Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

__16APR20____

Dennis Nichols Date

Approval Sheet

A Spatial Analysis of Aerosolized Antibiotic Resistance Genes and Waste Flows in La Paz, Bolivia: Environmental and Built Environment Characteristics

By

Dennis Nichols

MPH

Hubert Department of Global Health

Thomas Clasen, MSc, PhD, JD

Committee Chair

Joe Brown, PhD, PE

Committee Member

Lance Waller, PhD

Committee Member

Abstract Cover Page

A Spatial Analysis of Aerosolized Antibiotic Resistance Genes and Waste Flows in La Paz, Bolivia: Environmental and Built Environment Characteristics

By

Dennis Nichols

B.S., Georgia State University, 2018

Thesis Committee Chair: Thomas Clasen, MSc, PhD, JD

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Global Health

2020

Abstract

A Spatial Analysis of Aerosolized Antibiotic Resistance Genes and Waste Flows in La Paz, Bolivia: Environmental and Built Environment Characteristics

By Dennis Nichols

Antimicrobial resistance (AMR) is a growing threat to Global Health. The transfer of antibiotic genes (ARGs) between bacterial populations through conjugation or direct uptake from the environment are among the major modes of AMR transmission. Surface water bodies may mediate this environmental transmission of AMR organisms or ARGs much as they do for diarrheal pathogens. The aerosolization of AMR organisms and ARGs from fluids or surfaces has been considered as another pathway of environmental AMR transmission. While some studies of aerosolized ARGs have been conducted in high-income countries, few have occurred in low-income settings with environmental risk factors such as poor water and sanitation infrastructure. Nor have these studies employed GIS technologies to spatially analyze the associations between aerosolized ARGs and environmental characteristics. In order to address these knowledge gaps, we conducted environmental surveillance for aerosolized ARGs near open waste flows in La Paz, Bolivia. A spatial analysis was then performed to assess associations between aerosolized concentrations of the ARG bla_{TEM} and various environmental and built environment parameters, specifically focusing on the proximity of sampling sites to waste flows and hospitals. We found significant ($p<0.05$) associations between log-transformed aerosolized bla_{TEM} concentrations and the distance of sample sites from the nearest river (waste flow) and the number of hospitals upstream of a given sample site. The strongest effect size we observed was a ~0.3 log unit increase in sampled bla_{TEM} concentrations associated with each additional hospital upstream. We also observed a diurnal pattern in the importance of model variables suggesting that complex meteorological factors may be influencing aerosolized ARG transport in La Paz. Our findings are consistent with previous studies which have demonstrated the ubiquity of beta-lactam resistance genes both in hospitals and the environment, and furthermore have demonstrated their presence in hospital effluent. This analysis demonstrates the need for further study of the aeromicrobiological pathway of AMR transmission in relation to hospitals and waste flows along with accurate meteorological measurements of sample site microclimates. Our results also show that proper disposal of hospital effluents could be an important mitigation measure in the struggle to contain AMR transmission.

Cover Page

A Spatial Analysis of Aerosolized Antibiotic Resistance Genes and Waste Flows in La Paz, Bolivia: Environmental and Built Environment Characteristics

By

Dennis Nichols

B.S., Georgia State University, 2018

Thesis Committee Chair: Thomas Clasen, MSc, PhD, JD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2020

Table of Contents

Table of Figures

Chapter 1: Introduction

The WHO has identified antimicrobial resistance (AMR) as one the top ten threats to global health in 2019 [1]. Antimicrobial resistance genes (ARGs) can become prevalent in a microbial population due to selection pressure from improper drug use in humans or antimicrobials discharged into the environment [2]. Similar pressures affecting bacterial populations in the natural environment predate human influence and as a result, 'accidental resistance genes' are thought to function in protecting bacterial cells from toxins and heavy metal exposure. [3] The 'intrinsic resistance genes' mentioned above can also be introduced through transmission by horizontal gene transfer wherein microbes directly trade ARGs via mobile gene elements or microbes take up free ARGs from the environment, thus leading to 'acquired resistance'. Environmental factors contributing to this transmission such as poor water, sanitation, and hygiene (WASH), lack of sewage treatment, and poor food safety are common in La Paz, Bolivia and AMR organisms along with ARGs are often identified in local waterbodies. [4]–[6]

Transmission, dissemination, and accumulation of ARGs that can give rise to antimicrobially resistant phenotypes among enteric infections have can be conceptualized through the classic F-diagram framework [7]. However, this framework does not consider potential transmission through the air. Exploring the bioaerosol route, wherein pathogens and their genetic material can travel along with suspended solids or liquids in the air, could add to our understanding of environmental AMR transmission.

Problem: There is a need to study the importance of the aerosol route in environmental transmission of AMR, particularly in urban settings with poor sanitation infrastructure.

Purpose: This study will examine the spatial patterns of aerosolized ARGs in La Paz, Bolivia in order to describe the environmental and built environment predictors of aerosolized AMR transmission.

Research Question 1: Is proximity to urban waste flows (rivers) associated with aerosolized ARG concentrations?

Research Question 2: What other environmental or built environment factors are spatially associated with aerosolized ARG concentrations?

Significance: In the city of La Paz, some sections of the urban river/waste flow system are covered but many others are open and in close proximity to public spaces like parks and playgrounds. Establishing the connections between waste flow proximity and ARG concentrations could motivate city administrators to install covers over more waste canals. Determining the importance of other environmental and built environment characteristics in aerosolized AMR transmission could inspire policy changes in other urban contexts. For example, if the spatial proximity of hospitals is revealed as a strong predictor of aerosolized AMR concentration, this could imply the need for stricter regulation of hospital waste disposal. Finally, we hope the use of spatial methods to contextualize aerosolized ARG sampling data will inspire further application of spatial tools to the study of environmental AMR transmission.

Chapter 2: Review of Literature

Burden of Disease from AMR

The WHO has identified antimicrobial resistance (AMR) as one the top ten threats to global health in 2019.[1] According to a recent interagency report, the current mortality from AMR is 700,000 deaths a year but the authors predict this tally could rise to as high as 10 million deaths a year by 2050. [2] The economic damage accompanying this health burden is estimated to reach up to $100.2 trillion in GDP losses. [8]

In another 2014 WHO report on the status of AMR, the authors took a more granular view of the burdens of drug-resistant infections. Systematic reviews were performed on the costs of AMR. The authors found that for patients carrying some resistant organisms such as *E. coli* resistant to 3rd generation cephalosporins (3GC) or fluoroquinolones there was a twofold rise in all-cause mortality and 30-day mortality. [9] For patients carrying *K. pneumoniae* resistant to 3GC or carbapenems, there were smaller but still significant increases to the above measures. Furthermore, patients with methicillin-resistant *S. Aureus* (MRSA) incurred significant increases in bacterium-attributable mortality, hospital lengths-of-stay, and septic shock. [9]

There is considerable uncertainty in estimating the current and future burden of AMR. Some scientists call into question the methodology the high-end future mortality estimates and debate just how AMR mortality and morbidity should be attributed.[10], [11] Notably, the 2014 WHO report concedes that confidence in their figures is limited by the fact that most of the studies performed on AMR burden are drawn from upper-

middle and high-income countries. [9] Some claim that arriving at a valid estimate for global AMR burden is not currently feasible with available data. [11]

Indeed, the collection, aggregation, and analysis of AMR data at national and global scales is still very much in early days. The first CDC threat report on AMR organisms of US concern was released in 2013, with an update due in 2020. [12] The WHO launched its Global Antimicrobial Resistance Surveillance System (GLASS) in 2015 and it was still in the early implementation phase as of 2019. [13]

While experts debate the extent of harm attributable to AMR, all agree on its seriousness with respect to public health.[10], [11] An example of the changes that growing resistance has brought about is that even patients without resistant infections are affected by the now common prescription of broad-spectrum antibiotics. Exposure to these drugs can hurt a person's beneficial gut flora, often incur additional expense to the patient, and may increase the unintended contamination with human produced anti-microbials. [14] Given the wide-ranging effects of AMR, it is incumbent on the scientific community to explore the causes of AMR development and spread in a multi-disciplinary "one health" fashion focusing on the interactions of humans, animals, and the environment. [2]

The development of AMR

The process by which microorganisms develop antibiotic resistance may be seen as a classic case of Darwinian selection. [15] The simplest story is that under external selection pressure, microbes carrying and expressing genes conferring resistance to

antimicrobial compounds are more likely to survive and comprise a larger share of the population as the generations pass. Antimicrobial resistance genes (ARGs) have several sources: sometimes they are already present in an organism and become phenotypically expressed under antibiotic pressure, some arise through mutation or genetic reassortment, often they are acquired by an organism through horizontal gene transfer (HGT), and they can even be picked up directly from the environment. [15]

Sources of selection pressure, i.e. antibiotics, are where humans come in. We apply this pressure in our own bodies, in those of our food animals, and increasingly in the environment at large. In 2010, 70 billion doses of antibiotics were estimated to have been consumed by humans, an increase of 35% over the previous decade. [16] Unfortunately, much of this antibiotic use is unnecessary. For example, a CDC study suggested that during the 2010-2011 period, 30% of antibiotic prescriptions in the US were improper. [17] Just as human consumption of antimicrobials is large and growing, so it is with our food animals. Studies disagree on exact estimates of current and future antimicrobial consumption in animals, but the consensus is that non-therapeutic use in livestock, for the purpose of growth enhancement, dwarfs human intake. [18]–[20] One report predicts that given increasing demand for animal protein, antibiotic use in animals may increase 67% by 2030. [18]

Although humans and livestock are the intended consumers, large quantities of antibiotics eventually end up in our water and soil. Granted, some microbes produce endogenous antibiotics, but the concentration of naturally produced antibiotic compounds in the environment is negligible and thus selection pressure in the environment for resistance is primarily anthropogenic. [15] The next section will

discuss the routes by which human-produced antibiotics are introduced to the environment and how the microorganisms affected by said antibiotics contribute to the AMR burden.

