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Surveillance of Antibiotic Resistance Determinants in Gaborone and Palapye Wastewater Treatment Facilities.

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Abstract

Antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) are globally considered emerging environmental contaminants that pose a serious concern to human health and the ecosystem. The objective of the study was to detect the abundance of bacteria, characterize their antibiotic resistance phenotypic and genotypic characteristics in Palapye wastewater treatment facility (PWWTF), Gaborone wastewater treatment plant (GWWTP) and the downstream environments. The two chosen study areas are different in terms of population, wastewater treatment infrastructures and uses of final effluent. Culture dependent and independent approaches were used to determine occurrence, diversity and abundance of potentially pathogenic and antibiotic resistant bacteria and ARGs in wastewater influent, effluent and downstream environments samples. In PWWTF, 39% antibiotic resistant bacteria were detected in the influent, 25% in the effluent and 35% were detected in downstream environment. In GWWTP, the bacteria isolated with resistance to antibiotics were 36% in the influent, 39% in the effluent wastewater and the downstream environment had 25%. The ARB bacterial species detected were; *Staphylococcus species*, *E.coli*, presumptive *E.aerogenes*, *Pseudomonas species*, *Brucella species*, *Salmonella species*, *Listeria species* and *Campylobacter species*. The study also detected ARGs; *tet*A (tetracycline), *mph*A (macrolide), *str*B (streptomycin), *sul1* (sulphonamide), *dfr* (trimethoprim) and *int*1 (mobile ARG cassette) in all the sample sources including the downstream environments. The results reveal occurrence and diversity of clinically relevant ARB and ARGs that accumulate in wastewater treatment facilities and subsequently disseminated to the downstream environments, particularly water sources. This research is critical in the development of new water quality monitoring schemes and implementation of policies on the use of effluent water for irrigation purposes.

Keywords—Wastewater, antibiotic resistance, genes, bacteria, environment

I. INTRODUCTION

The misuse of antibiotics has recently been characterized as one of the major causes of antibiotic resistance. Initially antibiotics were developed for treatment of bacterial infections and improving human, animal and plant health but recently their use has widely increased particularly in agriculture as growth promoters in commercial livestock industry (Aminov, 2009).

Nearly all consumed antibiotics are excreted unchanged into the environment because the body partially metabolizes them. This together with disposal of unused antibiotics has led to the frequent detection of their residues in different environment such as wastewater treatment plants (Jelic *et al*., 2011). Wastewater treatment plants receives all sorts of discharges from hospitals, industries and domestic waste which make them an interface between different environments and, therefore, provide an opportunity for antibiotics in wastewater to select for resistant bacteria and promote the transfer of resistance genes in mobile genetic elements (e.g. plasmids, integrons) among the microbial communities (Szczepanowski *et al*., 2009). Antibiotic resistance genes can be disseminated among bacterial species through horizontal gene transfer mechanisms. (Luo *et al*., 2014).

Pathogens together with other non-pathogenic bacteria carrying resistance genes from the wastewater pose a threat to public health by migrating into groundwater or travelling off-site into surface water, soil and plants through the discharge of effluent wastewater and use in irrigation of crops. Different methods of waste treatment have been developed for public health safety, which aim at reducing pathogens, organic and inorganic components such as antibiotics in sewage prior to discharge. However, the effluent discharges still pose a major concern to the environment because many of these contaminants are not effectively removed.

The World Health Organization (WHO), the European Commission and the United States Centre for Disease Control and prevention (CDC) have acknowledged the importance of studying the emergence of resistance genes as well as the need for control strategies to minimise the development and spread of antibiotic resistance(Oteo *et al*., 2005).

This study was to focus primarily on determining the occurrence, abundance diversity and potential spread of pathogenic and antibiotic resistant bacteria, and antibiotic resistance genes in different wastewater treatment facilities and the environment receiving effluent waste water. The study also seek to understand the antibiotic resistance dynamics by considering Botswana's socio-economic and ecological factors.

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II. METHODOLOGY

A. Description of study area

The study areas were 1) Gaborone; Glen valley waste water treatment plant (GWWTP), downstream Notwane River receiving final effluent from GWWTP, 2) Palapye; Palapye wastewater treatment facility (PWWTF) and downstream man-made pond receiving the final effluent from PWWTF. Unless specified as influent, effluent or downstream, GWWTP and PWWTF acronyms will be used generally to differentiate the study areas Gaborone and Palapye respectively. The two chosen areas are different in terms of population (urban and rural), infrastructure (different treatment strategies) and different uses of the final effluent.

