

Assessment of Airborne Bacterial Diversity and Antibiotic Resistance Patterns in Wastewater Treatment Plants, Hospitals and Public Transport

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Abstract—There is presently insufficient information on the atmospheric microbial level in Botswana, the occurrence and diversity of airborne microbes in Botswana is not well understood. In addition, there is also growing concern in the global spread of antimicrobial resistant bacterial pathogens that continue to emerge and pose a huge challenge to human health. This study, being the first of its kind in the country was aimed at understanding the occurrence, distribution and relative diversity of bacteria in the atmosphere surrounding wastewater treatment plants, in hospitals and public transport. The focus was also to further understand the effects of atmospheric conditions, temperature and humidity on the concentration of airborne bacteria. The highest level of culturable bacteria was detected in aerosols (up to 1.25×10^3 CFU/m³) wastewater treatment plant downwind. The atmospheric bacterial population is directly affected by temperature and relative humidity; the highest airborne microbial load was recorded during autumn followed by spring while the lowest was observed during winter season. *Pseudomonas* species was the most frequently detected (27.1%) bacterium followed by *Brucella* (15.3 %), *Listeria* (10.7 %) and *Staphylococcus* (8.9 %) species. Diversity of genes encoding resistance to various antibiotics was also detected in airborne bacteria captured in various environments. This study remains important to better understand and monitor the atmospheric basal microbial levels in Botswana.

Keywords— *airborne bacterial diversity, antibiotic resistance, wastewater treatment plant, hospital, public transport*

I. INTRODUCTION

Although ambient air is not a suitable growth medium for microorganisms, it is considered as an inanimate transmitting vehicle by carrying biological agents from one host to another. In recent years, scientists are beginning to be aware of the diversity of environmental and health problems potentially caused by airborne microorganisms. These microorganisms (bacteria, fungi, bacterial and fungal spores, yeast, viruses, microbial toxins and other microbial fragments) exist as aerosols of biological origin (bioaerosols).

The major sources of bacteria maintained in suspension are among others the soil, water, human beings, animals, plants (Jeon *et al.*, 2011; Soto, *et al.*, 2009) whereas their survival and distribution highly depend on the microorganisms cell structure and the meteorological conditions (Lighthart, 1997). Wastewater treatment plants act as reservoirs of airborne bacteria. Burge and Rogers, (2000) and Kumar *et al.*, (2011) indicated that the atmospheric microbial concentration depends on abundance of source and factors controlling the release of airborne bacteria and dispersal from the surface boundary layer. Bioaerosols are dispersed over considerable distances depending on the physicochemical and meteorological air conditions, landscape features, the time of the day, the season, and the type of treatment technology (Bauer *et al.*, 2002; Karra and Katsivella, 2007). Variations in quantity and concentration of microorganisms in the atmosphere are temporal as well as spatial, meaning the concentration not only vary among areas but also over various time-scales, including seasons, months, days and hours (Chen *et al.*, 2012; Jensen *et al.*, 1994).

Pathogenic aerosol-associated microorganisms cause a wide range of diseases in human beings, animals and cause vegetation destruction (Chen *et al.*, 2012; Lee *et al.*, 2003). The atmospheric microbial contamination in hospital structures, mainly in operating theatre wards had continued to increase prevalence of nosocomial infections resulting in high mortality rates among hospitalized patients going through post operative surgery that is inseparable from Surgical Site Infection (SSI) (Hailemariam *et al.*, 2016; Weigelt *et al.*, 2010). Very few studies have highlighted the airborne microbial diversity and the ecology of bioaerosols in public transport, instead the bus stations and metro/subway stations have been more studied than the actual modes of transport. Zhou and Wang (2013) revealed that *Staphylococci* are the most common airborne strains isolated in metro stations likewise multiple-resistant *Staphylococcus aureus* reported on public transportation system in Portland (OR, USA). Therefore there are high chances that bacterial pathogens and antibiotic

resistant strains can be spread through passenger flow in buses and trains.

