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

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Summary

State of art metagenomics were used to investigate the microbial population, antibiotic resistance genes and plasmids of medical interest in wastewater used for urban agriculture in Ouagadougou (Burkina Faso). Wastewater samples were collected from three canals near agricultural fields in three neighbourhoods. Assessment of microbial population diversity revealed different microbial patterns among the different samples. Sequencing reads from the wastewaters revealed different functional specializations of microbial communities, with the predominance of carbohydrates and proteins metabolism functions. Eleven pathogen-specific and 56 orthologous virulence factor genes were detected in the wastewater samples. These virulence factors are usually found in human pathogens that cause gastroenteritis and/or diarrhoea. A wide range of antibiotic resistance genes was identified; 81 are transmissible by mobile genetic elements. These included seven different extended spectrum β-lactamase genes encoding synthesis of four enzyme families, including two metallo-β-lactamases ($bla_{\text{AIM-1}}$ and $bla_{\text{GES-21}}$). Ten different incompatibility groups of *Enterobacteriaceae* plasmid replicons (ColE, FIB, FIC, FII, P, Q, R, U, Y, and A/C), and 30 plasmid replicon types from Gram-positive bacteria. All are implicated in the wide distribution of antibiotic resistance genes. We conclude that wastewater used for urban agriculture in the city represents a high risk for spreading bacteria and antimicrobial resistance among humans and animals.

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

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.

Wastewater use for urban agriculture has been shown to expose humans and animals to enteric diseases caused by pathogenic bacteria, protozoa, and helminths (Dickin et al. 2016). To reduce the spread of antimicrobial resistant bacteria to humans, the role of wastewater, sanitation and hygiene must be understood and addressed (Graham et al. 2011; Wuijts et al. 2017).

Genomics allows us to understand the factors that drive ARGs transfer between bacteria and the mechanisms conferring resistance (Amos et al. 2014). The use of a metagenomic approach provides an opportunity to provide essential information so that the risks of untreated wastewater and impact upon human and animal health can be defined. In this study, we used metagenomics to assess the presence of virulence factor genes (VFs), antibiotic resistance genes (ARGs) and plasmids of medical interest in wastewater used for urban agriculture in Ouagadoudou (Burkina Faso). The aim was to measure the potential of urban agriculture to disseminate antibiotic resistance in the city.

Material and Methods

Wastewater sampling

Experiments were conducted with samples collected from Ouagadougou (Burkina Faso, West Africa) in October 2015, at the end of the rainy season, with three precipitation events during the month. Ouagadougou is the capital city of Burkina Faso, it occupies a surface area of 219.3 km², with a population of about 2.7 million inhabitants. The city is in the Sudano-Sahelian area, with a rainfall of about 800 mm per year, and temperatures ranging from 16 °C to 43 °C during the rainy and dry seasons, respectively. Wastewater samples used for urban agriculture were collected in three neighborhoods from three canals near agricultural fields. The sampling sites were selected based on their different characteristics. These canals are open-air water drainage and collection points of different transects (Figure 1). They were constructed to protect the city from floods, and receive hand-draining sludge, solid waste, and wastewater. They do not have regular cleaning and are used to irrigate agricultural fields which are found along its way. Passpanga canal is 5 km long, it receives effluent from the university teaching hospital, the city and biggest market of the town; these are the main sources of pollution. Its outlet is dam N^{o} 3. Zogona canal, which is 4.4 km long; it receives effluent from 1200 houses and the University of Ouagadougou as the main sources of pollution; its outlet is in Bangreweogo urban park. Dassasgho canal is 4.8 km long, it receives effluent from the city prison town and slaughterhouse as the main sources of pollution; its outlet is in Bangreweogo. The geographical coordinates of the sampled canals were Passpanga (12^{o}23' N, 1^{o}30' W), Zogona (12^{o}23' N, 1^{o}31' W), and Dassasgho (12^{o}23'N, 1^{o}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^oC, 8.27, 20.2 and 30.8, respectively); Zogona (29.5^oC, 7.20, 13.5 and 4.46, respectively) and Dassasgho (31.5^oC, 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

trimmed reads were submitted to annotation on MG-RAST metagenomics analysis server version 4.0.3 (Glass et al. 2010). They were further processed for taxonomic, functional annotations, and plasmids identification.

