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Wastewater used for urban agriculture in West Africa as a reservoir for antibacterial resistance dissemination

Bougnom, Blaise P.; Zongo, Cheikna; McNally, Alan; Ricci, Vito; Etoa, François X.; Thiele-Bruhn, Sören; Piddock, Laura J V

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1   **Wastewater used for urban agriculture in West Africa as a reservoir for**

2   **antibacterial resistance dissemination**

3   Blaise P. Bougnom [a c]; Cheikna Zongo [b]; Alan McNally [a]; Vito Ricci [a]; François X. Etoa [c]; Sören Thiele-Bruhn

4   [d]; Laura J.V. Piddock [a]*

5   [a] Institute of Microbiology and Infection, University of Birmingham, B15 2TT, UK

6   [b] Department of Biochemistry and Microbiology, University Ouaga I Pr Joseph KI-ZERBO, 03 BP 7021

7   Ouagadougou 03, Burkina Faso

8   [c] Department of Microbiology, Faculty of Science, University of Yaounde 1, P.O. Box 812 Yaounde, Cameroon

9   [d] Department of Soil Science, University of Trier, D-54286 Trier, Germany

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

13   **Summary**

14   State of art metagenomics were used to investigate the microbial population, antibiotic resistance genes and

15   plasmids of medical interest in wastewater used for urban agriculture in Ouagadougou (Burkina Faso).

16   Wastewater samples were collected from three canals near agricultural fields in three neighbourhoods.

17   Assessment of microbial population diversity revealed different microbial patterns among the different samples.

18   Sequencing reads from the wastewaters revealed different functional specializations of microbial communities,

19   with the predominance of carbohydrates and proteins metabolism functions. Eleven pathogen-specific and 56

20   orthologous virulence factor genes were detected in the wastewater samples. These virulence factors are usually

21   found in human pathogens that cause gastroenteritis and/or diarrhoea. A wide range of antibiotic resistance

22   genes was identified; 81 are transmissible by mobile genetic elements. These included seven different extended

23   spectrum β-lactamase genes encoding synthesis of four enzyme families, including two metallo-β-lactamases

24   (bla_{AIM-1} and bla_{GES-21}). Ten different incompatibility groups of *Enterobacteriaceae* plasmid replicons (ColE,

25   FIB, FIC, FII, P, Q, R, U, Y, and A/C), and 30 plasmid replicon types from Gram-positive bacteria. All are

26   implicated in the wide distribution of antibiotic resistance genes. We conclude that wastewater used for urban

27   agriculture in the city represents a high risk for spreading bacteria and antimicrobial resistance among humans

28   and animals.

29   **Keywords**: Antimicrobial resistance, metagenomics, urban agriculture, low and middle-income countries

30

Introduction

There is an increasing number of bacteria that are multi-resistant against common antibiotics and thus cannot be treated by current therapies (Leopold et al. 2014). Antibiotic resistance has led to the need for more expensive drugs, which many cannot afford, resulting in increased morbidity and mortality (Laxminarayan et al. 2016). Selection of drug-resistant bacteria and proliferation occurs at sub-inhibitory concentrations of antibiotics (Drlica, 2003; Andersson and Hughes, 2010). It has been reported that approximately 50 - 90 % of antibiotics administered to humans and animals are excreted via urine and faeces, as a mixture of parent drug and metabolite forms, and thus significant levels of active drug end up in the environment, where they may persist in soil and aquatic ecosystems (Kümmerer, 2009). To combat the presence of antibiotics in ecosystems, bacteria have evolved a plethora of different resistance genes of which many are mobile and can easily spread between species including human pathogens (Groh et al. 2007; Wellington et al. 2013; Zhang et al. 2016). Consequently, environmental bacteria serve as a reservoir of resistance genes that can be transmitted to pathogenic species (Allen et al. 2009). That is of concern in low and middle-income countries (LMICs) where populations are often directly exposed to untreated wastewater due to a lack of water and sanitation services including in urban areas (UN, 2017; Bougnom and Piddock, 2017).