The development of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) that lessen the therapeutic potential against bacterial pathogens (Rizzo, 2013; Zhang *et al.*, 2009b) is related with the intensification of antibiotic use for medical and agricultural purposes. This can be considered a major anthropogenic environmental threat affecting the natural environment (Cabello, 2006; Hawkey, 2008; Segawa *et al.*, 2013), where they are distributed into the surrounding environment by water and atmospheric circulation. The occurrence and diversity of airborne bacteria in Botswana is not well understood; therefore this study, being the first of its nature in the country is aimed at understanding the occurrence, distribution and relative diversity of bacteria in the atmosphere of selected areas; WWTPs (upwind and downwind air), hospitals (paediatric wards and operating wards) and public transport (bus and mini bus). The study is also aimed at identifying the antibiotic resistance patterns of the bacterial isolates by detecting antibiotic resistance phenotypes and genotypes in bacteria captured from the atmosphere. Furthermore, the study aims to find correlation between the different atmospheric conditions and the bacterial concentrations in the air.

II. METHODOLOGY

The distribution and relative diversity of airborne bacteria in WWTPs (upwind and downwind air), hospitals (paediatric wards and operating wards) and public transport (bus and mini bus), their correlation between atmospheric temperature and relative humidity were evaluated. The meteorological conditions; atmospheric temperature and relative humidity were recorded at each sampling site during the sampling periods. Antibiotic resistance patterns of the bacterial isolates were detected by identifying antibiotic resistance phenotypes and genotypes in bacteria captured from the atmosphere.

A. Experimental Setting/ Study Sites

Clinical Environment: Bacteria suspended in the indoor air of operating theatre wards and paediatric wards of Palapye Primary Hospital (PPH, Palapye), Nyangabwe Referral Hospital (NRH, Francistown) and Letsholathebe II Memorial Hospital (LMH, Maun) were assessed. An operating theater is one of the hospital's sterile facilities without windows and feature controlled temperature and humidity, where surgical operations are performed. The paediatric wards were assessed as one of the hospital units where the risk of infection in children have been reported to be the highest in most of the hospitals in the country due to the previously reported cases of *Salmonella*, *Escherichia coli* and other outbreaks. The operating theatre rooms were assessed because of high exposure of internal spaces of human body to possible infection during surgeries. The link between surgical site infection (SSI) or postoperative infection and operating theatre air quality in these environments warrants investigation.

Non-Clinical Environment: The airborne bacteria of public transport, shared passengers transport service available for commuting by the general public: bus (72 seater) and mini bus (26 seater) were assessed. Public transport bus services that were selected for this study are those confined to the country's main road (A1) circulating between Francistown and Palapye. In addition, the wastewater treatments facilities (Palapye, Gaborone and Maun) as the main reservoirs for airborne bacteria were also assessed.

B. Meteorological data

Temperature and relative humidity (RH) and wind speed were recorded at each sampling site with a handheld Thermocron iButtons (Dallas Semiconductors, Model DS1920). Temperature and relative humidity were then reported as an average of 1 hour 30 minutes sampling period for each sampling.

C. Air sampling

The principle of air impaction was applied for the quantitative determination of airborne bacteria. Air was directed against the media plates using a portable Microbial Air Sampler MAS-100 NT® device (Merck, Germany), at a flow rate of 100 L/min. The air sampler was placed 1.5 m above ground level during the wastewater treatment plants and hospital wards sampling, whereas in public transport the air sampler was placed at heights that mimic the average height of seated passengers (about 0.91 m). Airborne bacteria were collected by impaction onto various selective and differential agar media; Mannitol Salt agar, Brucella agar, Pseudomonas Agar F, Campylobacter agar, Listeria agar, Salmonella agar, Chromocult agar and *E. coli* agar. Cycloheximide (1µg/µL, Sigma-Aldrich Co., St. Louis, MO, USA), previously shown not to affect bacterial counts (Dybwad *et al.*, 2012) was added to each media to inhibit growth of fungi. All 8 media were used simultaneously to collect aerosolized bacteria due to the inherent biases caused by media selection, with each plate exposed to airborne bacteria collection for 10 minutes.