Factors in Environmental Transmission

Davies and Davies provide an excellent visual summary of the presumed pathways of antibiotics and AMR organisms through human and animal compartments, to the environment, and back again (*Figure 1*). [15] In the past, most of the links between compartments were assumed to be unidirectional from human settings into the environment. Following from this logic, AMR infections in humans were thought to result from exposure to other human compartments such as hospitals and long-term care facilities.

Now it is recognized that some portion of the AMR burden in humans stems from environmental exposure to resistant pathogens and transfers of resistance genes from environmental microbes into pathogenic microbe populations which affect humans. [20]–[23]While there is confidence in the contributions of the environment to the burden of resistance, quantification of these contributions is still a work in progress. [22] For example, in a review of studies on AMR and the role of the environment, the authors point out that the *presence* of resistant bacteria was detected at the majority of exposure-relevant sites and every wastewater source examined. However, few of the reviewed studies produced quantitative estimates of resistant bacteria and only one linked human and environmental AMR isolates in space and time. [21]

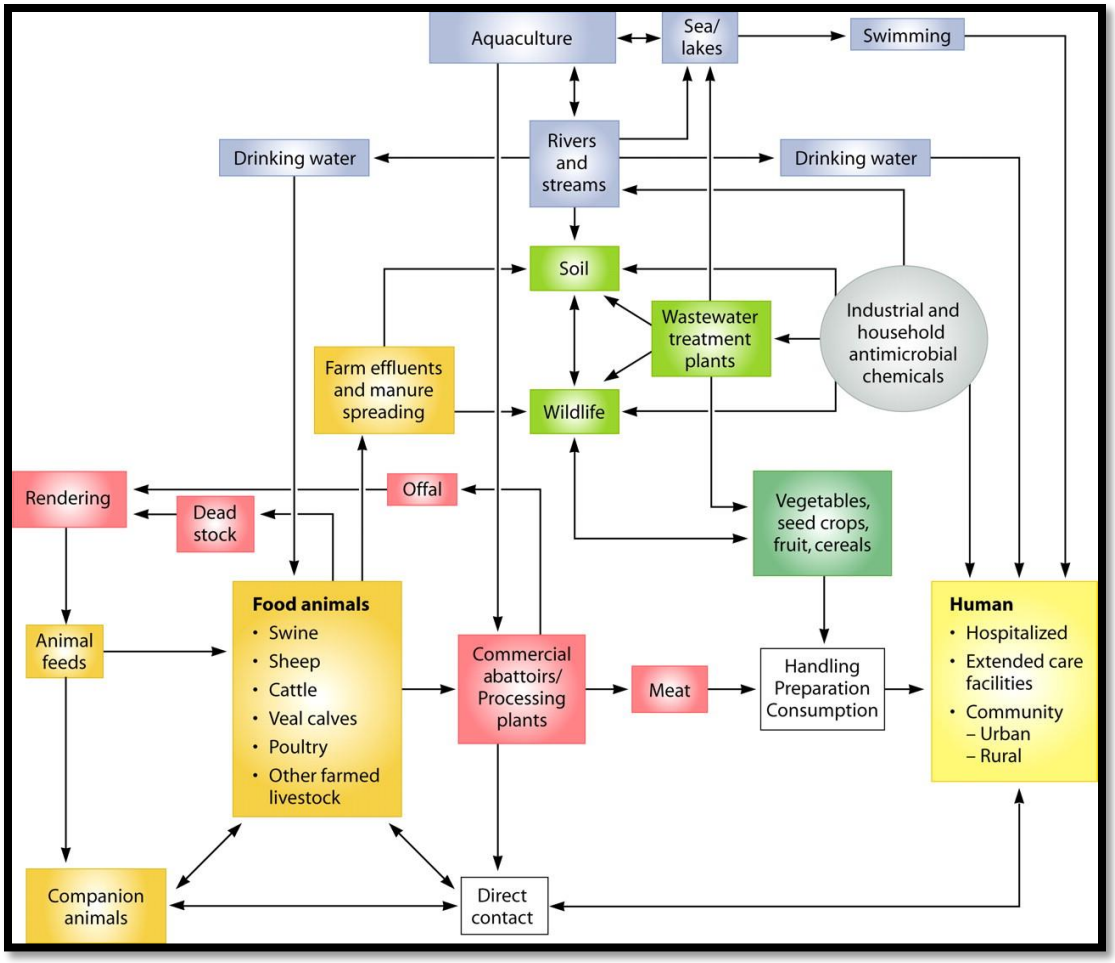

Figure 1: Dissemination of antibiotics and AMR within the human, agricultural, and natural environment. From Davies and Davies, 2010 [15]

Perhaps of greater concern than environmental transmission of resistant pathogens is the transmission of AMR genes, because these may lead to the rise of resistance in previously susceptible microbial species and populations. A significant example is the CTX-M class of β-lactamase genes which appears to have originated in the *Kluyvera* spp. bacteria that exist in human gut biomes as well as independently in the environment. [23] First identified in the 1980s, by the 2000s CTX-M had displaced other variants to become the dominant extended spectrum beta-lactamase in countries where testing occurred. [24], [25]Other clinically relevant ARGs of putative

environmental heritage include the *qnr* gene family which confers mild resistance to fluoroquinolones. These genes, often found in *Enterobacteraciae* afflicting humans, are thought to have originated in several aquatic species of bacteria like *Shewanella algae* and *Vibrio splendidus.* [23], [26]

As demonstrated by *Figure 1* and the aforementioned studies, the links between various human, animal, and environmental compartments are often facilitated by water bodies.[26] Rivers, waste canals, lakes, etc. provide a convenient mixing medium for antibiotics, resistant pathogens, and environmental populations of bacteria. [26] Several studies provide evidence for the role of water bodies as reservoirs and transmitters of resistance. In one longitudinal study of the Mhlathuze River in South Africa, the authors found a very high correlation ($r = 0.97$) between the resistance profiles of bacteria from diarrhea patients and bacteria found in the river.[27] Furthermore, a plurality of bacteria from environmental samples in this study carried class one integrons, a type of gene element which is important for the transfer of other resistance genes. As a note of caution, the authors in the above paper state that the levels of environmental resistance they found were considerably higher than those seen in other studies. [28] Other studies in locations as disparate as Colorado and China have found concentrations of free ARGs in rivers and reservoirs. [29], [30]Sometimes to find clinically relevant AMR in the environment, one need not sample major water bodies. After the emergence of NDM-1, a highly significant carbepenemase, researchers tested pools of water in streets and found the NDM-1 gene in 51 of 171 samples. [31]

Some of the major AMR contamination sources to our watersheds are urban sewage and hospital waste. A study in Austria of sewage treatment plants and resistant

E. coli found that the highest rates of resistance were present at the site which received both urban and hospital sewage. [32] Of concern, this study and others have found that sewage treatment does not fully remove resistant bacteria and ARGs, and that sometimes ARGs can be found in drinking water. [29], [31], [32]Furthermore, there is a possibility that these plants can amplify the proportion of resistance in a population of bacteria. [33]

Given the importance of waterbodies and diarrheal pathogens in the world of AMR, the field of water, sanitation, and hygiene (WASH) can provide useful perspective on environmental transmission. The classic F-diagram has served as a model for the passage of fecal-derived disease through the environment for decades and it is a simple but powerful conceptual model. [7] However, one possible pathway that this diagram leaves out is the aerosol route (*Figure 2)*, also known as the aeromicrobiological pathway. In the following section, the evidence for bioaerosols as another link the AMR transmission chain will be examined.