The urban population in sub-Saharan Africa is projected to grow by 3.5 % per annum from now until 2030, and the number of urban residents is expected to rise from 400 million in 2010 to 1.26 billion in 2050 (UN, 2012). This rapid increase in the urban population raises significant challenges, related to urban poverty, public health, housing, food security and environmental pollution. Urban and peri-urban agriculture is defined as the production of crops and livestock within and around cities (UNDP, 1996). It has been developed by urban dwellers to meet their food demand, as well as a source of employment and income. The importance of urban agriculture in terms of contributing to food security and poverty alleviation has been recognised and promoted (Martellozzo et al. 2014). Two hundred million urban dwellers are reported to be engaged in urban agriculture worldwide, producing in some cases up to 90 % of cities' demand for perishable vegetables (UN, 2013). It is reported that more than 80% of the domestic and industrial wastewater generated in LMICs is discharged untreated into the environment, and because of its low cost, availability, and nutrient content, urban agriculture hugely relies on wastewater for irrigation, (Mateo-Sagasta et al. 2013). As water is a vehicle for microbial dissemination, wastewater for agricultural irrigation represents a very serious health risk, not least as it increases exposure to faecal pathogens. Ingestion of faecally-contaminated water and/or food by opportunistic and/or pathogenic microorganisms is one of the major reasons for the high number of water-related diseases in LMICs.

61   Wastewater use for urban agriculture has been shown to expose humans and animals to enteric diseases caused

62   by pathogenic bacteria, protozoa, and helminths (Dickin et al. 2016). To reduce the spread of antimicrobial

63   resistant bacteria to humans, the role of wastewater, sanitation and hygiene must be understood and addressed

64   (Graham et al. 2011; Wuijts et al. 2017).

65   Genomics allows us to understand the factors that drive ARGs transfer between bacteria and the

66   mechanisms conferring resistance (Amos et al. 2014). The use of a metagenomic approach provides an

67   opportunity to provide essential information so that the risks of untreated wastewater and impact upon human

68   and animal health can be defined. In this study, we used metagenomics to assess the presence of virulence factor

69   genes (VFs), antibiotic resistance genes (ARGs) and plasmids of medical interest in wastewater used for urban

70   agriculture in Ouagadoudou (Burkina Faso). The aim was to measure the potential of urban agriculture to

71   disseminate antibiotic resistance in the city.

72

73   **Material and Methods**

74   *Wastewater sampling*

75   Experiments were conducted with samples collected from Ouagadougou (Burkina Faso, West Africa)

76   in October 2015, at the end of the rainy season, with three precipitation events during the month. Ouagadougou

77   is the capital city of Burkina Faso, it occupies a surface area of 219.3 km², with a population of about 2.7

78   million inhabitants. The city is in the Sudano-Sahelian area, with a rainfall of about 800 mm per year, and

79   temperatures ranging from 16 °C to 43 °C during the rainy and dry seasons, respectively. Wastewater samples

80   used for urban agriculture were collected in three neighborhoods from three canals near agricultural fields. The

81   sampling sites were selected based on their different characteristics. These canals are open-air water drainage

82   and collection points of different transects (Figure 1). They were constructed to protect the city from floods, and

83   receive hand-draining sludge, solid waste, and wastewater. They do not have regular cleaning and are used to

84   irrigate agricultural fields which are found along its way. Passpanga canal is 5 km long, it receives effluent from

85   the university teaching hospital, the city and biggest market of the town; these are the main sources of pollution.