Taxonomic and functional annotations

MetaPhlAn 2.0 (Metagenomic Phylogenetic Analysis) was used to profile the composition of microbial communities with species level resolution in the datasets with 75% of nucleotide identity threshold, using default parameter settings, and the sensitive local Bowtie2 alignment step. MetaPhlAn 2.0 program relies on ~1 million unique clade-specific marker genes identified from ~17,000 reference genomes (~13,500 bacterial and archaeal, 3,500 viral, and 110 eukaryotic) (Segata et al., 2012). The additional analysis type and argument was "-t rel_ab_w_read_stats" to profile bacterial metagenomes to estimate the number of reads coming from each clade. A cladogram was generated to visualize microbial abundances (circle size relating to microbial abundance) on a tree of life using GraPhlAn (Asnicar et al. 2015). The functional classification of the metagenomic data were annotated against SEED subsystems in MG-RAST (Glass et al. 2010). The functional profiles were generated using the E-value cut-off 10^{-5}, a minimum identity of 80%, and a minimum alignment length of 20 amino acids.

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

Antibiotic resistance genes (ARGs) and virulence factors (VFs) from metagenome reads were identified and quantified using ShortBRED (Short Better Representative Extract Dataset) (Kaminski et al. 2015). ShortBRED profiles protein family abundance in metagenomes in two-steps: (i) *ShortBRED-Identify* isolates representative peptide sequences (markers) for the protein families, and (ii) *ShortBRED-Quantify* maps metagenomic reads against these markers to determine the relative abundance of their corresponding families. Fragment length $\geq$ 30 amino acids and $\geq$ 95% identity is considered a valid hit. ARG and VF markers were generated from the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al. 2013) and the Virulence Factor Database (VFDB) (Chen et al. 2005), respectively, and using UniRef50 as a reference protein database. Using the antibiotic resistance ontology (ARO) numbers in CARD and VFDB, respectively, ARG and VF markers were aggregated, annotated and associated to the corresponding resistance family or predicted pathogen. Pathogen-specific and common VF genes were identified. Heatmaps were generated to visualize tabular abundance of ARGs and VFs in the different canals with hclust2 (Asnicar et al. 2015), using Bray-Curtis as the distance measure both between samples and between features.

Identification of plasmid amplicons of clinical relevance

Plasmid replicon sequences belonging to *Enterobacteriaceae* (Gram-negative) and to *Enterococcus, Staphylococcus* and *Streptococcus* (Gram-positive) were downloaded from the PlasmidFinder database 1.3 (https://cge.cbs.dtu.dk/services/PlasmidFinder) (Carattoli et al. 2014), and aligned against the metagenomic reads using BLAT. A read was assigned to a plasmid replicon if the BLAT hit had a sequence identity of ≥ 80%, to detect both large and small plasmids (Carattoli et al. 2014), using the E-value cut-off 10^{-5}.