B. Sample collection

The sampling sources from Gaborone were influent wastewater (before treatment), effluent (after treatment), and Dws1 and Dws2; (downstream of GWWTP along the Notwane river). In Palapye, samples were also from influent, effluent and downstream of man-made pond. Samples were collected to cover all the four seasons; spring (September), summer (January), autumn (March) and winter (June) and were collected once per each month. Three samples per site were taken using grab sampling method, in the mornings between 8am and 10am. Samples were collected as previously described by Yuan *et al*. (2015). Briefly 750ml water samples each collected in a 250 ml portions from three different points of the sites in sterile polystyrene bottles were collected. Samples were placed in a cooler with ice-packs and transported to the laboratory and analyzed within 12 hrs.

C. Bacterial isolation and quantification

Fresh samples were analysed exactly as described by Yuan & colleagues (2015). Briefly a 0.1ml aliquot of water samples from each of the ten-fold serial dilutions were spread plated on different selective agar media targeting different bacterial species that are of public health concern; (Harlequin pseudomonas agar base(LabM laboratories), Harlequin salmonella™ ABC agar base(LabM laboratories), Lab 112 campylobacter selective media (LabM laboratories), Mannitol salt agar,(Biolab laboratories), Brucella agar(Conda laboratories), Chromo cult agar (Merk laboratories) and Listeria selective media (sigma-Aldrich)). The plates were incubated at 37^0C for 24hrs. Only plates with 30 to 300 colonies were considered quantifiable. Isolates growing in respective selective media were randomly picked and confirmed by gram stain procedure. An average of eight bacterial colonies in different media were then randomly picked and further sub-cultured to obtain a pure culture. The pure culture isolates were grown in nutrient broth and subsequently stored in nutrient broth containing 50% glycerol (ratio, 1:1) at -80^0C for further antibiotic resistance characterization. For statistical analysis, an average counts from triplicate plates were used to obtain the mean and standard deviation which were imported into Graph Pad Prism software (7^{th} edition) and the difference between treatments plants were determined by t-Test with P values less than 0.05 considered being significant

D. Antibiotic resistance characterization

Resistance analysis was done as previously described by Yuan *et al.*, (2015). Frozen isolates were thawed at room temperature and each bacterial isolate was aseptically sub-cultured into a number of nutrient agar plates containing different classes of clinically relevant antibiotics using a sterile tooth pick. Isolates were also sub-cultured on a nutrient agar without antibiotic as a control. The selected antibiotics and concentrations used were defined as the Minimum Inhibition Concentration (MIC) of bacteria listed in Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS, 2015). Antibiotics and concentrations used were; Penicillin(PEN)(16µg/ml),Ampicillin(AMP)(32µg/ml), ciprofloxacin(CIP)(4µg/ml),tetracycline(TET)(16µg/ml), streptomycin(STR)(64µg/ml),erythromycin(ERY)(8µg/ml), cephalosporin(CEP) (32µg/ml), meropenem(MEM) (4µg/ml), sulfamethoxazole(SMX)(512µg/ml) and trimethoprim(TMP) (64µg/ml). Cyclohexamide (75µg/ml) was also supplemented to the plates for fungal growth control. The plates were then incubated for 24 hrs at 37^0C and resistances of isolates were recorded on the basis of growth in the presence of antibiotic. Percentage values and graphs of isolated bacteria including resistant bacteria were calculated and designed using Microsoft Excel 2010 edition.

E. Molecular characterization of antibiotic resistance

DNA was first extracted from the chosen bacterial isolates (volume of 1ml) as previously described by Mirmohammadsadeghi *et al*., (2013), with few modifications. The yield of extracted DNA was measured using a nano drop spectrophotometer at an absorbance of 260nm. The DNA quality was checked by gel electrophoresis (0.8% agarose gel (3µl DNA plus 2µl loading dye)). Antibiotic resistance genes were identified using qualitative PCR with primers specific for each gene. The primers used are as follows; *mp*hA(Macrolides),*sul*1(Sulfamethoxazole),*int*1(Class1Integrase),*dfr*(Trimethoprim),*str*A(Streptomycin),strB(Streptomycin), *tet*A(Tetracycline),*tet*B(Tetracycline),*erm*A(Erythromycin),(*erm*B)(Erythromycin), (*erm*C)Erythromycin, (*qnr*)Quinolones, (catB3)Chloramphenicol and (*cat*A1)Chloramphenicol.