86   Its outlet is dam N° 3. Zogona canal, which is 4.4 km long; it receives effluent from 1200 houses and the

87   University of Ouagadougou as the main sources of pollution; its outlet is in Bangreweogo urban park.

88   Dassasgho canal is 4.8 km long, it receives effluent from the city prison town and slaughterhouse as the main

89   sources of pollution; its outlet is in Bangreweogo. The geographical coordinates of the sampled canals were

90   Passpanga (12°23' N, 1°30' W), Zogona (12°23' N, 1°31' W), and Dassasgho (12°23'N, 1°29' W). From each

canal, samples were collected from the upper, middle, and lower reaches of the waterways. At each point, five samples of 100 ml were collected around $2m^2$, 15-20 cm below the surface, in sterile bottles. The five replicate samples were combined to give one sample of 500 ml. The combined samples from each point were pooled together to minimize the variations that may occur from one site to another, the representative sample of the canal was1500ml. The samples were transported on ice and frozen (-80°C) until processing. The values of temperature, pH, total organic carbon and total nitrogen were for Passpanga (30°C, 8.27, 20.2 and 30.8, respectively); Zogona (29.5^{o}C, 7.20, 13.5 and 4.46, respectively) and Dassasgho (31.5^{o}C, 7.39, 11.7 and 8.95, respectively).

DNA extraction and quantification

To extract total genome DNA from the wastewater, samples (500 mL each) from each canal were centrifuged at 2268 × g for 15 minutes to pellet the microbial cells. DNA extraction was conducted using the FastDNA Soil Kit (MP Biomedicals, CA, USA), according to the manufacturer's instructions. Total extracted DNA was quantified using the Quant-iT PicoGreen dsDNA Assay Kit, and the Qubit™ 3.0 Fluorometer (Qubit, Life Technologies, USA).

High-throughput sequencing

DNA samples from each of the three canals were sent to Edinburgh Genomics (Edinburgh, Scotland) for high-throughput sequencing using Illumina Hiseq4000 (Illumina, Inc, USA). Libraries were prepared by TruSeq DNA Nano gel free library using 350 bp insert. The sequencing strategy was index PE150+8+150 cycle (Paired End sequencing, 150-bp reads and 8-bp index sequence); it generated 76.3 Gb of raw sequence reads. The metagenomic data have been deposited at National Center for Biotechnology Information (NCBI), Sequence Read Archive (SRA) under project accession number PRJNA358310 (SRR5123280; SRR5123281; SRR5123278; SRR5123291; SRR5123282; and SRR5123287).

Quality reads filtering

Raw fastq data were uploaded into GALAXY Cloudman, using the Cloud Infrastructure for Microbial Bioinformatics (CLIMB) platform (Connor et al. 2016). Reads were assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)(Andrews, 2014). Reads of bad quality were filtered out by removing adapters, N bases below quality 3, a cutoff value of Phred score > 30, length sorted at ≥ 70 bases, using Trimmomatic (Bolger et al. 2014). Passpanga, Zogona and Dassasgho had 83, 724, 220; 84, 013, 504 and 88, 861, 498 raw reads, for 64, 536, 398; 63, 920, 619 and 69, 695, 966 trimmed reads respectively. The

120 trimmed reads were submitted to annotation on MG-RAST metagenomics analysis server version 4.0.3 (Glass et

121 al. 2010). They were further processed for taxonomic, functional annotations, and plasmids identification.

122 *Taxonomic and functional annotations*

123 MetaPhlAn 2.0 (Metagenomic Phylogenetic Analysis) was used to profile the composition of microbial

124 communities with species level resolution in the datasets with 75% of nucleotide identity threshold, using

125 default parameter settings, and the sensitive local Bowtie2 alignment step. MetaPhlAn 2.0 program relies on ~1

126 million unique clade-specific marker genes identified from ~17,000 reference genomes (~13,500 bacterial and

127 archaeal, 3,500 viral, and 110 eukaryotic) (Segata et al., 2012). The additional analysis type and argument was

128 "-t rel_ab_w_read_stats" to profile bacterial metagenomes to estimate the number of reads coming from each

129 clade. A cladogram was generated to visualize microbial abundances (circle size relating to microbial

130 abundance) on a tree of life using GraPhlAn (Asnicar et al. 2015). The functional classification of the

131 metagenomic data were annotated against SEED subsystems in MG-RAST (Glass et al. 2010). The functional

132 profiles were generated using the E-value cut-off 10^{-5}, a minimum identity of 80%, and a minimum alignment

133 length of 20 amino acids.