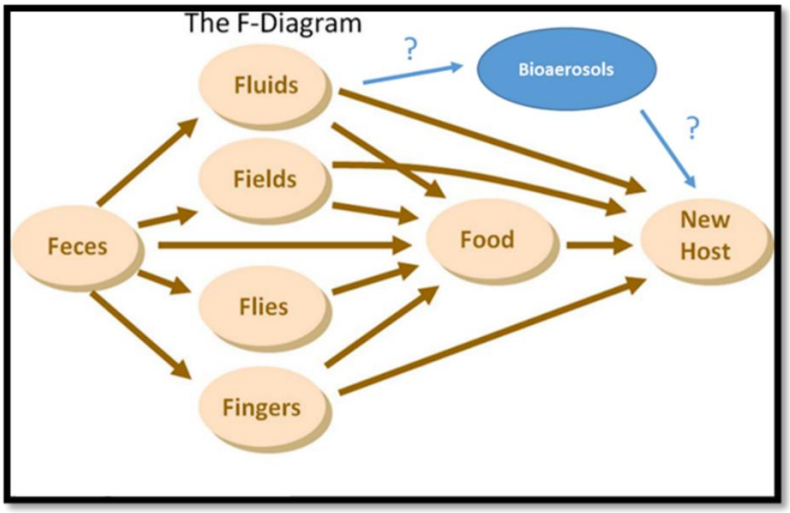

Figure 2: An updated version of Wagner and Lanoix's F-diagram. Adapted from Brown, Cairncross, and Ensink 2013. Note that this graphic is a simple representation, as discussed below, bioaersolization is possible for soils and surfaces in addition to fluids.

The Aeromicrobiological Pathway

Bioaerosols may be defined as "aerosolized particles with a biological origin. These particles originate from all types of organisms and can be dispersed into the air by a variety of abiotic and biotic mechanisms."[34] Following from this definition, aerosolized ARGs may be considered bioaerosols. Aerosols from liquid sources can arise due to evaporation and condensation, turbulence and condensation, or atomization by spraying such as in high-pressure cleaning applications. [35] The length of time for which particles can stay suspended in the air is directly related to their diameter squared, according to Stokes' law, with larger particles settling out of the air more quickly. [36]

Microbial viability in the aerosolized state may also be related to particle size. A recent study of bioaerosols along a New York City waterfront found that most samples

returning culturable bacteria or fungi were associated with particle sizes of 2.1 microns and greater.[37] Viruses and genes exist on the nanoscale and are much less limited than most bacteria species in terms of their aerosol transport potential. For example, one experiment involving the inoculation of a pig population with a modified virus was able to recover virus from the air at up to 4.7 km from the experimental site. [38] While it is not certain whether ARGs are differently susceptible to degradation at different particle sizes, their concentration does appear to increase along with particle diameter. [39]

Most studies of ARGs and air have focused on sewage treatment plants, hospital settings, and agricultural operations. At wastewater treatment plants (WWTPs), high concentrations of ARGs and the presence of resistant pathogens have been identified in air within their indoor spaces. [40] Furthermore, testing of the air up and downstream of WWTPs has demonstrated gradients in ARG concentrations which suggest these plants could be emission sources. [41], [42]Hospital studies have predominately examined airborne resistant bacteria and genes in indoor spaces. Several studies have identified multidrug resistant pathogens in the air of intensive care units (ICUs) and burn wards, with *A. baumannii* a recurring finding among isolated bacteria, consistent with its known ability to spread via respiratory droplets and persist in air. [43]–[45]These investigations also identified OXA-type beta-lactamases, which can metabolize carbapenem antibiotics, as prominent ARGs in airborne hospital isolates.[43], [44]

Similar to sewage treatment sites, studies of agricultural locations like concentrated feeding operations of poultry and pigs have identified high concentrations

of ARGs both on and off-site. Several studies establish these operations as possible contamination sources by showing that ARG concentrations are higher within the animal feeding operations compared to outdoor air as well as that concentrations downwind are higher than those upwind. [39], [46], [47]One poultry farm investigation was able to more comprehensively establish a chain of transmission by matching resistance patterns in bacteria isolated from chicken feces, indoor air, and outdoor air. [47]Several studies in North Carolina have shown a final link from exposure to industrial livestock operations which use antibiotics to human carriage of AMR organisms. A comparison of *S. aureus* and MRSA prevalence among workers at livestock operations and their household members found that livestock-associated strains of MRSA and multi drug-resistant *S. aureus* (MDRSA) were only present in the people exposed to operations which used antibiotics. [48] A later study found that adults exposed to industrial hog operations had significantly higher carriage of *S. aureus* and that children in their households had higher carriage of *S. aureus,* MRSA, and MDRSA when compared to community controls. [49]

In contrast to the aforementioned sites, less work has been done to test for aerosolized ARGs and pathogens in the environment outside of agricultural contexts. One recent study contrasted outdoor air samples with those of several different indoor environments, including hospitals, and found the highest ARG concentration in outdoor air. [50] Furthermore, a recent study of urban air in 18 cities worldwide showed large variances in the ARG concentrations detected with a nearly 2 log difference between the highest and lowest measured concentrations. This study also examined longitudinal data where it was available and showed that in Xi'an, China, where PM2.5 pollution is

monitored, aerosolized ARGs in the same area increased in relative abundance over 10 years even though PM concentrations remained the same. [51]

Up to this point in my review, it has been shown for AMR studies generally and for aerosol studies in particular that a majority of investigations have been done in high and upper-middle income countries. Likewise, the research settings typically have been either point sources of contamination like WWTPs or broad surveys of ARGs in connection with air pollution like the Li et. al 2018 study. This leaves a need for studies in lower income countries and for examinations of line sources like sewage-contaminated canals and rivers since many people are potentially exposed in these settings – for example, 500 million people live in the highly contaminated Ganga river basin. [52] We submit that our study of aerosolized ARGs around contaminated rivers in La Paz, Bolivia provides a needed exploration of current gaps in environmental AMR transmission literature.

Bolivia AMR Burden and La Paz environmental AMR evidence

There is limited comprehensive data on the AMR burden in Bolivia, but what evidence exists suggests that the proportion of resistant bacteria is high and increasing. [53], [54] In Bolivia, as of 2010, 47% of all *E. coli* isolates were fluroquinolone resistant, 49% of *K. pneumoniae* were 3GC resistant, and 49% of *S. Aureus* were methicillin resistant. [9] A study of commensal bacteria in Bolivian children from urban settings found greater than 90% resistance rates to ampicillin, tetracycline, and streptomycin. [55] Based on the data from the Pan American Health Organization, the most commonly isolated resistant bacteria in Bolivia are *E. coli, S. aureus,* and *Klebsiella spp.*

[56] Major increases in some resistance genes have been observed, with the CTX-M-type ESBL genes being found in 97% of beta-lactamase producing bacteria. [53]

Several factors suggest that environmental transmission of AMR plays a significant role in Bolivia as a whole and La Paz in particular. First, multiple studies have found significant proportions of AMR in the gut microbiomes of otherwise healthy individuals who have not or are very unlikely to have had contact with hospital settings. [55], [57]–[59]In a 2018 study of 337 children from the rural Chaco region of Bolivia, investigators found that 38.3% carried the *mcr-1* gene which can confer resistance to colistin, a last line antibiotic against gram-negative bacteria. Additionally, of the *E. coli* isolates gathered by the study, 24% produced ESBLs, mostly of the CTX-M type. Only 4 of the children had previously been prescribed antibiotics and the authors called the high carriage of potential colistin resistance "unexpected".[60]

The second piece of evidence for environmental transmission of AMR in La Paz is environmental sampling itself. One potential source which, due to its proximity to densely populated areas and importance in local agriculture, has received academic areas attention over the years is the Rio Choqueyapu, sometimes called the La Paz River. A 1997 study of river water samples found high bacterial counts even in downstream sections with less populated surrounds, several species of pathogenic *E. coli* and *Salmonella*, as well as two ampicillin-resistant isolates. [6] The next study, almost twenty years later in 2016, was able to demonstrate even wider spread contamination from AMR organisms and diarrheal pathogens. In this investigation, 4 sites were examined. The control site was a mountain reservoir, while the other 3 sites were impacted by urban sewage contamination, with one site chosen to test effects of river

proximity on agricultural contamination. The authors found that every single surface water sample impacted sites had at least one enteropathogen present with half of all isolated pathogens being resistant to at least 2 antibiotics. [5] A follow-up study of the same sites, this time using qPCR methods, was published this year. In this iteration, the researchers demonstrated the presence of enteropathogens, particularly enterotoxigenic *E. coli,* in river water, agricultural soils, and on vegetables. This paper also marked the first report of ESBL producing bacteria from environmental samples in Bolivia. [4]

Given the evidence for AMR organisms and genes in the waste flows of La Paz and investigations of environmental AMR elsewhere in the world, we have two hypotheses: First, that sampled aersolized ARG concentrations will decrease as the distance of a given site from the river increases. Second, we expect that since hospital effluent is known to contribute AMR organisms and genes into waterbodies, sampled aersolized ARG concentrations will decrease as the distance a given site from the nearest hospital increase.