D. Quantification and isolation of total airborne bacteria

After each sampling session, the culture media plates were incubated for 24 hours at 37°C for enumeration of viable airborne bacteria. To estimate airborne concentrations, the number of colonies present were counted and related to the volume of the air sampled. Microbial concentrations were expressed as mean values of colony-forming units (CFU) per m³, CFU/ m³. Morphologically distinct colonies were randomly selected and sub-cultured in to fresh nutrient agar plates to obtain pure cultures. Morphological analysis of airborne bacteria was performed through macroscopic and microscopic analysis of all isolates. All the isolates were stored at -80 °C in nutrient broth with 50% glycerol solution prior to antibiotic resistance typing and DNA extraction.

E. Antibiotic Susceptibility Test

Antibiotic susceptibility was assessed qualitatively by designating isolates as being resistant or sensitive based on the growth or no growth of colony growth on nutrient agar plates supplemented with various antibiotics. All isolates were assayed for susceptibility against following antibiotics and concentrations; Trimethoprim (16 µg/ml), Ampicillin (32 µg/ml), Cephalosporin (32 µg/ml), Penicillin (16 µg/ml), Erythromycin (8 µg/ml), Sulfonamide (512 µg/ml), Meropenem (4 µg/ml), Tetracycline (16 µg/ml), Ciprofloxacin (4 µg/ml) and Streptomycin (30 µg/ml). The plates were incubated at 30°C for 48 hours.

F. Detection of Antibiotic Resistance Genes

A total of 30 antibiotic resistant strains were selected for detection of various antibiotic resistance genes. The strains were sub cultured to obtain pure culture prior to DNA isolation. Genomic DNA was extracted using the method described by Neela et al., (2015) with slight modification. The pure culture isolates were inoculated on nutrient broth and incubated for 24 hours at 37 °C. The overnight cultured bacterial cells were harvested by centrifugation at 13000 rpm for 10 seconds, the pellet was resuspended in 600µl of Tris-HCl (50mM Tris hydrochloride - 10mM EDTA, pH8.0), the solution was then incubated for 5 minutes at 80 °C then left to cool at room temperature. RNase solution (3 µl) was added, and then mixed by inverting the tube 25 times followed by incubation at 37 °C for 30 minutes. The sample was cooled at room temperature and 200µl potassium acetate solution was added, followed by vortexing for 20s. The sample was then centrifuged at 13000 rpm for 3 minutes to pellet the protein, and the supernatant was transferred into a new tube and 600 µl of isopropanol added followed by centrifugation at 13000 rpm for 1 minute to obtain the pellet with the DNA. The extracted DNA was dissolved in TE (10 mM Tris-HCl, pH8.0, 1 mM EDTA) buffer.

The antibiotic resistance genes were detected by polymerase chain reaction (PCR). The bacterial isolates were detected using specific primers for the following resistance genes; Sulfonamide (*sul1* and *sul2*), tetracycline (*tetA* and *tetB*), erythromycin (*ermA*, *ermB* and *ermC*), streptomycin (*strA* and *strB*), quinolone (*qnrA*), *dfr1* resistant genes, integrons (*int1*). The PCR amplification was performed with a PCR Thermal Cycler (Proflex PCR system, Applied Biosystems). The PCR mixture contained: 2 µl of template DNA, 12.5 µl premix (EmeraldAmp® PCR Master Mix, TAKARA BIO INC), 1.5 µl of each of the primers (forward and reverse) and 7.5 µl deionised water. The following thermocycler parameters were used: initial denaturation at 95 °C for 5 min followed by 30 cycles of 1 minute at 98 °C, annealing for 40 seconds to 1 minute (the annealing temperature varied among the primers), this was followed by elongation at 72 °C for 1 minute with a final extension for 1 minute at 72 °C. The PCR products were then subjected to gel electrophoresis analysis. PCR products were analyzed on 1.0% (w/v) agarose gel

stained with ethidium bromide. Electrophoresis of the DNA was carried out at a constant voltage of 60V for about 1 hour 30 minutes in a horizontal tank in 0.5X concentration of Tris-Borate-EDTA (TBE) buffer. After electrophoresis, the gels were visualized on UV transillumination. PCR products were sub-cloned and confirmed by DNA sequencing.

G. Statistical analysis

The variations in total bacterial in clinical and non-clinical environment were assessed using an analysis of variance (ANOVA) test. The one-way ANOVA was used to evaluate the difference between the bacterial concentrations in hospitals, WWTPs and public transport. Results with a p-value less than or equal to 0.05 ($p \leq 0.05$) were considered to be statistically significant whereas those with greater than 0.05 p-value were considered statistically not significant. The tests were performed with the software Graphpad Prism 7.0.