134 *Identification and quantification of antibiotic resistance and virulence factor gene markers.*

135 Antibiotic resistance genes (ARGs) and virulence factors (VFs) from metagenome reads were

136 identified and quantified using ShortBRED (Short Better Representative Extract Dataset) (Kaminski et al.

137 2015). ShortBRED profiles protein family abundance in metagenomes in two-steps: (i) *ShortBRED-Identify*

138 isolates representative peptide sequences (markers) for the protein families, and (ii) *ShortBRED-Quantify* maps

139 metagenomic reads against these markers to determine the relative abundance of their corresponding families.

140 Fragment length $\geq$ 30 amino acids and $\geq$ 95% identity is considered a valid hit. ARG and VF markers were

141 generated from the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al. 2013) and the

142 Virulence Factor Database (VFDB) (Chen et al. 2005), respectively, and using UniRef50 as a reference protein

143 database. Using the antibiotic resistance ontology (ARO) numbers in CARD and VFDB, respectively, ARG and

144 VF markers were aggregated, annotated and associated to the corresponding resistance family or predicted

145 pathogen. Pathogen-specific and common VF genes were identified. Heatmaps were generated to visualize

146 tabular abundance of ARGs and VFs in the different canals with hclust2 (Asnicar et al. 2015), using Bray-Curtis

147 as the distance measure both between samples and between features.

148 *Identification of plasmid amplicons of clinical relevance*

149   Plasmid replicon sequences belonging to *Enterobacteriaceae* (Gram-negative) and to *Enterococcus,*

150   *Staphylococcus* and *Streptococcus* (Gram-positive) were downloaded from the PlasmidFinder database 1.3

151   (https://cge.cbs.dtu.dk/services/PlasmidFinder) (Carattoli et al. 2014), and aligned against the metagenomic

152   reads using BLAT. A read was assigned to a plasmid replicon if the BLAT hit had a sequence identity of $\geq 80\%$,

153   to detect both large and small plasmids (Carattoli et al. 2014), using the E-value cut-off 10^{-5}.

154

155   **RESULTS AND DISCUSSION**

156   *Microbial composition, population structure and functional classification*

157   Microbial population diversity was assessed to provide an overview on the diversity and relative

158   abundance of the different taxonomy groups, bacterial *phyla* and families of interest. The relative abundance of

159   bacteria, archaea and viruses is shown in Table 1. The relative abundance of *Bacteria* and *Archaea* domains of

160   Woese and viruses' group is shown in Table 1. The relative abundance of *Archaea* in samples from Passpanga

161   and Dassasgho were about 20 and 13-fold higher than the abundance in Zogona, respectively (Table S1). There

162   were 10 different bacteria phyla identified in Dassasgho, nine in Passpanga, and six in Zogona (Table 1).

163   *Proteobacteria* was the most prevalent *phylum* in all the wastewater samples tested, followed by *Actinobacteria*

164   and *Firmicutes* (Figure 2). Our results are in accordance with previous studies that showed that these *phyla* are

165   dominant in wastewaters (Mlejnkova and Sovova, 2010; Bengtsson-Palme et al. 2014). The microbial

166   community structure is significantly dependent on the level of water pollution, and abundance of *Proteobacteria*

167   or *Bacteroidetes* is reported to be a consequence of water pollution by human activities (Mlejnkova and Sovova,

168   2010; Suriya et al. 2017). Our data suggests that the sampled canals are impacted by anthropogenic pollution.

