air and Soil Pollution* 223, 5243-5257.

452 Mshana, S.E., Imirzalioglu, C., Hossain, H., Hain, T., Domann, E., Chakraborty, T. 2009. Conjugative IncFI  
453 plasmids carrying CTX-M-15 among *Escherichia coli* ESBL producing isolates at a University hospital in  
454 Germany. *BMC Infect Dis* 9, 97.

455 Müller, P., Alber, D.G., Turnbull, L., Schlothauer R.C., Carter D.A., Whitchurch C.B., Harry, E.J., 2013.  
456 Synergism between Medihoney and rifampicin against methicillin-resistant *Staphylococcus aureus* (MRSA).  
457 *PLoS ONE* 8, e57679.

458 McKinney, C.W., Dungan, R.S., Moore, A., Leytem, A.B., 2018. Occurrence and abundance of antibiotic  
459 resistance genes in agricultural soil receiving dairy manure. *FEMS Microbiol. Ecol.* 94, fyy010.

460 Nacke, H., Fischer, C., Thürmer, A., Meinicke, P., Daniel, R., 2014. Land use type significantly affects  
461 microbial gene transcription in soil. *Microb. Ecol.* 67:919-930.

462 Nesme, J., Simonet, P., 2015. The soil resistome: A critical review on antibiotic resistance origins, ecology and  
463 dissemination potential in telluric bacteria. *Environ. Microbiol.* 17, 913-930.

464 Ocimati, W., Bouwmeester, H., Groot, J. C., Tittonell, P., Brown, D., Blomme, G., 2019. The risk posed by  
465 *Xanthomonas* wilt disease of banana: Mapping of disease hotspots, fronts and vulnerable landscapes. *PloS one*,  
466 14 (4).

467 Onyenwoke, R.U., Brill, J.A., Farahi, K., Wiegand, J., 2004. Sporulation genes in members of the low G+C  
468 Gram-type-positive phylogenetic branch (*Firmicutes*). *Arch. Microbiol.* 182,182-192.

469 Pan M., Chu, L.M., 2018. Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater  
470 irrigation areas in the Pearl River Delta region, southern China. *Sci. Total Environ* 624, 145-152.

471 Piddock L.J., 2012. The crisis of no new antibiotics—what is the way forward? *Lancet Infect Dis.* 12, 249-253.

472 Poindexter, J.S., 1981. The Caulobacters: Ubiquitous Unusual Bacteria. *Microbiol Rev*, 45: 123-179.

473 Rawat, D., Nair, D., 2010. Extended-spectrum  $\beta$ -lactamases in Gram negative bacteria. *J. Glob. Infect. Dis.* 2,  
474 263-74.

475 Sentchilo, V., Mayer, A.P., Guy, L., Miyazaki, R., Tringe, S.G., Barry, K., Malfatti, S., Goessmann, A.,  
476 Robinson-Rechavi, M., van der Meer, J.R., 2013. Communitywide plasmid gene mobilization and selection.  
477 ISME J. 7, 1173–1186.

478 Sharma, M., Reynnells, R., 2016. Importance of Soil Amendments: Survival of Bacterial Pathogens in Manure  
479 and Compost Used as Organic Fertilizers. *Microbiol. Spectrum* 4, PFS-0010-2015

480 Thiele-Bruhn, S., Seibicke, T., Schulten, H.-R., Leinweber, P., 2004. Sorption of sulfonamide pharmaceutical  
481 antibiotics on whole soils and particle-size fractions. *J. Environ. Qual.* 33, 1331-1342.

482 Von Wintersdorff, C.J.H., Penders, J., van Niekerk, J.M., Mills, N.D., Majumder, S., van Alphen, L.B.,  
483 Savelkoul, P.H.M., Wolfs, P.F.G., 2016. Dissemination of Antimicrobial Resistance in Microbial Ecosystems  
484 through Horizontal Gene Transfer. *Front. Microbiol.* 7.