III. RESULTS

A. Bacterial quantification

Different bacterial species were detected at different quantities in different environments.

Detected *Salmonella species* from Gaborone were significantly higher in influent ($2.82x10^5$CFU/mL) followed by effluent ($9.53x10^2$CFU/mL), species significantly decreased further in the downstreams environments, environment 1 ($1.56x10^2$CFU/mL) and environment 2 ($1.57x10^2$CFU/mL). *Listeria species* were significantly higher in influent ($1.40x10^8$CFU/mL), significantly decreased in the effluent ($5.6x10^4$CFU/mL) and further significant increase in the downstreams, environment1 ($4.27x10^6$CFU/mL) and

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environment2 ($3.70x10^5$CFU/mL). *Staphylococcus species* were lower in the influent ($1.14x10^4$CFU/mL), then increased significantly in the effluent ($2.78x10^4$CFU/mL) and further significant increase in downstream1 ($2.78x10^6$CFU/mL) and downstream environment2 ($3.7x10^4$CFU/mL). In *Campylobacter species*, isolates were significantly higher in influent ($2.16x10^6$CFU/mL) followed effluent ($9.30x10^3$CFU/mL), then significant decrease in downstream environment1 ($1.85x10^3$CFU/mL) and environment2 ($3.7x10^2$CFU/mL). *Brucella species* were significantly higher in influent ($1.95x10^6$CFU/mL) than effluent ($2.04x10^4$CFU/mL).There was then a significant increase of the species in the downstream environment1 ($2.50x10^8$CFU/mL) and a decrease in environment2 ($4.69x10^7$CFU/mL). *E.coli* bacteria were significantly higher in influent ($8.80x10^6$CFU/mL) than effluent ($1.03x10^1$CFU/mL) and downstream environment1 ($1.00x10^1$CFU/mL). *E.coli* bacteria were non-quantifiable in downstream environment2. In *E.arogenes* bacteria, isolates were significantly higher in influent ($8.30x10^6$CFU/mL) followed by effluent ($4.1x10^4$CFU/mL), then evironment1 ($8.23x10^2$CFU/mL) and environment2 ($4.60x10^2$CFU/mL).There was no difference between effluent and downstream environments. *Pseudomonas species* were significantly higher in influent ($3.93x10^6$CFU/mL) than effluent ($1.01x10^3$CFU/mL).A significant increase of species in the downstream environment1 ($6.62x10^3$CFU/mL) & environment2 ($2.22x10^4$ CFU/mL) was then recorded **(Figure 1).**

In Palapye, *Salmonella species* were significantly higher in influent ($7.06x10^3$CFU/mL) than effluent ($2.67x10^3$CFU/mL).There was a further significant increase in the downstream environments ($2.08x10^4$CFU/mL). In *Listeria species*, isolates were significantly higher in influent ($1.75x10^5$CFU/mL), then a significant decrease in effluent ($4.2x10^3$CFU/mL).The species significantly increased further in the downstreams ($4.10x10^4$CFU/mL). *Staphylococcus species* were almost equal between influent ($5.53x10^2$CFU/mL), effluent ($2.13x10^2$CFU/mL) and the downstreams ($5.16x10^2$CFU/mL). *Campylobacter species* were significantly higher in influent ($7.16x10^4$CFU/mL) than effluent ($6.8x10^3$CFU/mL). There was then a further significant increase in downstreams ($3.73x10^5$CFU/mL). Detected *Brucella species* were lower in the influent ($1.17x10^5$CFU/mL), significantly increased in the effluent ($2.09x10^6$CFU/mL) and the downstream ($4.23x10^6$CFU/mL). *E.coli* bacteria were higher in influent ($7.53x10^5$CFU/mL), the bacteria was non-quantifiable in effluent. Quantifiable species were then recorded in the downstream ($1.03x10^4$CFU/mL).In *E.aerogenes*, isolates were significantly higher in influent ($2.83 x10^4$CFU/mL) than effluent ($3.53x10^3$CFU/mL) and downstream ($2.27 x 10^4$CFU/mL). In *Pseudomonas species*, isolates were significantly higher in influent ($2.82x10^{11}$CFU/mL) than effluent ($2.74x10^3$CFU/mL).There was then a significant further increase in downstream environments ($2.69x10^{11}$CFU/mL) (**Figure 1).**