RESULTS AND DISCUSSION

Microbial composition, population structure and functional classification

Microbial population diversity was assessed to provide an overview on the diversity and relative abundance of the different taxonomy groups, bacterial *phyla* and families of interest. The relative abundance of bacteria, archaea and viruses is shown in Table 1. The relative abundance of *Bacteria* and *Archaea* domains of Woese and viruses' group is shown in Table 1. The relative abundance of *Archaea* in samples from Passpanga and Dassasgho were about 20 and 13-fold higher than the abundance in Zogona, respectively (Table S1). There were 10 different bacteria phyla identified in Dassasgho, nine in Passpanga, and six in Zogona (Table 1). *Proteobacteria* was the most prevalent *phylum* in all the wastewater samples tested, followed by *Actinobacteria* and *Firmicutes* (Figure 2). Our results are in accordance with previous studies that showed that these *phyla* are dominant in wastewaters (Mlejnkova and Sovova, 2010; Bengtsson-Palme et al. 2014). The microbial community structure is significantly dependent on the level of water pollution, and abundance of *Proteobacteria* or *Bacteroidetes* is reported to be a consequence of water pollution by human activities (Mlejnkova and Sovova, 2010; Suriya et al. 2017). Our data suggests that the sampled canals are impacted by anthropogenic pollution. Natural water bodies such as rivers and lakes are less impacted and low nutrients content, thus favouring the survival of autochthonous bacteria, which are mainly autotrophs (Sigee, 2004; Zeglin et al. 2015). The higher bacterial diversity in the city canals could be explained by an increased nutrient load due to organic matter pollution by anthropogenic activities, favouring the establishment of more allochthone bacteria communities (Hewson et al. 2003; Qin et al. 2016). *Chlorobi, Fusobacteria, Spirochaetes* and *Verrucomicrobia phyla* have members known to live in anaerobic or micro-anaerobic conditions (Nelson, 2015). Their presence in wastewater could be a consequence of a decrease in oxygen availability following intense organic matter degradation by microorganisms in the water bodies. *Fusobacteria*, *Spirochaetes*, and *Verrucomicrobia* are commonly found in human and animal faeces, viruses are a major cause of waterborne diseases (Nelson, 2015;

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

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We assessed families of bacteria that carry contaminating plasmids ARGS and those that are autochthonous bacteria in water ecosystems. The relative prevalence of the families *Enterobacteriaceae*, *Enterococcaceae*, *Streptococcaceae*, *Staphylococcaceae*, *Comamonadaceae* and *Chroococcaceae* in the water samples is shown in Table 1. Taking into consideration the wide standard deviation, the proportion of nitrate-reducing bacteria (e.g. *Comamonadaceae*) and photosynthetic oxygenic microorganisms (e.g. *Chroococcaceae*) was found to be similar to those bacteria containing antibiotic resistant plasmids. That indicates anthropogenic pollution and ecological disturbances as suggested previously by others (Balmonte et al. 2016).

The functional categories of the microbial communities were distributed into 28 level 1 subsystems of SEED database (Figure 3). The predominant functions were clustering-based subsystems (14.4%), carbohydrates (13.4%), miscellaneous (10.8%), amino acids and derivatives (7.81%) and protein metabolism (7%). Virulence, disease and defence accounted for 2.44%; potassium metabolism (0.28%), dormancy and sporulation (0.20%) and photosynthesis (0.19%) were the less prevalent. Carbohydrates, amino acids and derivatives, and protein metabolism were among the most prevalent subsystems in the city wastewaters. Our results are in line with previous studies which have shown the predominance of those functions in wastewaters (Wang et al. 2013; Chao et al. 2013; Bäumlisberger et al. 2015). Microbial communities residing in those waters are well skilled to degrade easily accessible carbon substrates such as soluble carbohydrates or polysaccharides, as well as amino acid derivatives and proteins (Uroz et al. 2013). This is consequent to a constant anthropogenic pollution of these waters with organic matter. The proportion of genes involved in virulence, disease and defence in the metagenome reads conformed with the range previously found in wastewater by others (Wang et al. 2013). The negligible proportion of function allocated to photosynthesis translates the disappearance of the bacterioplankton.