III. RESULTS AND DISCUSSION

A. Abundance and Diversity of Airborne Bacteria in Various Environments

In all the sites studied in each of the three hospitals, all the paediatric wards showed the highest level of bacterial contamination, Letsholathebe Memorial Hospital (LMH) had the highest (6.74×10^2 CFU/m³) bacterial load, followed by Palapye Primary Hospital (PPH) (4.62×10^2 CFU/m³) lastly Nyangabwe Referral Hospital (NRH) (2.07×10^2 CFU/m³). The operating theatre rooms had the lowest airborne bacteria concentrations; the highest bacterial load was isolated from LMH (3.46×10^2 CFU/m³), followed by PPH (3.4×10^2 CFU/m³) and the least at NRH (6×10 CFU/m³). Fig 1 reveals a significant difference ($p \leq 0.05$) between bacterial concentrations in operating theatre rooms and paediatric wards among all the bacterial isolates from hospitals.

The PPH operating theater room had high airborne bacterial concentration, it is the hospital's only major operating room thereby it experiences large volumes of activities daily. The hospital is old (more than 30 years old) therefore the design and ventilation system may have a significant impact on the cleaning of the unit consequently giving rise to the air bacterial contamination. The operating unit is poorly arranged, the entrance is located next to the washroom that is easily accessible to the building's pathways hence easy exchange with the outdoor air. Many studies have reported that the sink drains are frequently colonized by large numbers of bacteria therefore serve as potential reservoir for aerosolized pathogens or opportunistic microorganisms (McBain et al., 2003; Moore et al., 2002).

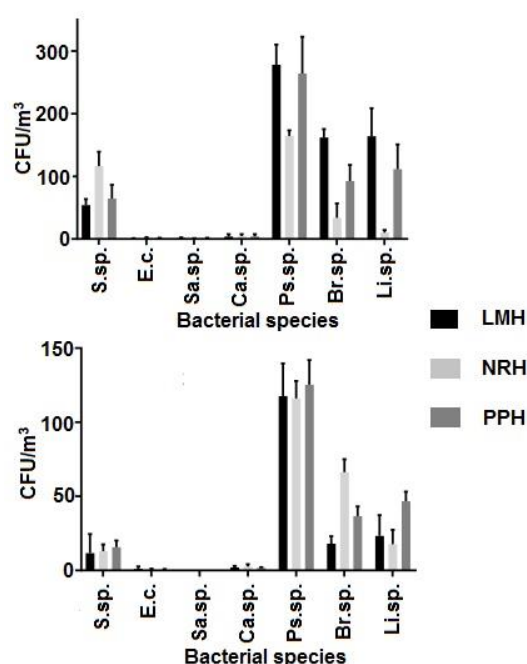


Fig. 1: Bacterial diversity of Isolates in Operating rooms versus in Paediatric wards.
S.sp. - *Staphylococcus* species, E.c - *Escherichia coli*, Sa.sp. - *Salmonella* species, Ca.sp.-*Campylobacter* species, Ps.sp.-*Pseudomonas* species, Br.sp.-*Brucella* species, Li.sp. -*Listeria* species, LMH-Letsholathebe II Memorial Hospital, NRH-Nyangabwe Referral Hospital, PPH-Palapye Primary Hospital

Pseudomonas species were the most abundant bacterial isolates from all the operating rooms and the paediatric wards, *Brucella*, *Listeria* and *Staphylococcus* species were also present in high numbers. Similar studies that involved bacteria isolation from air samples in hospitals are in accordance with our results (Borrego *et al.*, 2008; Gilbert *et al.*, 2010; Qudiesat *et al.*, 2009), their findings highlighted the high abundance of potentially pathogenic bacteria in the hospital air such as *Staphylococcus* sp., *Pseudomonas* sp., and *Streptococcus* sp. Other bacteria genera that were isolated in this study such as *Listeria* were also observed in similar studies conducted in hospitals such as Sarica *et al.*, (2002), where airborne *Bacillus* and *Listeria* were reported to be predominant in various areas of a hospital under study.