Chapter 3: Methodology

1. Sampling Site Selection

We downloaded the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Global Digital Elevation Model (GDEM) version 2 data for the area including La Paz, Bolivia. Using this DEM and a hydrographic model in ArcGIS, we generated the major streamlines for the La Paz metro area. From this set of waterbodies, we selected four rivers of interest to comprise our study window: the Choqueyapu, Orkojahuira, Irpavi, and Achumani. Next, we used ArcGIS to randomly select 50 points,

at a minimum distance of 150 m from the nearest point, within 200 m of our modeled streamlines *(Figure 3).*

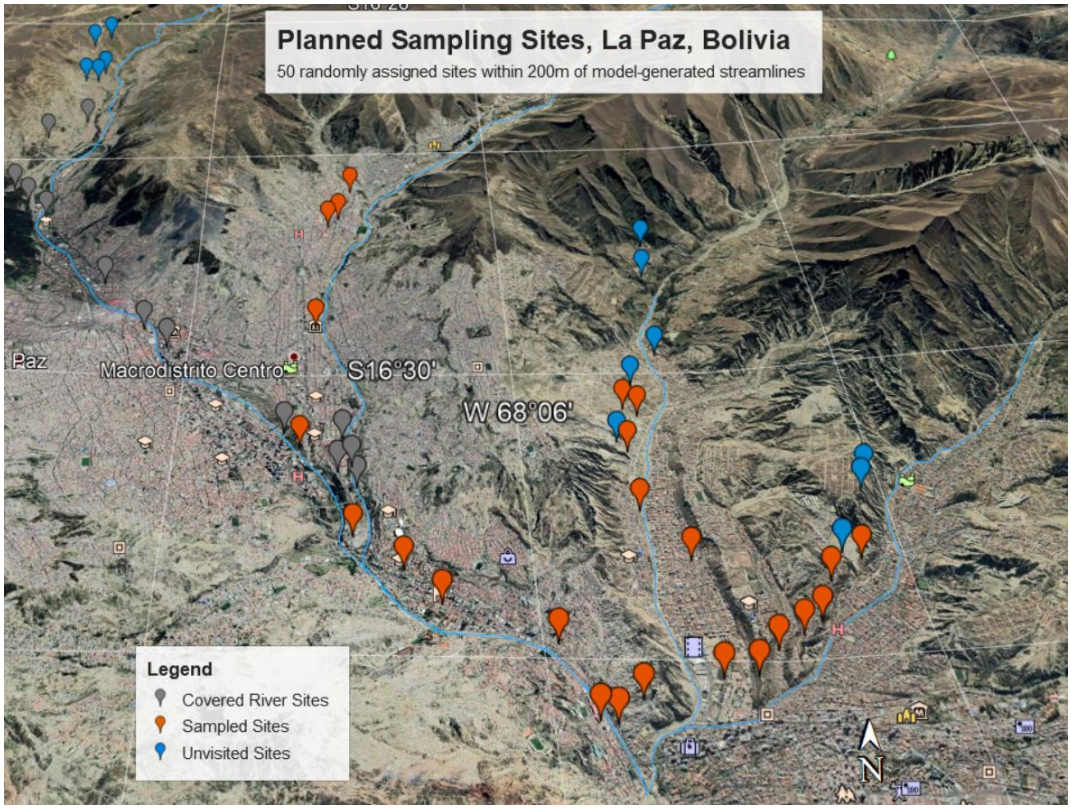

Figure 3: Map of Planned Sample Sites

Throughout the course of our study period we sampled once during the morning and once in the afternoon at 24 out of 50 possible sites, with 7 Choqueyapu sites, 4 Orkojahuira sites, 6 Irpavi sites, and 7 Achumani sites. Additionally, one control sample was taken about 25 km north of the study area at Chacaltaya mountain, a site assumed to be relatively free of human fecal contamination. When we chose our points from the randomly generated list, we had several aims. First, we often chose points that were near sites previously visited by the Ginn et. al team. [61] After this priority, we attempted to relatively evenly spread sites between the rivers in our sampling frame.

Next, an effort was made to maintain contiguity of the study frame vice sampling at more dispersed sites. Finally, the most distant sites were omitted due to the logistical constraints of travelling through the city. Thus we selected about half of the original random sample for measurements, based on access and coverage. Some additional guidelines we used for selecting points were as follows: When a site was inaccessible (due to being in a building, within private property, or on a cliff face) sampling was conducted at the nearest accessible point in a riverward direction. When the river proximate to a site was covered (most common for the Choqueyapu and Orkojahuira), that site was omitted.

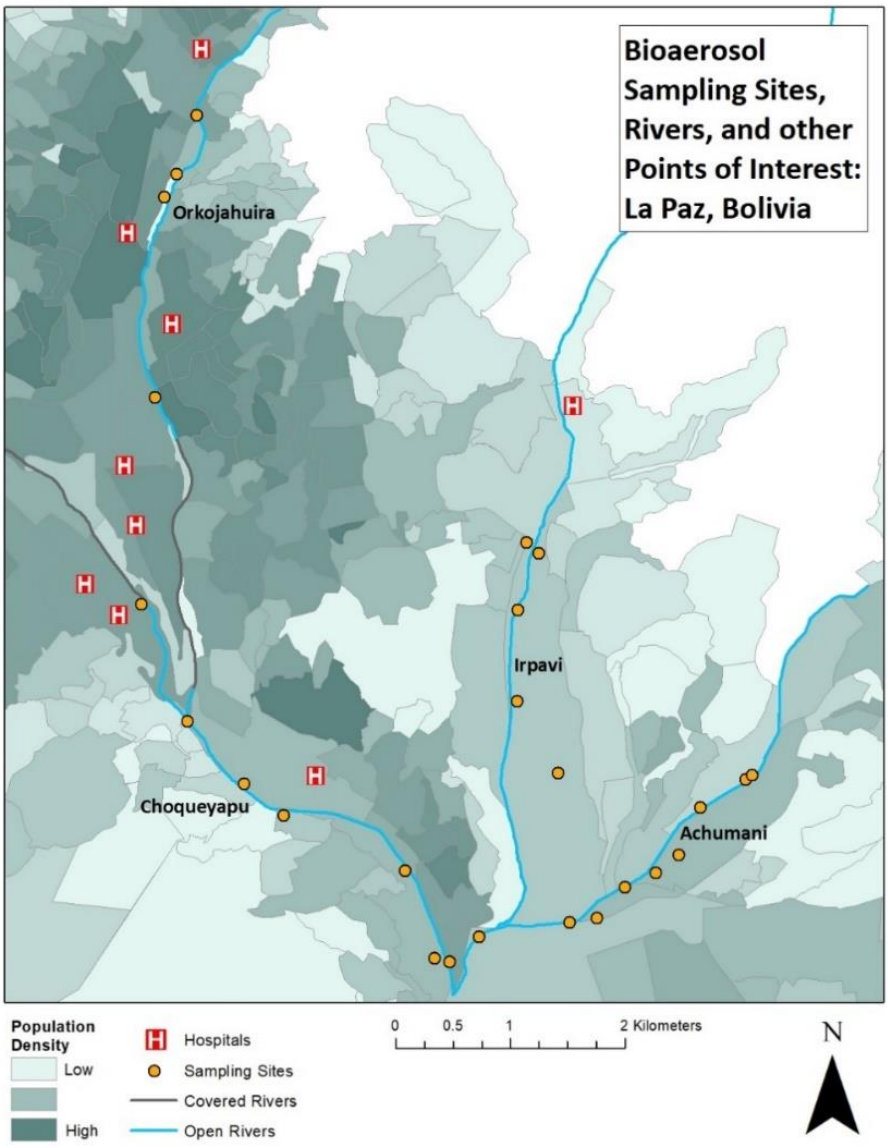

Figure 4: Map of Selected Bioaerosol Sampling Sites

2. Sample Collection

The ACD-200 BobCat Dry Filter Continuous Air Sampler (InnovaPrep, Drexel, MO, USA) (*Figure 5)* was used at each site to collect two 4-hour long bioaerosol samples at a flow rate of approximately 150 L/min. [61] Each BobCat sample collected bioaerosols on a 52 mm filter which was flushed with an elution kit from the

manufacturer to produce about 6 mL of liquid eluate.[62] Manufacturer's testing of this system's aerosol recovery efficiency in a variety of conditions yielded results ranging from ~100% for synthetic 1 μm particles with fresh filters to ~35% for *B. atropheus* spores. [62] Finally, the eluate was mixed at a 1:1 ratio with universal extraction lysis buffer (UNEX; Microbiologics, St. Cloud, MN, USA), an extraction medium determined by the CDC to have acceptable nucleic acid recovery compared to commercially available kits, and portioned into 1 mL cryovials for transportation to the Georgia Tech laboratory in Atlanta, Georgia.[61], [63]

Figure 5: ACD-200 Bobcat

3.Biological Processing

ARG detection and analysis were performed by the Brown Water Group (School of Civil & Environmental Engineering, Georgia Institute of Technology) for *bla*$_{TEM}$*, intl1, tetA,* and *flor* using droplet digital PCR (ddPCR) as described by Ginn et al. [61].