169   Natural water bodies such as rivers and lakes are less impacted and low nutrients content, thus favouring the

170   survival of autochthonous bacteria, which are mainly autotrophs (Sigee, 2004; Zeglin et al. 2015). The higher

171   bacterial diversity in the city canals could be explained by an increased nutrient load due to organic matter

172   pollution by anthropogenic activities, favouring the establishment of more allochthone bacteria communities

173   (Hewson et al. 2003; Qin et al. 2016). *Chlorobi, Fusobacteria*, *Spirochaetes* and *Verrucomicrobia phyla* have

174   members known to live in anaerobic or micro-anaerobic conditions (Nelson, 2015). Their presence in

175   wastewater could be a consequence of a decrease in oxygen availability following intense organic matter

176   degradation by microorganisms in the water bodies. *Fusobacteria*, *Spirochaetes*, and *Verrucomicrobia* are

177   commonly found in human and animal faeces, viruses are a major cause of waterborne diseases (Nelson, 2015;

178   Nieuwenhuijse et al. 2017). Their presence indicates that the water in these canals presents a high risk of

179   transmitting infectious diseases.

180   .

181   We assessed families of bacteria that carry contaminating plasmids ARGS and those that are

182   autochthonous bacteria in water ecosystems. The relative prevalence of the families *Enterobacteriaceae*,

183   *Enterococcaceae*, *Streptococcaceae*, *Staphylococcaceae*, *Comamonadaceae* and *Chroococcaceae* in the water

184   samples is shown in Table 1. Taking into consideration the wide standard deviation, the proportion of nitrate-

185   reducing bacteria (e.g. *Comamonadaceae*) and photosynthetic oxygenic microorganisms (e.g. *Chroococcaceae*)

186   was found to be similar to those bacteria containing antibiotic resistant plasmids. That indicates anthropogenic

187   pollution and ecological disturbances as suggested previously by others (Balmonte et al. 2016).

188   The functional categories of the microbial communities were distributed into 28 level 1 subsystems of

189   SEED database (Figure 3). The predominant functions were clustering-based subsystems (14.4%),

190   carbohydrates (13.4%), miscellaneous (10.8%), amino acids and derivatives (7.81%) and protein metabolism

191   (7%). Virulence, disease and defence accounted for 2.44%; potassium metabolism (0.28%), dormancy and

192   sporulation (0.20%) and photosynthesis (0.19%) were the less prevalent. Carbohydrates, amino acids and

193   derivatives, and protein metabolism were among the most prevalent subsystems in the city wastewaters. Our

194   results are in line with previous studies which have shown the predominance of those functions in wastewaters

195   (Wang et al. 2013; Chao et al. 2013; Bäumlisberger et al. 2015). Microbial communities residing in those waters

196   are well skilled to degrade easily accessible carbon substrates such as soluble carbohydrates or polysaccharides,

197   as well as amino acid derivatives and proteins (Uroz et al. 2013). This is consequent to a constant anthropogenic

198   pollution of these waters with organic matter. The proportion of genes involved in virulence, disease and

199   defence in the metagenome reads conformed with the range previously found in wastewater by others (Wang et

200   al. 2013). The negligible proportion of function allocated to photosynthesis translates the disappearance of the

201   bacterioplankton.

202   *Virulence factors*

203   To further assess the presence and relative abundance of bacteria pathogenic to humans in the

204   wastewaters, we assessed the presence of VF genes as pathogenic indicators. Sixty-one different VF genes were

205   identified in the samples (Figure 4). Eleven were pathogen-specific VFs and 60 are orthologous commonly

206   found in both pathogens and non-pathogens. The genes encoding different mechanisms of virulence were (in

207   order of relative abundance): secretion systems, toxins, exoenzymes, adherence, iron uptake, biofilm formation,

208 stress response, antiphagocytosis, manganese uptake, a type IV secretory protein, and plasminogen activator.

209 The identified VF genes are commonly carried by *Escherichia coli* (17)*, Shigella* spp (14), *Clostridium*

210 *perfringens* (6) and *Mycobacterium tuberculosis* (6). The pathogen-specific VFs belonged to *Streptococcus*

211 *agalactiae*, C. *perfringens*, M. *tuberculosis*, *Legionnela pneumophila*, *Shigella* spp, S. *flexneri*, *Yersinia*

212 *enterocolitica*, and *Bartonnella henselae*. All the pathogen-specific VFs were present in Passpanga, those

213 belonging to C. *perfringens*, S. *flexneri*, Y. *enterocolitica*, and B. *henselae* were present in Dassasgho, and the

214 one of Y. *enterocolitica* in Zogona.