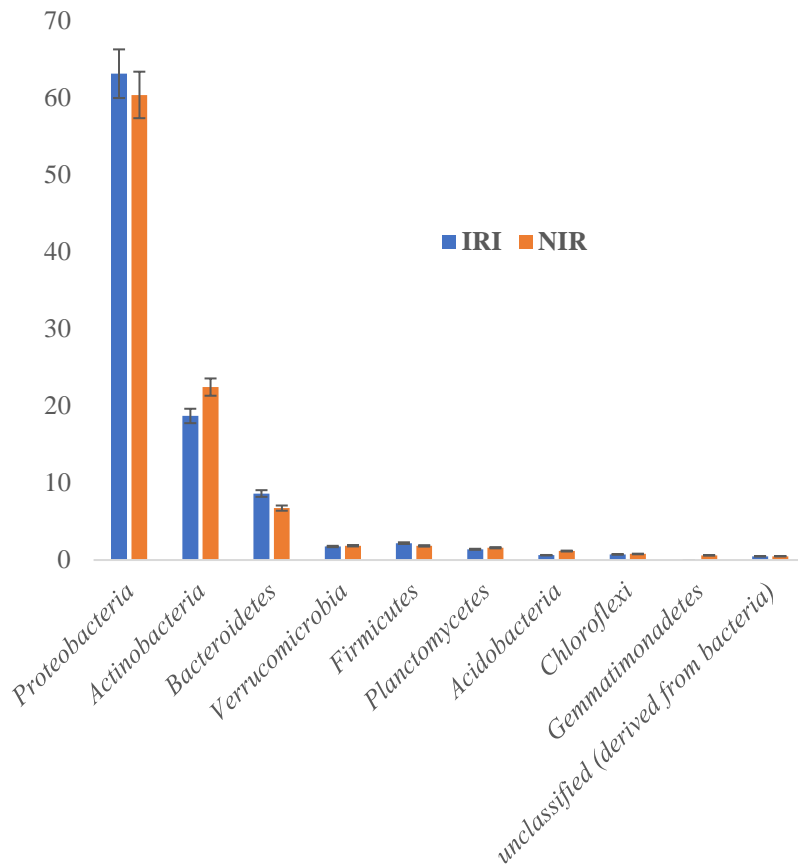
485 Wang, F., Qiao, M., Su, J.Q., Chen, Z., Zhou, X., Zhu, Y.G. 2014. High throughput profiling of antibiotic  
486 resistance genes in urban park soils with reclaimed water irrigation. *Environ. Sci. Technol.* 48, 9079-9085.

487 Wellington, E.M.H., Boxall, A.B.A., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S.,  
488 Jones, D.L., Lee, N.M., Otten, W., Thomas, C.M. and Williams, A.P., 2013. The role of the natural environment  
489 in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect Dis* 13, 155-165.

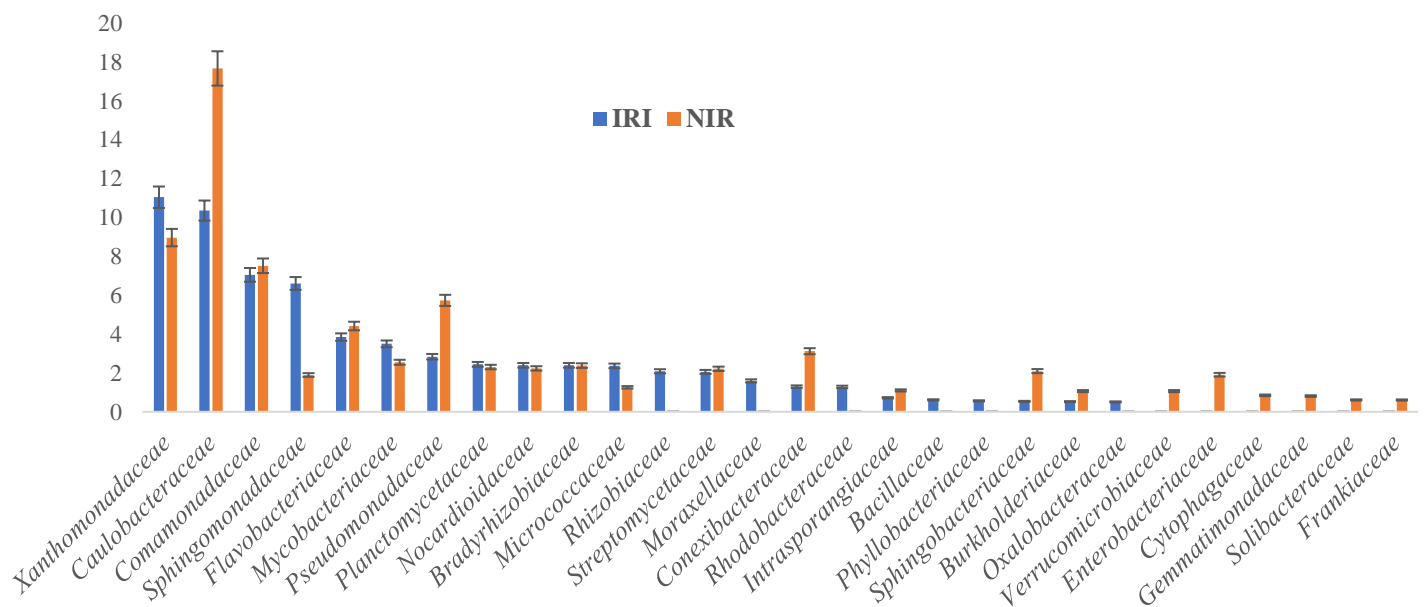
490 World Health Organization., 2014. Antimicrobial resistance: global report on surveillance. Geneva: World  
491 Health Organization.