4.Spatial Variable Acquisition

4.a. Distance to Nearest River

A spatial lines file comprising the major urban river system in La Paz was produced using digitization from satellite imagery in both ArcGIS and Google Earth Pro. [64], [65] This system includes the Choqueyapu, Orkojahuira, Irpavi, and Achumani rivers. Next, a spatial points file containing each sample site was constructed using GPS coordinates collected via Google Maps and uploaded into ArcGIS. The distance from each point (in meters) to the nearest river segment (*river_distance)* was then found using the *spatial join* function in ArcGIS. One site (34) was found to be much further (~450 m) than within the intended 200 m buffer and removed from further analysis. After this, the *river_distance* variable was found to have a median value of 27.9m with a distribution as denoted by *Figure 6.*

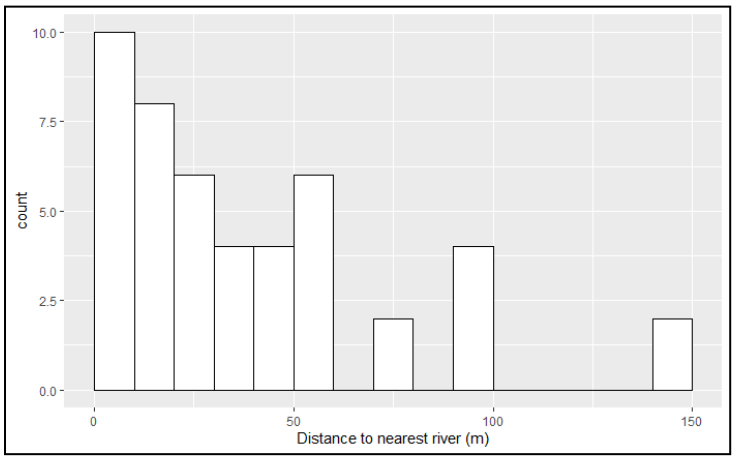

Figure 6: Distribution of Sampling Site Distances from the Nearest River

This original *river_distance* measurement is the simple straight-line Euclidean distance between the points on a two-dimensional surface. However, the city of La Paz

has considerable variation in its topography and there were large differences in elevation between the sampling sites and nearest rivers on some occasions. In order to account for this influence of topography on the true distance between features of interest, an adjustment was calculated using the ASTER GDEM V2 (NASA LP DAAC) and ArcGIS according to the following procedure:

Points were generated along the river line file at 1 m intervals and the elevation values of these points were extracted from the DEM raster. Similarly, the elevation values for the sample sites were also extracted. Then, a spatial join was performed to assign the elevation value of the nearest river point to each sampling point. The next step was to calculate the adjusted distances in RStudio using *Equation 1* and *Equation 2* as illustrated in *Figure 7*.

Equation 1

$$\boldsymbol{\theta = \tan^{-1}\left(\frac{abs(a-b)}{c}\right)}$$

Equation 2

$$\boldsymbol{d = \frac{c}{\cos\theta}}$$

a = site elevation, b= river elevation

c = unadjusted distance, d= adjusted distance

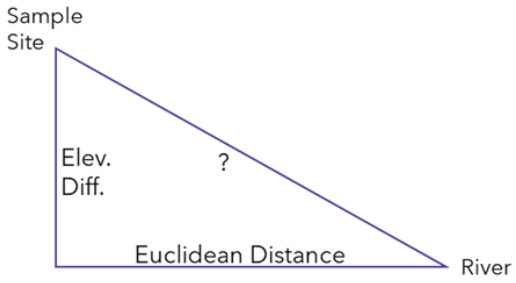

Figure 7: River Distance Adjustment Calculation

In the end, the adjusted distance diverged little from the raw distance, with a mean difference of only 0.1 m between the two measurements. This may be explained by the relatively coarse resolution (~30 m) of freely available DEMs. It is likely that in many cases, the sample points and river points fell on the same DEM raster cell and were assigned the same elevation value, even if they were different.

4.b Population Density at the Sample Site

Population density (persons/km2) at each sample site was estimated using a polygon map of population density in 2013 for neighborhood scale administrative districts in the municipality of La Paz (*Mapa de densidad poblacional del Municipio de La Paz, 2013).* With this original polygon map of population, there can be sudden large changes in density at the borders of individual polygons. The real-life variation in population density across the city is probably less abrupt than in this scenario. In order to produce more accurate density estimates, a kernel density estimation (KDE) function was used in ArcGIS to construct a smoothed raster of population density (*Figure 8)*. This function was run with search windows of 500 m, 750 m, and 1000 m. The 750 m

window raster was chosen to extract population density to the sample points because it produced the best balance between over-smoothing and bullseye effects from the KDE function.

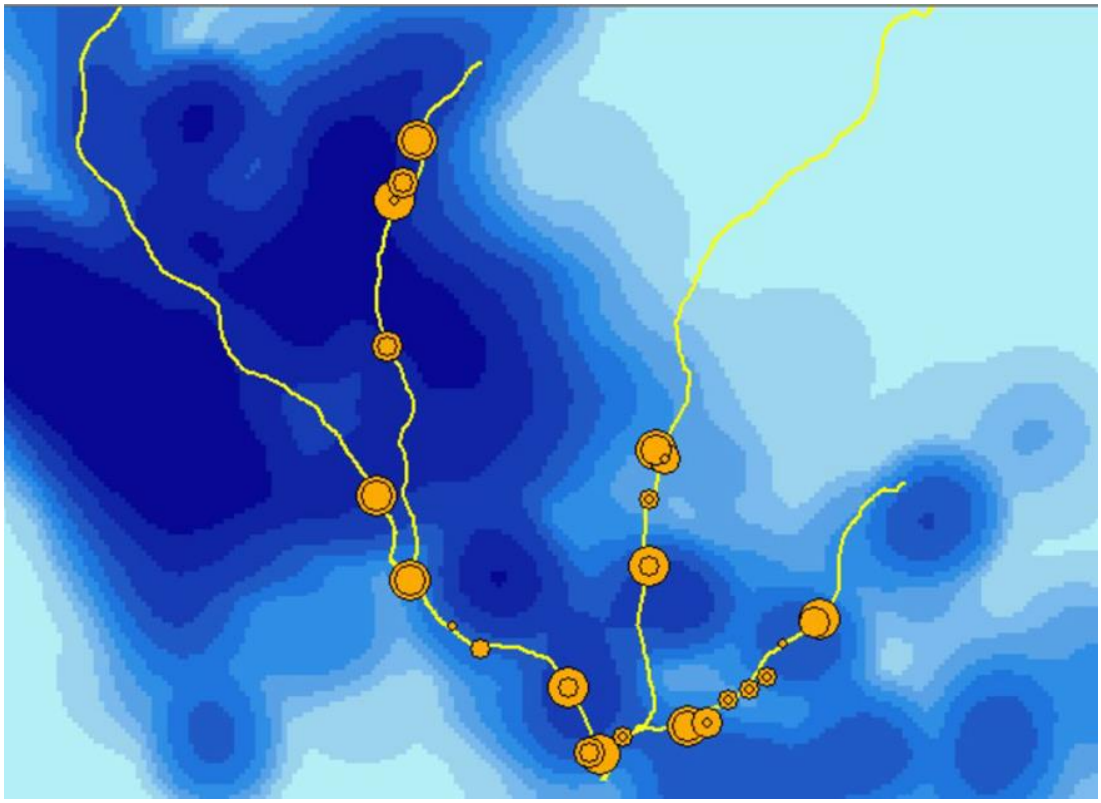

Figure 8: Population Density Raster using a 750 m search window and KDE

4.c. Sample Site to Hospital Distance

A list of the hospital locations in city of La Paz was acquired with the *openstreetmap* package in RStudio. This list was then filtered to hospitals within 500 m of the river network (*Figure 9)*. Finally, each sample site was assigned the distance of the nearest hospital via a spatial join operation in ArcGIS.