Bacterial concentrations were higher in the downwind air, this could be due to constant flow of raw wastewater through the aerated grit chamber and the inflow on raw wastewater into the sedimentation tank, in which small droplets of bioaerosols were produced and dispersed to the air by wind. Downwind air also had the highest abundance and diversity of bacteria, with the most dominant microorganisms being *Pseudomonas*, *Brucella*, *Listeria* and *Staphylococcus* species. The data obtained from this study revealed the existence of a significant level of bacterial contamination in public transport in

Botswana. Considering that the human's daily intake of air (more than 10.000 liters, Soto *et al.*, 2009) and that a mean value of 7.2×10^2 CFU/ m³ was obtained, this study reveals that one public transport passenger may inhale a total estimate of no less than about 7.0×10^3 microorganisms from the air per hour on each trip made. The high bacterial percentages in buses and mini buses may be as a result of the build-up of airborne bacteria shed by the human body due to high numbers of passengers confined to a small space. Some pathogenic bacteria like *Staphylococcus aureus*, can survive for several months in dust particles (Qudiesat *et al.*, 2009), thereby the lack of cleaning and poor ventilation (windows are usually closed throughout the trip) of the public transport may result in the accumulation of airborne bacteria. Passengers using public transport are therefore at high risk of infectious diseases. The airborne microbial data in public transport has been under-studied, not only in Botswana but the entire world.

B. Temporal Variation of Airborne Bacterial Communities

Botswana's climate is characterized by four seasons; hot and wet summers (November, December and January), wet autumns (February, March and April), cold dry winters (May, June and July) and arid windy springs (August, September and October). Fig. 2 below shows the total microbial load of the detected airborne bacteria (CFU/m³) in PWWTP downwind air was highest in autumn (March) and at the beginning of winter season (May) compared to the end of the winter season (July). Whereas at GWWTP downwind air, the bacterial concentration in the atmosphere was the highest in spring (August) followed by autumn (March) and the lowest in winter (May), while at MWWTP microbial load was much higher in spring (August) and autumn (March) and the lowest in winter (May). The results of this study indicate that in all the three seasons, total bacterial concentration was highest during the autumn and spring.

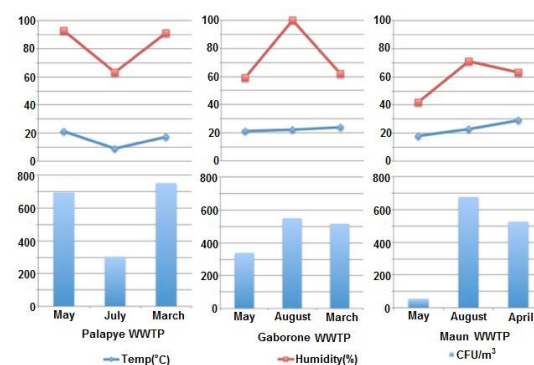


Fig. 2: Seasonal distribution of bacterial concentration isolated from WWTP (wastewater treatment plant) downwind versus temperature and humidity. During the study, temperature ranged between 9 and 29°C, RH ranged between 42 and 100%.

The analysis of the correlation of atmospheric temperature and humidity and total bacterial load revealed that levels of airborne bacterial pollution depend on climatic conditions, a

greater number of bacteria were observed in the months of lower temperatures (autumn-spring), high temperatures have the potential to significantly reduce bacterial concentrations in the air. This study is consistent with the work by Michalkiewicz *et al.*, (2011) in Kostrzyń WWTP premises, where large numbers of bacteria were recorded in autumn (3×10^3 CFU/m³), which made the air severely polluted at that time and lesser airborne bacteria concentrations in spring and winter. Gotkowska-Płachta *et al.*, (2008) and Korzeniewska *et al.*, (2008) indicated that the largest number of airborne bacteria was recorded at the WWTPs during early spring. The bacterial cells are found to absorb water from the atmosphere when the relative humidity ranges between 20% and 95% thus explaining the abundance of airborne bacteria within that range.