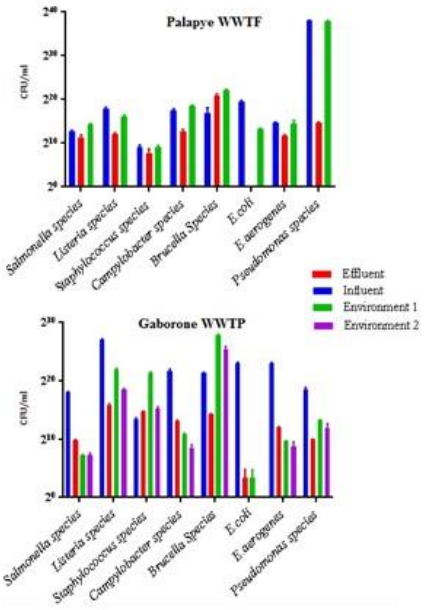

Figure 1: Quantification of bacterial isolates in the treatment facilities and downstream environments. Environment 1 and 2 are site Dsw1 and Dsw2 respectively

B. Antibiotic resistant bacteria

A total of 973 isolates from Gaborone wastewater treatment plant (GWWTP) 566 (58%), influent, effluent and downstream and Palapye wastewater treatment plant (PWWTF) 407 (42%) influent, effluent and downstream were analysed for antibiotic resistance. In PWWTF, 127 (39%) antibiotic resistant bacteria were detected in the influent, (82) 25% in the effluent and (113) 35% were detected in downstream environment. The bacteria isolated with resistance to antibiotics from GWWTP, were 191 (36%) in the influent, 204 (39%) in the effluent wastewater and the downstream environment had 131 (25%) **(Figure 2).**

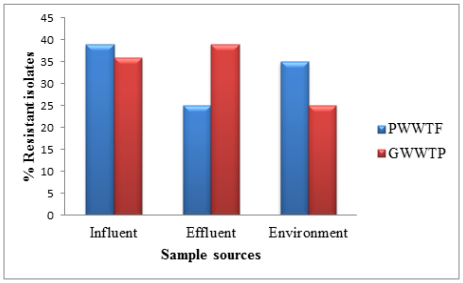

Figure 2: Antibiotic resistant bacteria isolated from different sources (influent, effluent and downstream environments) in GWWTP and PWWTF.

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In both wastewater treatment facilities, antibiotic resistant bacteria were frequently resistant against erythromycin, cephalosporin, trimethoprim, ampicillin, sulfamethoxazole, penicillin, tetracycline as well as ciprofloxacin. Streptomycin and meropenem were the least detected.