Virulence factors

To further assess the presence and relative abundance of bacteria pathogenic to humans in the wastewaters, we assessed the presence of VF genes as pathogenic indicators. Sixty-one different VF genes were identified in the samples (Figure 4). Eleven were pathogen-specific VFs and 60 are orthologous commonly found in both pathogens and non-pathogens. The genes encoding different mechanisms of virulence were (in order of relative abundance): secretion systems, toxins, exoenzymes, adherence, iron uptake, biofilm formation,

stress response, antiphagocytosis, manganese uptake, a type IV secretory protein, and plasminogen activator. The identified VF genes are commonly carried by *Escherichia coli* (17), *Shigella* spp (14), *Clostridium perfringens* (6) and *Mycobacterium tuberculosis* (6). The pathogen-specific VFs belonged to *Streptococcus agalactiae*, C. *perfringens*, M. *tuberculosis*, *Legionnela pneumophila*, *Shigella* spp, S. *flexneri*, *Yersinia enterocolitica*, and *Bartonnella henselae*. All the pathogen-specific VFs were present in Passpanga, those belonging to C. *perfringens*, S. *flexneri*, Y. *enterocolitica*, and B. *henselae* were present in Dassasgho, and the one of Y. *enterocolitica* in Zogona.

The widespread presence of VFs usually found in human pathogenic bacteria in wastewater using a high-throughput shotgun sequencing technique has been reported in many countries (Lu et al. 2013; Ye and Zhang, 2013; Kumaraswamy et al. 2014). However, in those studies the wastewater was neither from an open-air canal nor used for irrigation of crops intended for human consumption. With regard to the number of pathogen-specific and common VFs, next generation sequencing (NGS) is recommended to efficiently assess the probable presence of pathogenic bacteria in wastewater. Its broader coverage allows the identification of microorganisms present in low numbers, which would not be detected with traditional molecular tools (Kumaraswamy et al. 2014). The presence of pathogen-specific and common VF genes in urban waterways and which will be used for urban agriculture indicates that such waters represents a risk to human health. Most of the detected VFs belong to pathogens that are transmitted by direct water exposure or ingestion of water/food contaminated with human or animal faeces and are commonly responsible for waterborne diseases. Therefore, it is not surprising that populations directly or indirectly exposed to these wastewaters could suffer from acute diarrhoea, chronic gastritis, and gastroenteritis (Nitiema et al. 2011; Traore et al., 2015). In LMICs 842 000 people die annually from diarrhoea (WHO, 2017); this is because of inadequate water, sanitation, and hygiene. Moreover, the presence of pathogen-specific VFs in these canals should be a source of concern, since the use of this water for urban agriculture is a reservoir for pathogens and ARGs and so represents a strong vehicle for transmission. Pathogen-specific VF belonging to M. *tuberculosis* was detected in Passpanga canal. M. *tuberculosis* is predominantly transmitted between humans by droplets of water by coughing and possibly sneezing. Its presence in the canal could be due to the high prevalence rate in the country; 5 866 cases of tuberculosis were reported in Burkina Faso in 2015 (WHO, 2016). Patients infected with tuberculosis can contaminate water and soil ecosystems, and M. *tuberculosis* can remain viable for extended periods of time (Velayati et al. 2015). The indirect transmission of M. *tuberculosis* might be facilitated with their persistence in the environment (Falkinham et al. 2015).

Antibiotic resistance genes analysis

In total, 129 ARGs were detected; 81 were determined to be transmissible by mobile genetic elements through horizontal gene transfer (HGT). The 81 transmissible ARGs confer resistance to 11 major classes of antibiotics and trimethoprim (Figure 5). The most prevalent resistance genes encode resistance to aminoglycosides (20 genes), tetracycline (19 genes), beta-lactams (13 genes) and macrolides (9 genes). ARG prevalence in Zogona was the lowest, while prevalence of ARGs in Passpanga was the highest. A heat map of the ARG relative abundance showed that Passpanga and Dassasgho clustered together; 69 ARGs were identified in Passpanga, 21 in Zogona, and 55 in Dassasgho. Eighteen ARGs were common to the three sampling sites; 25 were common to Passpanga and Dassasgho; 25 and 10 resistant genes, respectively were found solely in Passpanga and Dassasgho. Zogona had no unique ARG. The top five most prevalent transmissible ARGs were *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, *sul*2, *aad*A13, *aad*A, and *aad*A15 in Dassasgho. Interestingly, seven different ARGs encoding extended-spectrum beta lactamases (ESBLs) ($bla_{\text{OXA-226}}$, $bla_{\text{OXA-256}}$, $bla_{\text{OXA-347}}$, $bla_{\text{OXA-46}}$, $bla_{\text{SHV-100}}$, $bla_{\text{GES-21}}$, and $bla_{\text{AIM-1}}$) were detected in the city wastewaters. All of these genes were present in Dassasgho. The $bla_{\text{SHV-100}}$ and $bla_{\text{AIM-1}}$ genes were not found in Passpanga, but the $bla_{\text{OXA-226}}$ and $bla_{\text{OXA-256}}$ were identified in water from Zogona.