Similarly, in public transport bus services, the results found in this study revealed that the total bacterial concentration was highest during the autumn, followed by summer and was the lowest in winter. Although most of the airborne bacteria in public transport are emitted by indoor sources like humans, some may enter either by means of passive ventilation or by means of ventilation systems from the surrounding areas such as bus stations. However, the local public transports systems such as buses, mini buses and taxis lack ventilation systems such as air conditioners that could regulate the indoor air temperature and humidity disabling a sweeping out effect. Thereby growth conditions like excessive humidity content in transportation systems is encountered on a more frequent basis, which in most cases is the contributing factor for microbial growth. The results of this study are in accordance with Bowers *et al.*, (2012) study that demonstrated that bacterial abundances varied by season with the highest concentrations in autumn and spring.

C. Antibiotic Resistance Patterns

Of all the 1204 bacterial isolates, 79.4 % were resistant to at least one antibiotic while only 20.6 % were sensitive to all the ten antibiotics). The figure below, Fig. 3 indicate that most airborne bacteria are resistant to widely used clinical antibiotics, the mean percentage of antibiotic resistant bacterial isolates from the public transport: bus and mini bus was 83.5 % and the mean percentage of antibiotic sensitive bacterial isolates was 16.6 %. In hospitals, both operating theater wards and paediatric wards the mean percentage of antibiotic resistant and sensitive bacterial isolates were 86.4% and 13.6 % respectively. Lastly, the wastewater treatments plants had a mean percentage of antibiotic resistant and sensitive bacterial isolates of 73.4% and 26.6 % respectively.

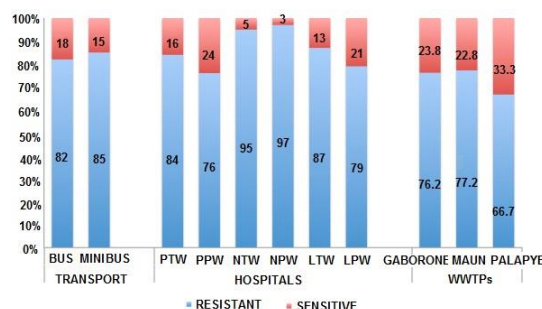


Fig. 3: The overall percentages of antibiotic resistant and sensitive bacterial isolates from public transport, hospitals and wastewater treatment plants.

Nyangabwe Referral Hospital had the highest antibiotic resistant bacterial population, both in paediatric wards (NRHPW) and operating theatre ward (NRHTW): 97.2 % and 95.3 % respectively. Similarly, other hospitals' wards had the high bacterial population with high antibiotic resistance, > 75%. The majority of hospitalized patients receive antibiotics for therapy or prophylaxis during their inpatient stay (Rehm *et al.*, 2009; Woldu *et al.*, 2013), and this is one of the factors driving antibiotic resistance in hospitals. Also, patients who enter hospitals for the treatment of resistant bacterial infections are a source of resistant bacteria and/or resistance-encoding genes. In Botswana, most of the broad-spectrum antibiotics are used indiscriminately and narrow-spectrum antibiotics are also used inappropriately. Woldu *et al.*, (2013), compared the usage and prescription of one or more antibiotics in hospitals and reported the highest in Ethiopia than from a study in Botswana by Fisher *et al.*, (2009), where the lowest percentage of antibiotic in prescriptions were recorded.

Public transportation has disturbing antibiotic resistant bacterial populations, and this could be due to the lower hygienic standard and higher number of commuters in confined space. Thereby antibiotic resistant bacterial strains released into the environment through the aerial route may contribute to antibiotic resistance development in wild-type strains of other bacterial strains. In wastewater treatment plants the antibiotic resistant bacterial load are mostly from the households, hospitals and other clinical settings (Baquero *et al.*, 2008), these bacterial strains then enter into the atmosphere where they are carried over long distances to various environments and resistance genes introduced into natural bacterial ecosystems. The reported multiple antibiotic resistant bacterial concentrations in hospitals, public transport and the wastewater treatment plants are a great threat to the inpatients and health workers, commuters, workers and nearby inhabitants.