215   The widespread presence of VFs usually found in human pathogenic bacteria in wastewater using a

216 high-throughput shotgun sequencing technique has been reported in many countries (Lu et al. 2013; Ye and

217 Zhang, 2013; Kumaraswamy et al. 2014). However, in those studies the wastewater was neither from an open-

218 air canal nor used for irrigation of crops intended for human consumption. With regard to the number of

219 pathogen-specific and common VFs, next generation sequencing (NGS) is recommended to efficiently assess

220 the probable presence of pathogenic bacteria in wastewater. Its broader coverage allows the identification of

221 microorganisms present in low numbers, which would not be detected with traditional molecular tools

222 (Kumaraswamy et al. 2014). The presence of pathogen-specific and common VF genes in urban waterways and

223 which will be used for urban agriculture indicates that such waters represents a risk to human health. Most of

224 the detected VFs belong to pathogens that are transmitted by direct water exposure or ingestion of water/food

225 contaminated with human or animal faeces and are commonly responsible for waterborne diseases. Therefore, it

226 is not surprising that populations directly or indirectly exposed to these wastewaters could suffer from acute

227 diarrhoea, chronic gastritis, and gastroenteritis (Nitiema et al. 2011; Traore et al., 2015). In LMICs 842 000

228 people die annually from diarrhoea (WHO, 2017); this is because of inadequate water, sanitation, and hygiene.

229 Moreover, the presence of pathogen-specific VFs in these canals should be a source of concern, since the use of

230 this water for urban agriculture is a reservoir for pathogens and ARGs and so represents a strong vehicle for

231 transmission. Pathogen-specific VF belonging to M. *tuberculosis* was detected in Passpanga canal. M.

232 *tuberculosis* is predominantly transmitted between humans by droplets of water by coughing and possibly

233 sneezing. Its presence in the canal could be due to the high prevalence rate in the country; 5 866 cases of

234 tuberculosis were reported in Burkina Faso in 2015 (WHO, 2016). Patients infected with tuberculosis can

235 contaminate water and soil ecosystems, and M. *tuberculosis* can remain viable for extended periods of time

236 (Velayati et al. 2015). The indirect transmission of M. *tuberculosis* might be facilitated with their persistence in

237 the environment (Falkinham et al. 2015).

Antibiotic resistance genes analysis

239   In total, 129 ARGs were detected; 81 were determined to be transmissible by mobile genetic elements

240   through horizontal gene transfer (HGT). The 81 transmissible ARGs confer resistance to 11 major classes of

241   antibiotics and trimethoprim (Figure 5). The most prevalent resistance genes encode resistance to

242   aminoglycosides (20 genes), tetracycline (19 genes), beta-lactams (13 genes) and macrolides (9 genes). ARG

243   prevalence in Zogona was the lowest, while prevalence of ARGs in Passpanga was the highest. A heat map of

244   the ARG relative abundance showed that Passpanga and Dassasgho clustered together; 69 ARGs were identified

245   in Passpanga, 21 in Zogona, and 55 in Dassasgho. Eighteen ARGs were common to the three sampling sites; 25

246   were common to Passpanga and Dassasgho; 25 and 10 resistant genes, respectively were found solely in

247   Passpanga and Dassasgho. Zogona had no unique ARG. The top five most prevalent transmissible ARGs were

248   *aad*A15, *aad*A13, *sul*1, *aad*A, and *tet*B in Passpanga; *sul*1, *sul*2, *aad*A13, *aad*A, and *Rbp*A in Zogona; and *sul*1,

249   *sul*2, *aad*A13, *aad*A, and *aad*A15 in Dassasgho. Interestingly, seven different ARGs encoding extended-

250   spectrum beta lactamases (ESBLs) ($bla_{OXA-226}$, $bla_{OXA-256}$, $bla_{OXA-347}$, bla_{OXA-46}, $bla_{SHV-100}$, bla_{GES-21}, and bla_{AIM-1})

251   were detected in the city wastewaters. All of these genes were present in Dassasgho. The $bla_{SHV-100}$ and bla_{AIM-1}

252   genes were not found in Passpanga, but the $bla_{OXA-226}$ and $bla_{OXA-256}$ were identified in water from Zogona.