Water ecosystems polluted by pathogenic bacteria and ARGs act both as a natural reservoir and a channel for the spread of clinically relevant antibiotic resistance traits. Previous studies have shown a correlation between pathogen prevalence and ARGs (Michael et al. 2013; Bengtsson-Palme et al. 2014; Zheng et al. 2017). Our study provides data that confirms those from previous studies, with greater relative prevalence of pathogen-specific VFs and ARGs found in the canals. The wide variety of detected resistance gene classes suggests that they might have been acquired under selection pressure from diverse antibiotics and/or other factors selecting for resistances mechanisms (Kristiansson et al. 2011; Bengtsson-Palme et al. 2014). The high density of bacteria in the canals, favours cell-to-cell contact, which likely contributes to ARG transmission (Tennstedt et al. 2003; Bengtsson-Palme et al. 2014). Hospital wastewater is known to contain high quantities of drug-resistant bacteria and antibiotic residues, and their interaction contributes to an increase of ARGs (Brechet et al., 2014; Lien et al. 2016). This could explain the highest ARGs diversity and relative abundance in Passpanga, which receives effluent from the university teaching hospital.

Aminoglycosides, sulphonamides, tetracyclines and beta-lactams are the most commonly administered antibiotics in human medicine in Burkina Faso (Krause et al. 1999; Ouedraogo et al. 2017). Tetracyclines and sulphonamides are commonly used as growth promoters and for disease prevention in animals (Zhang et al.

2015). This could explain the high prevalence of genes encoding resistance against these antibiotics in the canals. Aminoglycoside-modifying enzymes (*aac*, *aad*, and *ant*) are plasmid and transposon associated; dihydropteroate synthase enzymes (*sul*1 and *sul*2) are plasmid encoded and found on class 1 integrons. These characteristics facilitate the dissemination and persistence of drug resistance phenotypes within the bacterial communities, and thus could explain their relative predominance in the canal water (Hall and Collis, 1995; Mingeot-Leclercq et al. 1999; Stoll et al., 2012). The relatively high prevalence and diversity of tetracycline resistance genes identified in our datasets could be related to their presence on mobile genetic elements and single strains carrying multiple drug-resistance genes. It was of concern to find seven different ESBL genes encoding synthesis of four enzyme families in these ecosystems. It was reported that 58% of *Enterobactericeae* responsible for infections in three main hospitals of Burkina Faso were ESBL producers (Ouedraogo et al., 2017). Our results suggest a link between resistance in clinical settings and in the environment like previously stated (Tacao et al. 2012; Forsberg et al. 2012). Two genes ($bla_{\text{AIM-1}}$ and $bla_{\text{GES-21}}$) encoding carbapenemase enzymes were found in the wastewater. Carbapenems are the last resort drugs for patients infected with ESBL-producing bacteria; they are expensive drugs, which many patients in LMICs cannot afford.