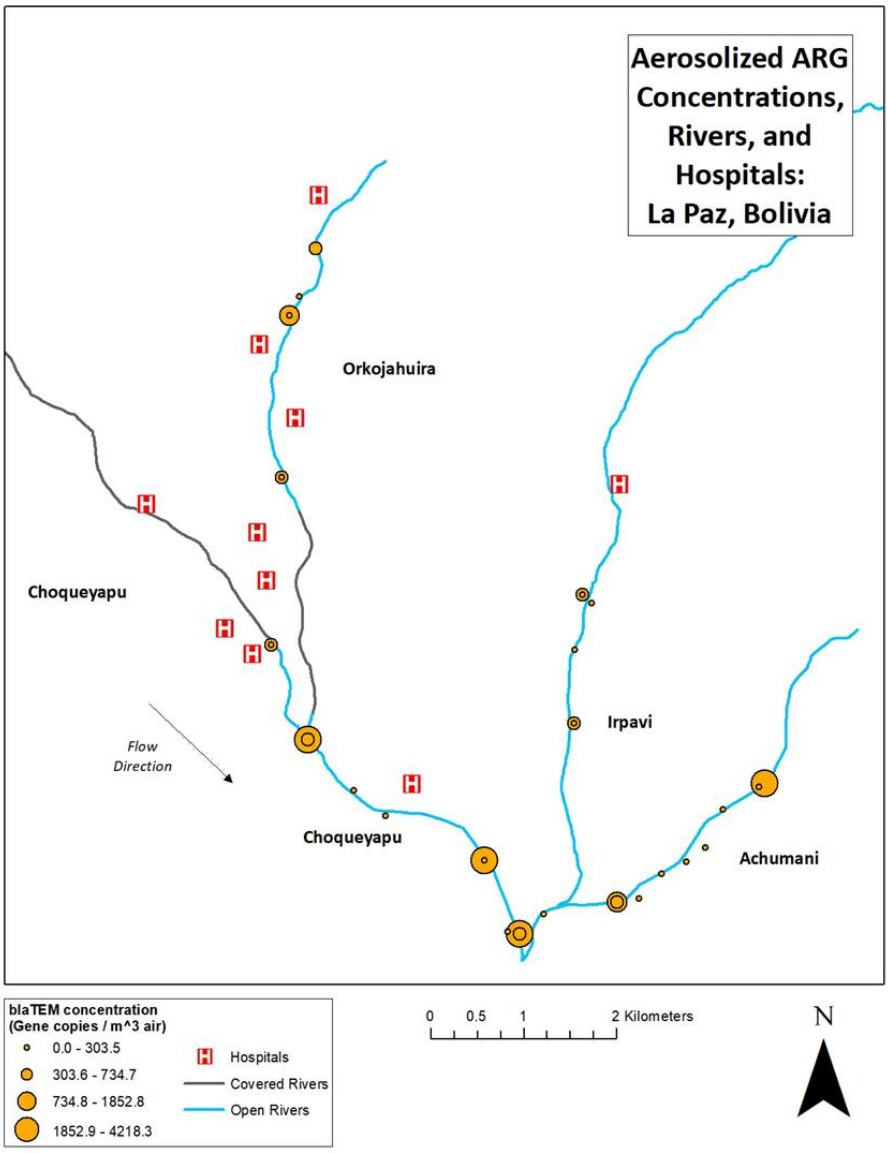

Figure 9: Map of bla_{TEM} *concentrations along with Hospital Locations*

4.d. Number of Hospitals Upstream

Using the list of hospitals generated in the previous step along with the sample points file, a variable containing the number of hospitals upstream of a given sample site

was manually generated. This variable was then joined with the sample points file in RStudio.

4. e. Sample site distance along River Network

Given the assumption that the upstream environment surrounding a river has a cumulative influence on sample sites further downstream, a proxy variable of the network distance of sample sites along the river system was constructed. Some basis for this assumption is found in studies showing significant positive correlations between population density near rivers and both pharmaceutical contamination and fecal indicator bacteria levels in surface water samples.[66], [67] In particular, the pharmaceutical study showed this relationship between concentrations and the *upstream* population density for each sample site.[66] Thus, with this motivation, we calculated the total meters of urban river upstream of each sample site using the following process:

First, the river system shapefile was extended using digitization in Google Earth Pro so that the start point of each river section was defined by the northernmost intersection of that river and the La Paz municipal boundary (*Figure 10).* Then, the river sections were separated into continuous river runs from their start point at the city border to their end point at the intersection off all four major rivers. Next, the position of each sampling site along its adjoining river was calculated using the *gProject* function of the *rgeos* package in RStudio. Finally, the total amount of river upstream of sites was calculated algebraically in RStudio for cases when a sample site was influenced by multiple upstream river sections (e.g. sites along the lower Choqueyapu river) (*Figure 11).*

Figure 10: River Start Points

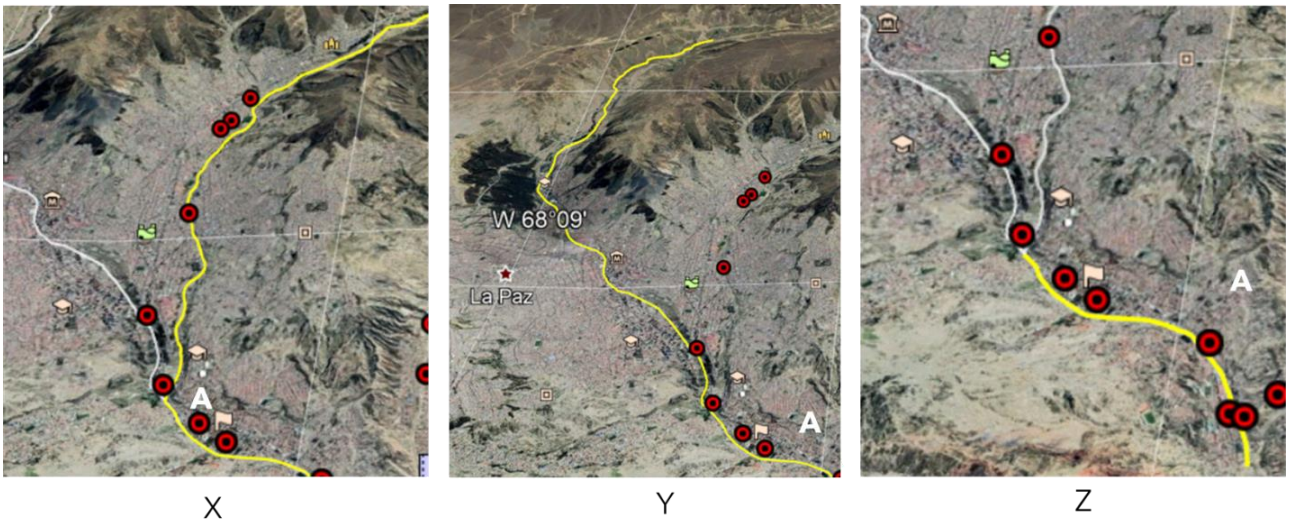

Figure 11: River Distance Calculation. The amount of urban river upstream of point A on the lower Choqueyapu is distance_X + distance_Y – distance_Z.

4. f. Daily Minimum and Maximum Temperature

Daily minimum and maximum temperature for La Paz during the study period were acquired from the Servicio Nacional de Meteorologia y Hydrologia (SENAMHI) website. June and July daily temperature data were downloaded for the "La Paz Centro" station. Finally, these data were joined by day with each sample observation in RStudio.

4. e. Sample Site Elevation

Sample site elevation was extracted from the ASTER GDEM V2 (NASA LP DAAC) for each site using ArcGIS.

5. Linear Regression Models and Global Moran's I Tests of Residuals

Multiple linear regression models of bla_{TEM} concentration (gene copies / m^3 air) were generated in RStudio. Due to the right skewed distribution of the dependent variable, models were run against both untransformed and log-transformed concentration in order to better meet the assumptions of parametric statistical tests. The best fitting models, as defined by adjusted R^2 values, were selected for reporting in the results section.

Next, we tested model residuals for spatial autocorrelation using the global Moran's I test. This allowed for the detection of spatial structure that could impact model performance. Since this test determines whether the concentrations at neighboring points are more similar (or dissimilar) than would be expected based on the global mean, we first had to choose a definition of 'neighbors'. In order to assure that only sample points along the same river segment would be considered neighbors, we

chose a k-nearest neighbors setting of 3 with a maximum distance for neighbors of 500 m.