253   Water ecosystems polluted by pathogenic bacteria and ARGs act both as a natural reservoir and a

254   channel for the spread of clinically relevant antibiotic resistance traits. Previous studies have shown a

255   correlation between pathogen prevalence and ARGs (Michael et al. 2013; Bengtsson-Palme et al. 2014; Zheng et

256   al. 2017). Our study provides data that confirms those from previous studies, with greater relative prevalence of

257   pathogen-specific VFs and ARGs found in the canals. The wide variety of detected resistance gene classes

258   suggests that they might have been acquired under selection pressure from diverse antibiotics and/or other

259   factors selecting for resistances mechanisms (Kristiansson et al. 2011; Bengtsson-Palme et al. 2014). The high

260   density of bacteria in the canals, favours cell-to-cell contact, which likely contributes to ARG transmission

261   (Tennstedt et al. 2003; Bengtsson-Palme et al. 2014). Hospital wastewater is known to contain high quantities of

262   drug-resistant bacteria and antibiotic residues, and their interaction contributes to an increase of ARGs (Brechet

263   et al., 2014; Lien et al. 2016). This could explain the highest ARGs diversity and relative abundance in

264   Passpanga, which receives effluent from the university teaching hospital.

265   Aminoglycosides, sulphonamides, tetracyclines and beta-lactams are the most commonly administered

266   antibiotics in human medicine in Burkina Faso (Krause et al. 1999; Ouedraogo et al. 2017). Tetracyclines and

267   sulphonamides are commonly used as growth promoters and for disease prevention in animals (Zhang et al.

268 2015). This could explain the high prevalence of genes encoding resistance against these antibiotics in the

269 canals. Aminoglycoside-modifying enzymes (*aac*, *aad*, and *ant*) are plasmid and transposon associated;

270 dihydropteroate synthase enzymes (*sul*1 and *sul*2) are plasmid encoded and found on class 1 integrons. These

271 characteristics facilitate the dissemination and persistence of drug resistance phenotypes within the bacterial

272 communities, and thus could explain their relative predominance in the canal water (Hall and Collis, 1995;

273 Mingeot-Leclercq et al. 1999; Stoll et al., 2012). The relatively high prevalence and diversity of tetracycline

274 resistance genes identified in our datasets could be related to their presence on mobile genetic elements and

275 single strains carrying multiple drug-resistance genes. It was of concern to find seven different ESBL genes

276 encoding synthesis of four enzyme families in these ecosystems. It was reported that 58% of *Enterobacticeae*

277 responsible for infections in three main hospitals of Burkina Faso were ESBL producers (Ouedraogo et al.,

278 2017). Our results suggest a link between resistance in clinical settings and in the environment like previously

279 stated (Tacao et al. 2012; Forsberg et al. 2012). Two genes (bla_{AIM-1} and bla_{GES-21}) encoding carbapenemase

280 enzymes were found in the wastewater. Carbapenems are the last resort drugs for patients infected with ESBL-

281 producing bacteria; they are expensive drugs, which many patients in LMICs cannot afford.

282 *Plasmid replicons*

283   The presence of plasmids, putative carriers and disseminative agents of ARGs by horizontal gene

284 transfer was assessed. Thirty-one plasmid replicons of medical interest from *Enterobacteriaceae* were identified

285 in the wastewater; 27 were present in Passpanga, four in Zogona and 18 in Dassasgho (Table S2). E. *coli* was

286 the main likely carrier. The identified plasmid replicons belonged to 10 different incompatibility groups, namely

287 ColE, FIB, FIC, FII, P, Q, R, U, Y, and A/C. The 10 groups were found in Passpanga and Dassasgho, while

288 only IncP and IncQwere identified in Zogona. The three most prevalent plasmid replicon families were IncFIB,

289 IncFII and IncQ in Passpanga, and IncQ, ColE and IncP in Dassasgho. Thirty plasmid replicon types from

290 Gram-positive bacteria were found in the canal water; all were present in Passpanga, 21 in Dassasgho, and none

291 in Zogona (Table S.3). In both the Passpanga and Dassasgho canals, based on the probe origin, *Staphylococcus*

292 spp was the most likely carrier, followed by *Enterococcus* spp. One plasmid replicon type in Passpanga was

293 associated with *Streptoccoccus* spp as a possible carrier.