Plasmid replicons

The presence of plasmids, putative carriers and disseminative agents of ARGs by horizontal gene transfer was assessed. Thirty-one plasmid replicons of medical interest from *Enterobacteriaceae* were identified in the wastewater; 27 were present in Passpanga, four in Zogona and 18 in Dassasgho (Table S2). E. *coli* was the main likely carrier. The identified plasmid replicons belonged to 10 different incompatibility groups, namely ColE, FIB, FIC, FII, P, Q, R, U, Y, and A/C. The 10 groups were found in Passpanga and Dassasgho, while only IncP and IncQwere identified in Zogona. The three most prevalent plasmid replicon families were IncFIB, IncFII and IncQ in Passpanga, and IncQ, ColE and IncP in Dassasgho. Thirty plasmid replicon types from Gram-positive bacteria were found in the canal water; all were present in Passpanga, 21 in Dassasgho, and none in Zogona (Table S.3). In both the Passpanga and Dassasgho canals, based on the probe origin, *Staphylococcus* spp was the most likely carrier, followed by *Enterococcus* spp. One plasmid replicon type in Passpanga was associated with *Streptoccoccus* spp as a possible carrier.

Plasmids can carry genes conferring resistance to one or more antibiotics, and their ability to transfer by HGT between bacteria make them key contributors to the emergence and dissemination of ARGs in human and animal medicine (Orlek et al. 2017). The great relative number of plasmid replicons in the canals is a consequence to plasmid production and acquisition (Wegrzyn et al. 2002; Kristiansson et al. 2011). The

diversity of plasmid incompatibility group and relative abundance in the different canals matched those of ARGs, suggesting likely association between the two. The identified plasmid incompatibility groups are carriers of ARGs encoding resistance to all common antibiotic classes. Mobile genetic elements are known to greatly contribute to the dissemination of ARGs via HGT and plasmids are responsible for much of the widespread distribution of clinically relevant ARGs (Gyles and Boerlin, 2014). The presence of such extensive plasmid incompatibility groups could contribute to their extensive acquisition by different bacterial species, and further spread. The plasmid replicons IncP and Q are frequently associated with the carbapenemases $bla_{\text{AIM-1}}$ and $bla_{\text{GES-21}}$, respectively (Carattoli, 2009; Leiros et al. 2012); all were found in both Passpanga and Dassasgho. E. *coli* is a faecal indicator, its presence indicates the possible occurrence of pathogenic bacteria and faecal contamination. E. *coli* was the main carrier of *Enterobacteriaceae* plasmid replicons of medical interest in the waters. Considering its plasticity, E. *coli* easily acquires and transfer ARGs to other strains and intensively promotes bacterial resistance dissemination (Rasheed et al. 2014). Thus, these wastewaters constitute a serious health risk. The multidrug resistant pathogens of special concern for both community and/or hospital acquired infections are E. *coli*, K. *pneumoniae*, S. *aureus*, S. *pneumoniae*, *Enterococci*, V. *cholerae*, and M. *tuberculosis*. Pathogen-specific and common VFs were found in the wastewaters along with clinically relevant plasmids and ARGs conferring resistance to all major classes of antibiotics. Consequently, these wastewaters can give rise to drug-resistant bacteria and so represent a potentially serious threat to public health in the town via direct contact or the food chain. It is estimated that vegetable production occupies more than 561 ha in the city, using part of the 620,000m^3 of both domestic and industrial wastewater that are discharged every year (Vezina, 2002; Dao et al. 2016).

Conclusion

Metagenomic exploration of wastewater in open-air canals, used for urban agriculture in Ouagadougou, revealed a wide functional specialization of microbial communities, a high prevalence of pathogen-specific and commonly found VFs, a variety of plasmid incompatibility types and multiple ARGs in this environment, suggesting that wastewater is a “hot spot” for formation and dissemination of antibiotic resistance. Our data showed that wastewater for urban agriculture in Burkina Faso might contribute to dissemination of bacterial resistance, including ESBLs and carbapenemases. That is of special concern for both community and hospital acquired infections. Further investigations are needed to determine the extent that exposed populations are

affected by this health issue. There is an urgent need to improve global access to clean water, sanitation, and hygiene in LMICs to prevent the rise and dissemination of bacterial resistance from the environment to people.

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Transparency declarations

The authors declare no competing financial interest.

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