Chapter 4: Results

Given the limited variability and low number of detections of other ARGs (*Figure 12)*, for this thesis, we chose to run models solely with bla_{TEM} concentration. *Table 1* lists the key summary statistics for the dependent and predictor variables used in these models. Of note, mean bla_{TEM} concentration was found to be 583.1 gene copies per m^3 of air (SD = 984.2). As previously mentioned, the concentration distribution is highly right-skewed. The distributions of unaltered and log-transformed bla_{TEM} concentration are shown in and *Figure 14 .* However, only 4 samples (8.7%) detected no bla_{TEM} concentration. Consideration was given to dropping these 4 zero concentration observations from the dataset to improve normality, but the resulting models performed poorly (Appendices, *Table 5*).

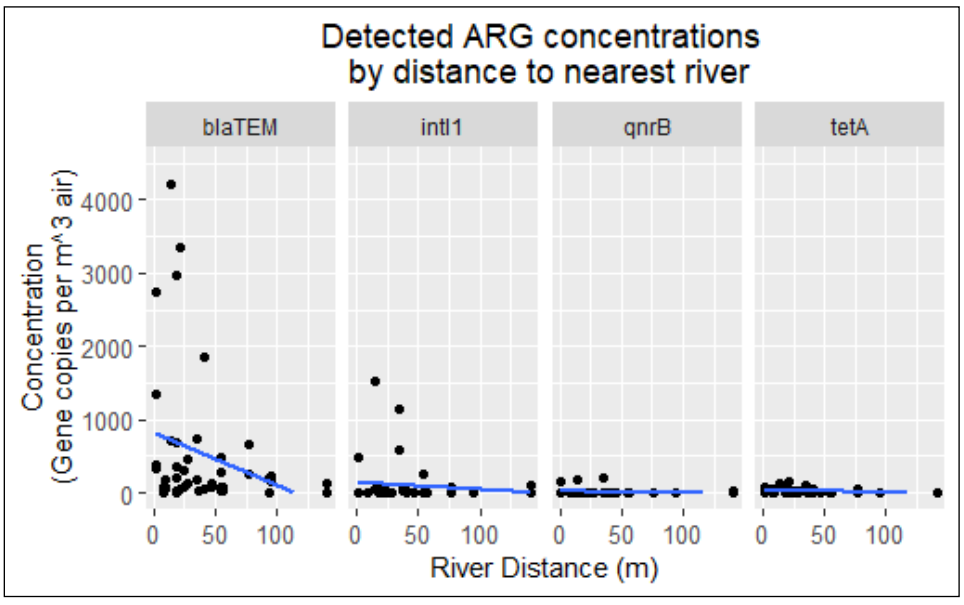

Figure 12: ARG concentrations by distance to nearest river

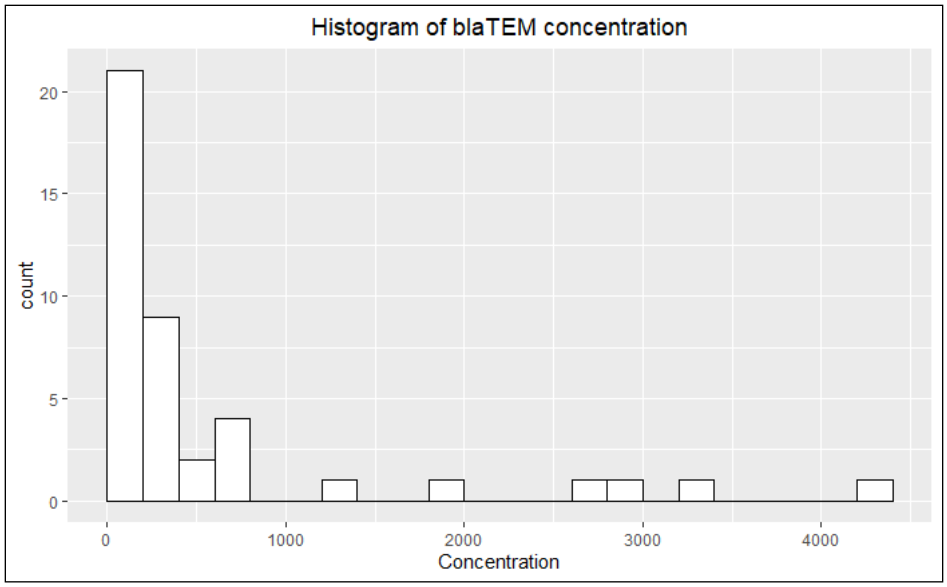

Figure 13: Histogram of bla_{TEM} concentration

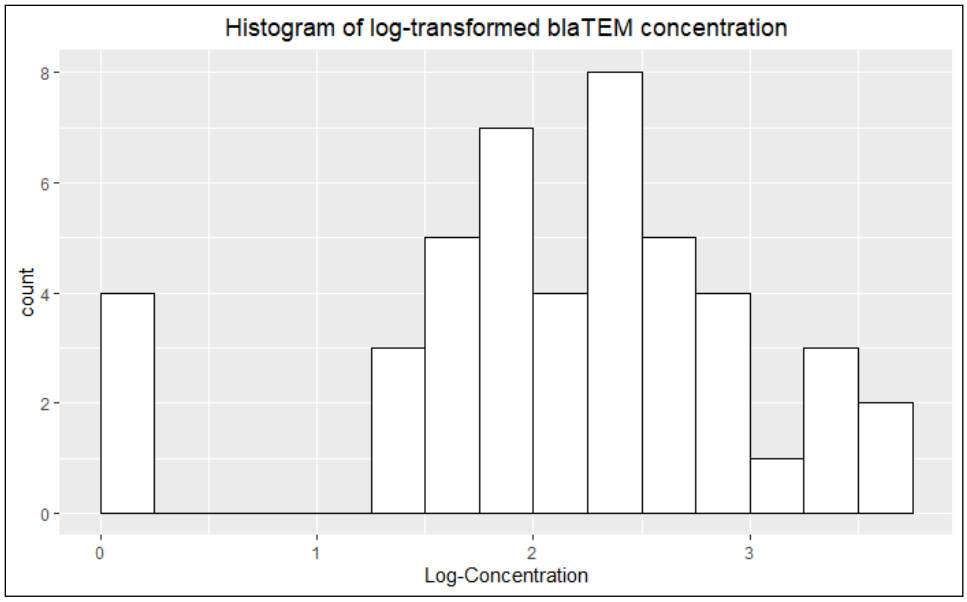

Figure 14: Histogram of log-transformed bla_{TEM} concentration

Variable	Mean	SD
bla_{TEM} Concentration (gc / m^3)	583.1	984.2
Log bla_{TEM} concentration	2.3	0.6
River Distance (m)	36.8	30.9
Network Distance (m)	$1.26x10^4$	$1.03x10^4$
Hospital distance (m)	$1.61x10^3$	$1.03x10^4$
Hospitals Upstream	3.5	4.0
Population Density (persons per km^2)	$7.27x10^3$	$5.64x10^3$
Maximum Daily Temperature (C)	20.3	1.2
Minimum Daily Temperature (C)	4.5	0.6
Elevation (m)	$3.40x10^3$	144.7

Table 1: Summary Statistics

The best-fitting regression model of the full transformed dataset (*Table 2)* showed that river distance, network distance, and the number of hospitals upstream were significantly associated ($p<0.05$) with log bla_{TEM} concentration. Examination of both transformed and untransformed models showed differential associations when separated by time of day. While population density was weakly associated ($p=0.07$) in the untransformed morning (AM) model (*Table **3)***, river distance ($p=0.04$), site to hospital distance ($p=0.02$), and number of hospitals upstream ($p=0.06$) were associated with concentration in the afternoon (PM) model. The largest effect size observed was for number of hospitals upstream in the afternoon model at 542 gene copies per m^3 per additional hospital.

	Full Model		AM Model		PM Model	
Variable	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
River Distance (m)	-0.007	0.040 **	-0.004	0.337	-0.006	0.349
Network Distance (100m)	-0.010	0.041 **	---	---	-0.018	0.047 **
Hospitals Upstream	0.299	0.016 **	0.035	0.301	0.611	0.018 **
Population Density (1000 persons per km^2)	---	---	0.047	0.062 *	---	---
Minimum Daily Temperature (C)	---	---	0.342	0.142	---	---
Elevation (m)	---	---	---	---	0.004	0.131
Hospital distance (10 m)	---	---	---	---	0.005	0.157
Adjusted R^2 =	0.169		0.330		0.162	

* = p < 0.10 | ** = p < 0.05

Table 2: Multiple Linear Regression Models of log-transformed bla_{TEM} *concentration*

	Full Model		AM Model		PM Model	
Variable	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
River Distance (m)	-7.68	0.063 *	---	---	-14.2	0.034 **
Network Distance (100m)	-3.59	0.536	2.32	0.127	-12.3	0.219
Hospitals Upstream	179.6	0.242	---	---	542.4	0.057 *
Population Density (1000 persons per km^2)	---	---	52.1	0.066 *	---	---
Maximum Daily Temperature (C)	---	---	---	---	217.0	0.319
Elevation (m)	---	---	---	---	3.64	0.147
Hospital distance (10 m)	2.10	0.188	---	---	8.83	0.022 **
Adjusted R^2 =	0.103		0.166		0.285	

* = p < 0.10 | ** = p < 0.05

Table 3: Multiple Linear Regression Models of untransformed bla_{TEM} *concentration*

No significant spatial autocorrelation of regression model residuals, as denoted by Global Moran's I value, was detected (*Table 4**Error! Reference source not found.**).* To be certain that it was not skewing the results, the 3-nearest neighbor structure was visualized in the spatial software GeoDa to confirm that neighbor connections did not inappropriately jump rivers (*Figure 15).* These results generally indicate a lack of significant spatial structure to our sampled aerosolized bla_{TEM} concentrations.