D. Detection of Antibiotic Resistance Genes

In the current study, the presence of Macrolide 2'-phosphotransferase A, *mph*(A) gene was identified in 9 of the 30 selected airborne bacterial isolates; *Pseudomonas* sp., *Listeria* sp., *Brucella* sp. and *Staphylococcus* sp. All the bacterial isolates screened for *mph* (A) gene were resistant to

erythromycin, however the percentage of the airborne bacterial isolates harboring the *mph(A)* gene was 30%. The *mph(A)* gene was identified in air samples isolated from the Palapye WWTP upwind, Maun WWTP upwind, Maun WWTP downwind, Gaborone WWTP downwind, Tsholofelo Extension, NRH paediatric ward, LMH operating theatre ward and the paediatric ward. Other macrolide resistance genes, *ermA* and *ermB* were not identified in any of the bacterial isolates whereas *ermC* was found in 10% of the airborne bacterial isolates. The bacterial strains with *ermC* were *Staphylococcus* sp. and two *Pseudomonas* sp. isolated from Palapye WWTP upwind, Gaborone WWTP downwind and LMH paediatric ward respectively. Only one bacterial strain harbored both the *mph(A)* and *ermC* resistance genes, and this was the *Pseudomonas* sp. from the Gaborone WWTP downwind. The *qnr* resistance gene was not detected in any of the bacterial isolates that were screened for the antibiotic resistance genes. Other authors (Pereira *et al.*, 2007) described the low frequency of isolation of the *qnr* gene. Furthermore, Jacoby *et al.*, (2003) evaluated 91 bacterial isolates and from all those, identified only one isolate harboring the *qnr* gene.

In the sulfonamide and trimethoprim resistance gene families, *sul1* and *sul2* resistance genes were absent from all the bacterial isolates that were screened whereas *dfr1* was abundant in most of the bacterial isolates (40%). Trimethoprim dihydrofolate reductase (*dfr*) resistance genes were widespread amongst pathogenic bacteria; these were identified from *Staphylococcus* sp. (LMH operating theatre ward and the paediatric ward, public transport), *Escherichia coli* (Palapye WWTP downwind), *Brucella* sp. (NRH operating theatre ward, Tsholofelo Extension), *Listeria* (Maun WWTP downwind) and *Pseudomonas* (PPH operating theatre ward and paediatric ward, Gaborone WWTP downwind). Most of these genes are detected in isolates that are related to the hospital and wastewater treatment plants downwind atmospheric air. According to Jury *et al.*, (2010), mostly are associated with integrons and use elaborate transfer mechanisms to laterally spread and proliferate within the bacterial community. They further explained the *dfr1* located on both Class1 and Class 2 integrons; particularly the Class 1 integrons (*int1*) that are predominant in gram-negative bacteria isolated from humans and animals hence the abundance of the gene in all the sampling areas involved in this study.

In the present study *tetA* and *tetB* resistance genes were not identified in any of the airborne bacterial isolates. Similarly, no *strA* resistance genes were detected in this study; *strB* resistance gene was however identified in only 1 bacterial isolate of the *Pseudomonas* sp. from the GWWTP downwind air. The results of this study are in accordance with those from a study by Zhang *et al.*, (2009), that tetracycline, macrolide and multidrug resistance genes are the more represented whereas other resistances, such as resistance to aminoglycosides and sulfonamides and are less frequent. The results of this study clearly indicate that hospitals and wastewater treatment plants are hotspots for antibiotic

resistance genes, which could be due to recurrent contamination with both antibiotic residues and resistant bacteria resulting in the further spread of multidrug resistant bacteria. In addition, public transport also has high abundances of antibiotic resistant bacteria, thereby the need to regularly monitor airborne biogenic material in hospitals, wastewater treatment plants, public transport and other areas where human population is high.

IV. CONCLUSION

Airborne bacteria are widely distributed in WWTPs (upwind and downwind air), hospitals (paediatric wards and operating wards) and public transport (bus and mini bus). High percentages of the airborne bacteria are resistant to clinically used antibiotics. Majority of the antibiotic resistant bacteria were found to harbor some antibiotic resistant genes, though in lower percentages, $\leq 30\%$. The results from this study revealed that most antibiotic resistant bacterial isolates were from the hospitals, followed by public transport and lastly the wastewater treatments plants. The results are not as expected, as it was hypothesized that the wastewater treatment plants would have the highest antibiotic resistant bacterial population followed by hospitals and lastly the public transportation.

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