When analysing individual species isolates in GWWTP, *Staphylococcus species* were highly resistant against erythromycin, cephalosporin, trimethoprim, tetracycline, sulfamethoxazole and ampicillin while resistance against penicillin, streptomycin and meropenem were lower. Ciprofloxacin resistant *Staphylococcus* was not detected. In effluent, *Staphylococcus species* were highly resistant against cephalosporin, erythromycin, penicillin, tetracycline, sulfamethoxazole and ampicillin. The species resistance against trimethoprim, streptomycin, meropenem and ciprofloxacin was lower. In the downstreams, *Staphylococcus species* resistance against all the antibiotics was lower except ciprofloxacin resistant *Staphylococcus species* which were not detected.
Presumptive *Pseudomonas species* in influent and effluent, had a lower resistance against all the antibiotics used, meropenem resistant *Pseudomonas species* was not detected. In the downstreams, the species had a higher resistance against erythromycin, cephalosporin, trimethoprim, penicillin, and sulfamethoxazole. The species had a lower resistance against tetracycline, ciprofloxacin, streptomycin and meropenem.
Presumptive *Salmonella species'* resistance against all the antibiotics was lower except against streptomycin, meropenem and ciprofloxacin in influent which were not detected. In effluent and downstreams, presumptive *Salmonella species* resistance was lower against all antibiotics used, except meropenem resistant *Salmonella species* which were not detected in both and ciprofloxacin resistant *Salmonella species* in the downstreams.
Presumptive *Campylobacter species* resistance against all the antibiotics used were lower except meropenem and ciprofloxacin resistant *Campylobacter species* in influent which were not detected. In effluent, presumptive *Campylobacter species* were highly resistant against tetracycline, penicillin, sulfamethoxazole, ampicillin, trimethoprim and cephalosporin. The species showed a lower resistance against erythromycin, ciprofloxacin, streptomycin and meropenem. In the downstream the presumptive *Campylobacter species* resistance were lower against all antibiotics.
Presumptive *Listeria species* from influent and effluent had a lower resistance against all the antibiotics used except streptomycin, meropenem and ciprofloxacin resistant *Listeria species* which were not detected. In the downstream environments, the species had a lower resistance against all the antibiotics used except Ciprofloxacin resistant *Listeria species* which were not detected.
Presumptive *Brucella species* from influent, effluent and downstream environments had a lower resistance against all the antibiotics. Ciprofloxacin resistant *Brucella species* were not detected in effluent.
Presumptive *E.aerogenes* from influent and downstreams environment had a higher resistance against erythromycin and lower resistance against all other antibiotics. Meropenem resistant *E.aerogenes* bacteria were not detected. In effluent, the species had higher resistance against penicillin and lower resistance against all other antibiotics except meropenem resistance which was not detected.
E.coli bacteria from influent had higher resistance against erythromycin, penicillin and trimethoprim. It had lower resistance against all other antibiotics. In effluent, the bacteria had a higher resistance against penicillin, cephalosporin, sulfamethoxazole, tetracycline and lower resistance against ampicillin, erythromycin, trimethoprim, streptomycin and ciprofloxacin. Meropenem resistant *E.coli* bacteria were not detected. In the downstreams the bacteria had a lower resistance against all the antibiotics used except meropenem resistant *E.coli* bacteria which was not detected.

In PWWTF, *Staphylococcus species* from influent was highly resistant against erythromycin, cephalosporin, penicillin ampicillin, trimethoprim and sulfamethoxazole. Resistance against tetracycline, ciprofloxacin and streptomycin were lower. Meropenem resistant *Staphylococcus species* were not detected. In effluent, *Staphylococcus species* were highly resistant against erythromycin, cephalosporin, ampicillin, trimethoprim, sulfamethoxazole and tetracycline. The species' resistance was lower against penicillin, ciprofloxacin, streptomycin and meropenem. In the downstream environments, the *species* resistance against all the antibiotics was lower except tetracycline resistant *Staphylococcus species* which were not detected. Presumptive *Pseudomonas species* from influent, effluent and downstream environments had a higher resistance against all antibiotics except meropenem, streptomycin and ciprofloxacin resistance which was lower. Tetracycline resistant *Pseudomonas species* was not detected in the downstreams. The *species* from influent and effluent were higher against penicillin and lower against all other antibiotics in influent and not detected in effluent. In the downstreams, it was lower against all antibiotics except tetracycline, streptomycin, meropenem and ciprofloxacin resistant P*seudomonas species* were not detected.
Presumptive *Campylobacter species* from influent had a higher resistance against erythromycin, trimethoprim, sulfamethoxazole, tetracycline and penicillin. The species resistance were lower against cephalosporin, ampicillin, ciprofloxacin and streptomycin .Meropenem resistant *Campylobacter species* were not detected. In effluent, the *species* resistance was lower against all the antibiotics except streptomycin, meropenem and ciprofloxacin resistant *Campylobacter species* which were not detected. In the downstream, the *species r*esistance were lower against all the antibiotics except tetracycline, meropenem and ciprofloxacin resistance which were not detected.
Presumptive *Listeria species* from influent were highly resistant against; erythromycin, penicillin, trimethoprim, cephalosporin, ampicillin and sulfamethoxazole. The species had a lower resistance against tetracycline ciprofloxacin and

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streptomycin Meropenem resistant *Listeria species* were not detected. In effluent presumptive *Listeria species* had a higher resistance against erythromycin, penicillin, trimethoprim, cephalosporin, ampicillin and sulfamethoxazole. Lower resistance was detected against tetracycline, ciprofloxacin and streptomycin. Meropenem resistant *Listeria species* was not detected. In the downstreams the *species* had a higher resistance against erythromycin and penicillin. It showed lower resistance against all other antibiotics except streptomycin and ciprofloxacin resistant *Listeria species* which were not detected. Presumptive *Brucella species* from influent and effluent were highly resistant against erythromycin, cephalosporin, penicillin, trimethoprim and ampicillin. Lower resistance of the species were detected against tetracycline, ciprofloxacin; streptomycin, and meropenem. In the downstream environments, the species showed a lower resistance against all the antibiotics.