294   Plasmids can carry genes conferring resistance to one or more antibiotics, and their ability to transfer

295 by HGT between bacteria make them key contributors to the emergence and dissemination of ARGs in human

296 and animal medicine (Orlek et al. 2017). The great relative number of plasmid replicons in the canals is a

297 consequence to plasmid production and acquisition (Wegrzyn et al. 2002; Kristiansson et al. 2011). The

298   diversity of plasmid incompatibility group and relative abundance in the different canals matched those of

299   ARGs, suggesting likely association between the two. The identified plasmid incompatibility groups are

300   carriers of ARGs encoding resistance to all common antibiotic classes. Mobile genetic elements are known to

301   greatly contribute to the dissemination of ARGs via HGT and plasmids are responsible for much of the

302   widespread distribution of clinically relevant ARGs (Gyles and Boerlin, 2014). The presence of such extensive

303   plasmid incompatibility groups could contribute to their extensive acquisition by different bacterial species, and

304   further spread. The plasmid replicons IncP and Q are frequently associated with the carbapenemases bla_{AIM-1}

305   and bla_{GES-21}, respectively (Carattoli, 2009; Leiros et al. 2012); all were found in both Passpanga and

306   Dassasgho. E. *coli* is a faecal indicator, its presence indicates the possible occurrence of pathogenic bacteria

307   and faecal contamination. E. *coli* was the main carrier of *Enterobacteriaceae* plasmid replicons of medical

308   interest in the waters. Considering its plasticity, E. *coli* easily acquires and transfer ARGs to other strains and

309   intensively promotes bacterial resistance dissemination (Rasheed et al. 2014). Thus, these wastewaters

310   constitute a serious health risk. The multidrug resistant pathogens of special concern for both community

311   and/or hospital acquired infections are E. *coli*, K. *pneumoniae*, S. *aureus*, S. *pneumoniae*, *Enterococci*, V.

312   *cholerae*, and M. *tuberculosis*. Pathogen-specific and common VFs were found in the wastewaters along with

313   clinically relevant plasmids and ARGs conferring resistance to all major classes of antibiotics. Consequently,

314   these wastewaters can give rise to drug-resistant bacteria and so represent a potentially serious threat to public

315   health in the town via direct contact or the food chain. It is estimated that vegetable production occupies more

316   than 561 ha in the city, using part of the 620,000m^3 of both domestic and industrial wastewater that are

317   discharged every year (Vezina, 2002; Dao et al. 2016).

318

319   **Conclusion**

320   Metagenomic exploration of wastewater in open-air canals, used for urban agriculture in Ouagadougou,

321   revealed a wide functional specialization of microbial communities, a high prevalence of pathogen-specific and

322   commonly found VFs, a variety of plasmid incompatibility types and multiple ARGs in this environment,

323   suggesting that wastewater is a "hot spot" for formation and dissemination of antibiotic resistance. Our data

324   showed that wastewater for urban agriculture in Burkina Faso might contribute to dissemination of bacterial

325   resistance, including ESBLs and carbapenemases. That is of special concern for both community and hospital

326   acquired infections. Further investigations are needed to determine the extent that exposed populations are

327 affected by this health issue. There is an urgent need to improve global access to clean water, sanitation, and

328 hygiene in LMICs to prevent the rise and dissemination of bacterial resistance from the environment to people.

329

337 **Transparency declarations**

338 The authors declare no competing financial interest.

339

340 **References**

341

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