492 Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013.  
493 Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. U. S. A.* 110,  
494 3435-3440.

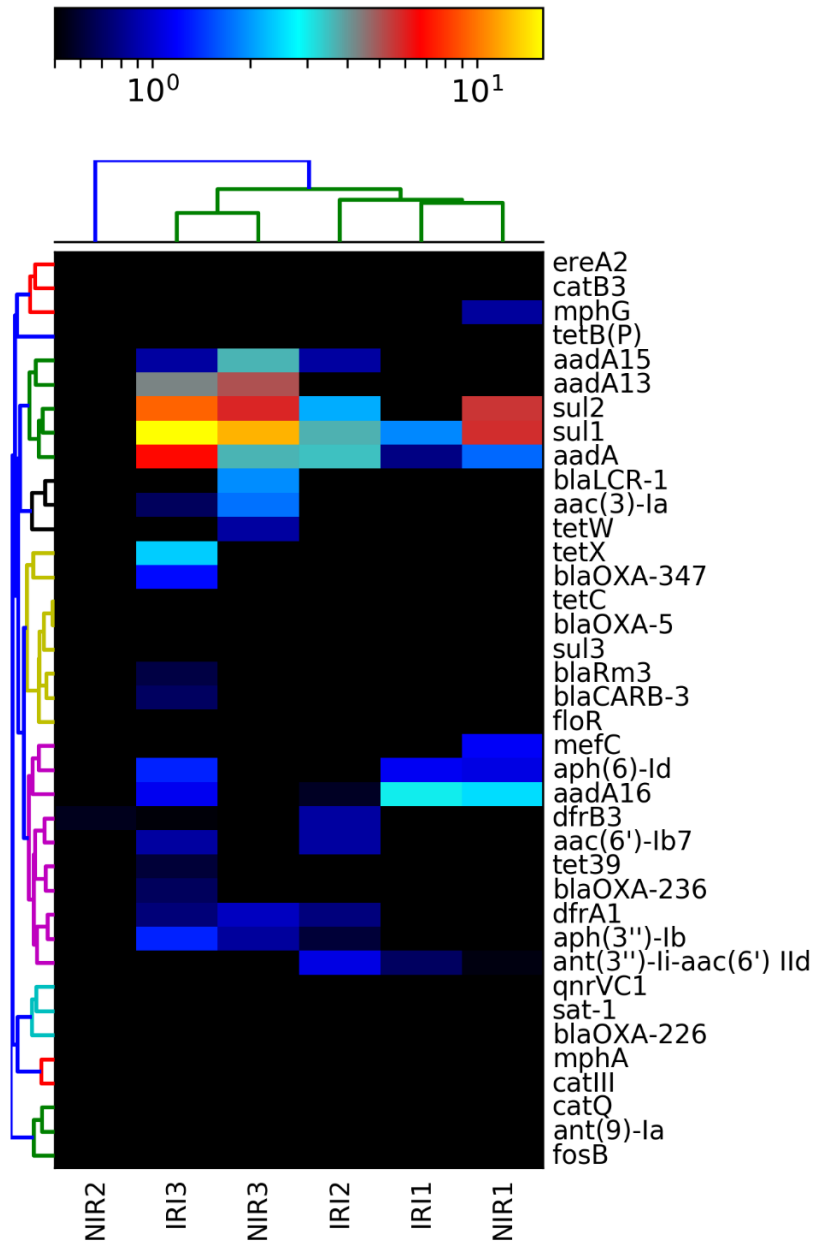
a)