E.coli bacteria from influent had higher resistance against erythromycin, ampicillin, cephalosporin and streptomycin. Lower resistance occurred against penicillin, trimethoprim, sulfamethoxazole, ciprofloxacin and tetracycline. Meropenem resistant *E.coli* was not detected. In effluent; *E.coli* had a lower resistance against penicillin, trimethoprim, ampicillin and erythromycin. Cephalosporin, streptomycin, sulfamethoxazole, ciprofloxacin, tetracycline and meropenem resistant *E.coli* were not detected. The downstreams had a lower resistance against penicillin, erythromycin, cephalosporin, trimethoprim and sulfamethoxazole. Tetracycline, streptomycin, ciprofloxacin and meropenem resistant *E.coli* bacteria were not detected. Presumptive *E.aerogenes* bacteria from influent had a higher resistant against cephalosporin, ampicillin, erythromycin and penicillin. The bacteria had lower resistance against tetracycline, trimethoprim, sulfamethoxazole and ciprofloxacin. Streptomycin and meropenem resistant *E.aerogenes* were not detected. In effluent, the bacteria showed lower resistance penicillin only, all other were not detected. In the downstreams the bacteria had a lower resistance against all the antibiotics except streptomycin and meropenem resistant *E.arogenes* bacteria which were not detected.

C. *Antibiotic resistant genes*

The frequently detected genes in Gaborone WWTP influent were *tet*A, *mph*A, *dfr, sul1* and *int*1. *str*B and *tet*B genes were not detected. In effluent, the frequently detected genes were *tet*A, *mph*A, *sul*1, *dfr*, and *int*1. *str*B gene was detected in lower quantity while *tet*B was not detected. In the downstreams, frequently detected genes were *dfr*, *mph*A and *tet*A. *sul*1, *int*l and *str*B had lower quantities. *tet*B was not detected.

In Palapye influent, *tet*A, *sul*1 and *dfr* were detected in higher quantities. Genes that had a lower detection were *mph*A and *str*B. *tetB* and *int*1 gene were not detected. In effluent, genes that were frequently detected were *dfr*, *sul*1, *int*1 and *tet*A. *mph*A was detected in lower quantities while *tet*B and *tet*A genes were not detected. In the downstreams, frequently detected genes were *dfr*, *int*1, *tet*A, *mph*A and *str*B. *tet*B was detected in lower quantities while *sul*1 gene was not detected.

In antibiotic resistance genes per individual species, *Staphylococcus species* from Gaborone WWTP influent and effluent had *tet*A gene only. There was no gene detection in the downstreams. In *Pseudomonas species* the species from influent, effluent and downstreams environment had *tet*A and *sul*1.*dfr* was also detected in effluent. *Campylobacter species* from influent had *tet*A, *dfr* and *mph*A genes. *mph*A gene was further found in the effluent. There was no gene detection in the downstreams. *Listeria species* from influent had *tet*A, *dfr* and *mph*A genes. *Int1* and *mph*A were detected in effluent. In downstream environments, *dfr* and *mph*A were detected. *Brucella species* from influent had *int*1 and *mph*A. *tet*A was found in the effluent and the downstream had *int*1, *strB* and *mph*A. *E.coli* bacteria from influent had *tet*A, *int1, dfr, sul1* and *mph*A, effluent species had *tet*A, s*ul*1 and *mph*A. *tet*A and *mph*A were further found in the downstreams. In *E.aerogenes* bacteria from influent, *tet*A, *dfr* and *mph*A were detected. Species from effluent had *tet*A, *int*1, *str*B, *dfr* and *mph*A. In the downstreams, only *dfr* gene was detected.

Staphylococcus species from Palapye WWTF influent and effluent had *sul*1 gene only. The downstreams had *dfr* gene only. *Pseudomonas species* from influent had *tet*A and *str*B, effluent had *dfr* and *sul*1.In downstreams, *tet*A, *int*1, *dfr* and *mph*A were detected. *Salmonella species* from influent had *sul*1 while effluent had *int*1 and *dfr*. No gene was detected in the downstream. In *Campylobacter* from influent, only *dfr* gene was detected, effluent had *tet*A, *int*1, *dfr* and *sul*1.In downstreams *tet*A, *str*B and *mph*A were detected. In *Listeria species*, no gene detection in influent and downstreams. *tet*A, *dfr*, *mph*A, *int*1 were detected in effluent. *Brucella species* from influent had *tet*A, *dfr*, *sul*1 and *mph*A. *dfr* was further found in effluent. In the downstreams, *tet*B, *int*1 and *dfr* were detected. In *E.aerogenes*, no gene was detected in influent and effluent. *tet*A and *dfr* genes were detected in the downstreams.

IV. DISCUSSION

This study examined the occurrence, diversity and abundance of potentially pathogenic and antibiotic resistant bacteria and resistance genes from PWWTF, GWWTP and their downstream environments. All of the species were quantifiable (*Staphylococcus, E.coli, Brucella, Campylobacter, Listeria, Salmonella, Pseudomonas, and E.aerogenes*) in all the sample sources of the treatment facilities except for *E.coli* bacteria that were not quantifiable in the effluent samples of both treatment facilities. The abundance of the different bacterial species in effluent and the downstream environments indicates contamination by effluent wastewater from the treatment facilities. All of these species are of clinically importance and may pose a serious concern to the human and livestock, contributing to the spread of infectious diseases such as Brucellosis caused by *Brucella species* (Corbel, 2006),Campylobacteriosis caused by *Campylobacter species* and Listeriosis by *Listeria species* (Robert, 2004).

Both treatment facilities showed presence and diversity of antibiotic resistant bacteria in treated wastewater and the

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downstreams, with GWWTP having higher abundance of the resistant bacteria. The targeted antibiotic resistant species from both treatment facilities and downstream environments showed resistance against all targeted antibiotics with higher abundance against clinically important antibiotics; erythromycin, cephalosporin, trimethoprim, ampicillin, sulfamethoxazole, penicillin, and tetracycline. Even though some at lower frequencies, occurrence of resistance to the last resort antibiotics like meropenem is notable particularly in downstream environments and may have serious public health implications if the bacteria causes infection that could be otherwise untreatable.

The study further looked at the genotypic characteristics of some of the isolates. Different antibiotic resistance genes; *tet*A, *mph*A, *dfr*, *lnt1*, *sul*1, *str*B and *tet*B were detected in many of the isolates and the most dominant being *tet*A, *mph*A and *dfr* gene. The genes were mostly found in the influent water followed by the effluent then the downstream. Abundance of genes in the influent samples may be because this is untreated wastewater which is a reservoir of antibiotic resistance.

Contaminated water carrying the various ARGs can further pose a threat to public health when discharged to the environments because ARGs can further be transferred to other bacteria found in downstream. These results confirms the study expectations and also supports a suggestion by Hong *et al*., (2013), that most municipal wastewater treatment design are unable to remove antibiotic resistant bacteria and their associated resistance genes entirely. The presence of antibiotic resistant bacteria in effluent wastewater and the downstreams in the present study is supported also by other studies; (Thomas & Nielsen, 2005; Ferreira da Silva *et al*., 2006) while those of genes are supported by (Ziembińska-Buczyńska *et al*., (2015; Miller *et al*., 2016).The differences in efficiency of the treatment facilities may be because of different treatment processes used between the treatment plants. PWWTF treats its final effluent with bio-filters then disinfect with chlorine while GWWTP uses activated sludge and maturation ponds. Chlorination has been found as an ideal disinfectant for inactivating bacteria in WWTP, from a study by Iwane *et al*. (2001). On the other hand, resistant analysis showed high rates among isolated strains in four treatment which uses activated sludge for treatment. The treatment water plant does not chlorinate the final effluent (Munir *et al*., 2011).

V. CONCLUSION

Culture dependent method confirmed the occurrence of emerging contaminants like ARB and ARGs in the wastewater effluents and downstream environments. Molecular analysis revealed diversity of ARGs that were not eliminated by the treatment processes leading to the dissemination to the downstream environments. Improvement in infrastructure of the treatment facilities is necessary to mitigate the problem of antibiotic resistance dissemination and potential spread to clinical pathogens. More research on the environment dimension of antibiotic resistance is warranted as it links together clinical and non-clinical environments, with serious implications to human health. This research is also critical in the development of new water quality monitoring schemes and implementation of policies on the use of effluent wastewater for irrigation purposes.

Botswana International University of Science and Technology

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