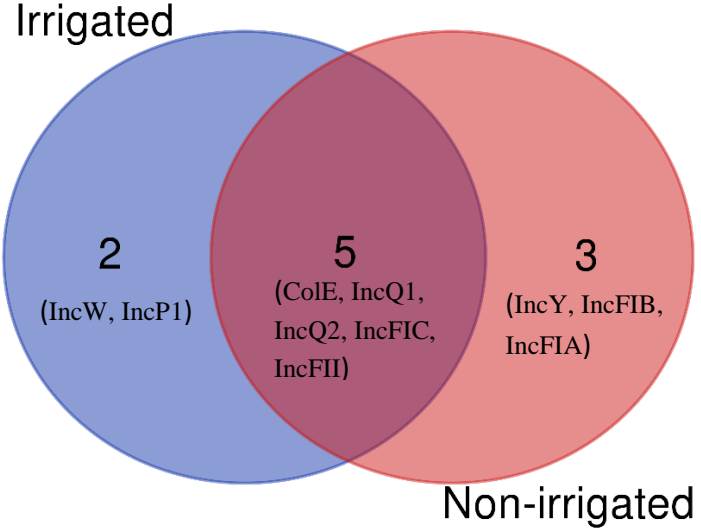
b)



**Figure 1.** Relative abundances (%) of soil a) bacterial phyla and b) families derived from the metagenomic reads in irrigated (IRI) and non-irrigated (NIR) fields. Bacterial phyla and families with average relative abundance > 0.5% are visualized. (n=3)



**Figure 2.** Heatmap representing the relative percentage (RPKM unit) of the different antibiotic resistance genes abundance in the agricultural soil samples. *RPKM* Reads Per Kilobase Million; *IRI* Irrigated; *NIR* Non-irrigated.



**Figure 3:** Venn diagram showing the identified *Enterobacteriaceae* plasmid replicons in the irrigated and non-irrigated agricultural fields. (n=3)

**Table 1.** pH, carbon, nitrogen content and concentration of antibiotic residues (ng. g<sup>-1</sup>) in the samples collected in irrigated and non-irrigated fields

Parameters	Irrigated fields				Non-irrigated fields			
	IRI1	IRI2	IRI3	Mean ( $\pm$ SD)	NIR1	NIR2	NIR3	Mean ( $\pm$ SD)
pH	7.26	6.49	7.00	6.92 ( $\pm$ 0.39)	7.00	5.29	5.78	6.02 ( $\pm$ 0.88)
C (% dw)	2.16	4.54	2.38	3.03 ( $\pm$ 1.31)	2.14	2.77	1.22	2.04 ( $\pm$ 0.78)
N (% dw)	0.15	0.33	0.19	0.22 ( $\pm$ 0.09)	0.17	0.22	0.11	0.17 ( $\pm$ 0.05)
Sulfadimidine	n.d.	2.64	0.07	0.90 ( $\pm$ 1.50)	n.d.	2.42	n.d.	0.81 ( $\pm$ 1.40)
Sulfadiazine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sulfamethoxazole*	n.d.	n.d.	0.54	0.18 ( $\pm$ 0.31)	n.d.	0.11	n.d.	0.04 ( $\pm$ 0.06)
Trimethoprim	0.04	0.33	0.38	0.25 ( $\pm$ 0.18)	0.25	0.25	0.44	0.31 ( $\pm$ 0.11)
Ciprofloxacin	0.44	n.d.	2.33	0.92 ( $\pm$ 1.24)	0.19	0.95	0.18	0.44 ( $\pm$ 0.44)
Enrofloxacin*	0.76	0.57	1.98	1.10 ( $\pm$ 0.77)	0.47	0.31	0.43	0.40 ( $\pm$ 0.08)
Oxytetracycline*	n.d.	0.26	n.d.	0.09 ( $\pm$ 0.15)	n.d.	n.d.	n.d.	n.d.
Chlortetracycline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tetracycline	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tylosin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

\* The distribution is significantly different between the two agricultural systems (Student's *t*-test,  $P < 0.05$ ). *dw* dry weight; *SD* Standard deviation; *n.d.* not detected; *IRI* irrigated; *NIR* non-irrigated. Detection limits (5 to 20  $\mu\text{g L}^{-1}$ ); Quantification limits (2 to 10  $\mu\text{g L}^{-1}$ ).

**Table 2.** Relative distribution of sequencing reads (RPKM units) in major level 1 subsystems in irrigated and non-irrigated fields. Metagenomic data were annotated against SEED subsystems in MG-RAST at a cut off of E-value < 10<sup>-5</sup>.

Functional subsystems	Agricultural fields							
	Irrigated				Non-irrigated			
	IRI1	IRI2	IRI3	Mean (±SD)	NIR1	NIR2	NIR3	Mean (±SD)
Carbohydrates	2.62x 10 <sup>6</sup>	2.35x 10 <sup>6</sup>	2.35x 10 <sup>6</sup>	2.44x 10 <sup>6</sup> (±1.56x 10 <sup>5</sup> )	2.51x 10 <sup>6</sup>	2.11x 10 <sup>6</sup>	2.59x 10 <sup>6</sup>	2.40x 10 <sup>6</sup> (±2.57x10 <sup>5</sup> )
Clustering-based subsystems*	2.55x 10 <sup>6</sup>	2.46x 10 <sup>6</sup>	2.48x 10 <sup>6</sup>	2.50x 10 <sup>6</sup> (±4.59x 10 <sup>4</sup> )	2.63x 10 <sup>6</sup>	1.90x 10 <sup>6</sup>	2.82x 10 <sup>6</sup>	2.45x 10 <sup>6</sup> (±4.88x 10 <sup>5</sup> )
Amino acids and derivatives	2.12x 10 <sup>6</sup>	2.08x 10 <sup>6</sup>	2.08x 10 <sup>6</sup>	2.09x 10 <sup>6</sup> (±2.49x 10 <sup>4</sup> )	2.14x 10 <sup>6</sup>	1.65x 10 <sup>6</sup>	2.38x 10 <sup>6</sup>	2.06x 10 <sup>6</sup> (±3.74x 10 <sup>5</sup> )
Miscellaneous	1.34x 10 <sup>6</sup>	1.33x 10 <sup>6</sup>	1.38x 10 <sup>6</sup>	1.35x 10 <sup>6</sup> (±2.58x 10 <sup>4</sup> )	1.41x 10 <sup>6</sup>	9.85x 10 <sup>5</sup>	1.61x 10 <sup>6</sup>	1.33x 10 <sup>6</sup> (±3.19x 10 <sup>5</sup> )
Protein metabolism	1.31x 10 <sup>6</sup>	1.21x 10 <sup>6</sup>	1.23x 10 <sup>6</sup>	1.25x 10 <sup>6</sup> (±5.10x 10 <sup>4</sup> )	1.32x 10 <sup>6</sup>	9.68x 10 <sup>5</sup>	1.40x 10 <sup>6</sup>	1.23x 10 <sup>6</sup> (±2.27x 10 <sup>5</sup> )
Cofactors, vitamins, prosthetic groups, pigments	1.24x 10 <sup>6</sup>	1.19x 10 <sup>6</sup>	1.20x 10 <sup>6</sup>	1.21x 10 <sup>6</sup> (±2.33x 10 <sup>4</sup> )	1.25x 10 <sup>6</sup>	9.46x 10 <sup>5</sup>	1.39x 10 <sup>6</sup>	1.20x 10 <sup>6</sup> (±2.27x 10 <sup>5</sup> )
RNA metabolism	8.85x 10 <sup>5</sup>	9.30x 10 <sup>5</sup>	9.76x 10 <sup>5</sup>	9.30x 10 <sup>5</sup> (±4.58x 10 <sup>4</sup> )	9.84x 10 <sup>5</sup>	6.15x 10 <sup>5</sup>	1.14x 10 <sup>6</sup>	9.13x 10 <sup>5</sup> (±2.70x 10 <sup>5</sup> )
Fatty acids, lipids and isoprenoids	8.01x 10 <sup>5</sup>	8.19x 10 <sup>5</sup>	7.60x 10 <sup>5</sup>	7.93x 10 <sup>5</sup> (±3.01x 10 <sup>4</sup> )	8.26x 10 <sup>5</sup>	6.38x 10 <sup>5</sup>	8.54x 10 <sup>5</sup>	7.73x 10 <sup>5</sup> (±1.17x 10 <sup>5</sup> )
DNA metabolism*	7.20x 10 <sup>5</sup>	7.23x 10 <sup>5</sup>	7.39x 10 <sup>5</sup>	7.27x 10 <sup>5</sup> (±1x 10 <sup>4</sup> )	7.60x 10 <sup>5</sup>	5.06x 10 <sup>5</sup>	8.48x 10 <sup>5</sup>	7.05x 10 <sup>5</sup> (±1.78x 10 <sup>5</sup> )
Cell wall and capsule	6.69x 10 <sup>5</sup>	7.23x 10 <sup>5</sup>	7.61x 10 <sup>5</sup>	7.18x 10 <sup>5</sup> (±4.62x 10 <sup>4</sup> )	7.38x 10 <sup>5</sup>	4.86x 10 <sup>5</sup>	8.76x 10 <sup>5</sup>	7.00x 10 <sup>5</sup> (±1.98x 10 <sup>5</sup> )
Respiration	4.84x 10 <sup>5</sup>	4.42x 10 <sup>5</sup>	4.34x 10 <sup>5</sup>	4.53x 10 <sup>5</sup> (±2.72x 10 <sup>4</sup> )	4.70x 10 <sup>5</sup>	3.81x 10 <sup>5</sup>	4.94x 10 <sup>5</sup>	4.48x 10 <sup>5</sup> (±5.93x 10 <sup>4</sup> )
Nucleosides and nucleotides*	4.54x 10 <sup>5</sup>	4.37x 10 <sup>5</sup>	4.46x 10 <sup>5</sup>	4.45x 10 <sup>5</sup> (±8.73x 10 <sup>3</sup> )	4.63x 10 <sup>5</sup>	3.45x 10 <sup>5</sup>	5.04x 10 <sup>5</sup>	4.37x 10 <sup>5</sup> (8.25x 10 <sup>4</sup> )
Virulence, disease and defense	4.36x 10 <sup>5</sup>	5.13x 10 <sup>5</sup>	5.85x 10 <sup>5</sup>	5.11x 10 <sup>5</sup> (±7.48x 10 <sup>4</sup> )	5.24x 10 <sup>5</sup>	3.12x 10 <sup>5</sup>	7.46x 10 <sup>5</sup>	5.27x 10 <sup>5</sup> (±2.17x 10 <sup>5</sup> )
Stress response*	4.06x 10 <sup>5</sup>	4.20x 10 <sup>5</sup>	4.23x 10 <sup>5</sup>	4.16x 10 <sup>5</sup> (±9.04x 10 <sup>3</sup> )	4.44x 10 <sup>5</sup>	2.93x 10 <sup>5</sup>	4.99x 10 <sup>5</sup>	4.12x 10 <sup>5</sup> (±1.07x 10 <sup>5</sup> )

\* The distribution is significantly different between the two agricultural systems (Student's *t*-test, *P* < 0.05). *SD* Standard Deviation. *IRI* Irrigated; *NIR* Non-irrigated.

**Table 3.** Positive correlations between relative abundances of antibiotic resistant genes and antibiotics concentrations. In each cell, the value represents Spearman's coefficient (*r*).

	<i>bla</i> <sub>CARB-3</sub>	<i>catIII</i>	<i>dfrA1</i>	<i>floR</i>	<i>bla</i> <sub>OXA-347</sub>	<i>bla</i> <sub>OXA-5</sub>	<i>bla</i> <sub>rm3</sub>	<i>sul3</i>	<i>tetC</i>	<i>tetX</i>
Sulfamethoxazole	0.979**	0.979**	-	0.864*	0.979**	0.979**	0.979**	0.979**	0.979**	0.946**
Ciprofloxacin	0.926**	0.926**	-	0.899*	0.926**	0.926**	0.926**	0.926**	0.926**	0.881*
Enrofloxacin	0.970**	0.970**	-	-	0.970**	0.970**	0.970**	0.970**	0.970**	0.936**
Trimethoprim	-	-	0.869*	-	-	-	-	-	-	-

\*Asterisk denoted significant correlation at  $p < 0.05$  level (2- tailed).

\*\*Double asterisk denoted significant correlation at  $p < 0.01$  level (2- tailed).