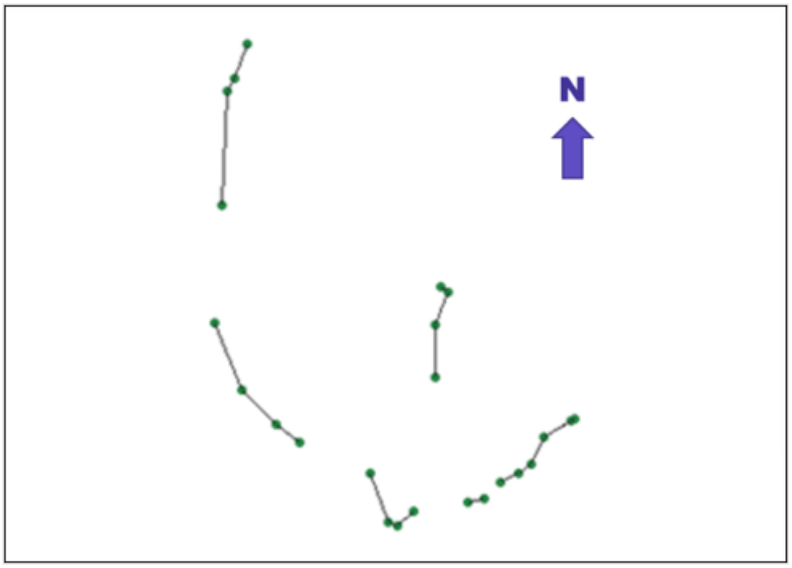

***Figure 15**: La Paz Sample Site Neighbor Connectivity Graph*

	Untransformed Models		Log-transformed Models	
Model	**Moran's I**	**p-value**	**Moran's I**	**p-value**
Full	-0.17	0.830	-0.11	0.702
AM	-0.26	0.862	-0.15	0.623
PM	-0.24	0.656	-0.29	0.818

Table 4: Model Moran's I Values

Chapter 5: Discussion

This spatial analysis demonstrates that proximity to urban waste flows is at least weakly associated with aerosolized *bla*$_{TEM}$ concentrations. The *bla*$_{TEM}$ assay used for this study covers 135 different resistance mechanisms, including ESBLs. [61] Associations between aerosolized *bla*$_{TEM}$ genes and waste flow proximity are consistent with recent findings of ESBL producing bacteria in the Choqueyapu River by Guzman-Otazo et al. (2019) and Poma et. al. (2016). [4], [5] One difference we observed in our study compared to that of Guzman-Otazo et. al was that we found *bla*$_{TEM}$ genes in 42 of 46 samples, whereas *bla*$_{TEM}$ was only identified in 5 of 101 isolates in the previous study.[4] This difference could be due to the challenge in isolating genes from whole organisms and the difference in molecular biology methods employed; qPCR was used by Guzman-Otazo et al. while our team, Ginn et. al, used ddPCR. [4], [61] Globally, *bla*$_{TEM}$ ARGs have been identified in riverine environments such as India, Nigeria, and Portugal using both culture-dependent and culture free methods.[68]–[70] However, we have been unable to find any literature on aerosolized ARGs in the context of urban waste flows or urban riverine environments, making our study a novel contribution to the field.

While the simple proximity of sample sites to the nearest river was weakly inversely associated with concentrations, the strongest predictor effect sizes we observed were for the number of hospitals upstream of a given site. Our finding is consistent with the possibility of hospital effluent contributions given that in a 2013 study in Cochabamba, Bolivia, every *A. baumanii* isolate tested possessed the *bla*$_{TEM}$ gene. [71] In the full log-transformed model, each additional hospital upstream was

associated with a ~0.3 log unit increases in sampled bla_{TEM} concentrations (p=0.02). This appears consistent with the identification of bla_{TEM} in hospital effluent (HE) by several studies in countries spanning a range of incomes. [72]–[74] Notably, concentrations of bla_{TEM} are often observed to be much higher in association with HE than in control sites. One study in India found that bla_{TEM} concentrations were 49.7-fold higher in hospital outlet pipe sediments than in those collected from sites in the receiving river basin. [73]

When we separated the analysis by time of day, some predictors varied in importance between the morning and the afternoon models. In the afternoon models, the dominant predictors of bla_{TEM} concentration were number of hospitals upstream and distance from nearest river. Whereas in the morning models, the only important variable, that we tested, seemed to be population density. We propose that this variation of predictor importance in our models of aerosol ARG concentration may be driven by many diurnal cycles. According to the *Aerosols Handbook*, "Diurnal patterns in bioaerosol concentrations are caused by changes in RH and air and surface temperatures as well as fluctuations in wind speed and turbulence, all of which affect the emission, suspension, and removal of … and other bioaerosols from the atmosphere." [75] The general factors listed above may also be joined by several others relevant to the urban riverine environment in La Paz. First, one process of liquid aerosolization is turbulence followed by condensation, with a key contributor to turbulence being flow rate.[35] The streamflow rates of the La Paz river system may vary diurnally due to environmental and anthropogenic causes. Environmental influences may include ice and snow melt that feed the river headwaters, as well as evapotranspiration driven flow variation.[76] On the anthropogenic side, sewage inflows cycles in other settings

demonstrate two peaks “resulting from morning and early evening water usage”. [77] The second important process of liquid aerosolization is evaporation followed by condensation, which may be expected to vary diurnally with solar radiation. [35]

Drawing from an additional review of the effects of meteorological factors on bioaerosol concentrations, the following may be an explanation for our observations: During the morning, recently dried surfaces proximal to our sampling sites release dust and other particles to be aerosolized as wind speeds increase during the day. [78] What evidence exists suggests that this wind driven aerosolization may be possible in La Paz. One study based on weather data from the La Paz-El Alto International Airport (LPB) found that average windspeeds (during a La Nina year) were typically ~3.5 m/s and rarely went below ~ 1.5 m/s. [79] Furthermore, freely available (for browsing, not download) historical data show that the highest windspeeds, measured at LPB, typically occurred from 3-6 PM during our study period (Weather Underground, weatherundergound.com).

Initially in the morning, without the input of much solar energy, these aerosols remain in a “shallow, undeveloped, mixing layer”. [78] During this timeframe, population density may be the most important predictor since the amount of ARGs in the general environment are assumed to be associated with human factors, or the animals which these humans keep. As the day proceeds, solar heating of the atmosphere increases and mixing takes place due to convection. This might diminish the importance of the environment local to our sample sites in the afternoon, but allow aerosol transport from more distant locales, i.e. the river/waste flow system. Reinforcing this

possibility, we might expect increased evaporative aerosolization from the rivers from noon through the afternoon due to increased solar energy.

This explanation, and my analysis in general, face several challenges from both our sampling design and potential confounders. The design of our study is nontraditional in that sampling at each site on separate days over a 40-day period it is not cross-sectional. By the same token, since each site was only sampled twice, the design is not longitudinal. Thus, it is best to frame our study as an exploratory analysis. The second main challenge arises from the potential confounding factors in our study area. Previous aerosol studies mentioned in this review have often focused on PM and we cannot ignore this as a possible source for any ARGs or pathogen genes we retrieve. La Paz has its fair share of mobile source pollution and, as our study was conducted in the dry season, windblown dust.[80] Finally, we did not obtain quality data on several of the important meteorological factors mentioned by the *Aerosols Handbook,* namely relative humidity, wind speed, and site specific air temperatures.[64]

Chapter 6: Implications and Recommendations

This spatial analysis implies that bioaerosols in ambient air surrounding urban waste flows may be an important factor in the environmental transmission of AMR. Our study particularly emphasizes this point for La Paz as it builds on previous water sampling studies of the Rio Choqueyapu. For example, Poma et. al's most contaminated site, chosen specifically for being hospital-impacted, corresponded almost exactly with the site where we recorded our 4th highest aerosolized *bla*$_{TEM}$ reading. [5]

Our spatial analysis is consistent with the existing theme in the literature that hospital effluents are a particularly important contributor to environmental AMR. Previous studies of hospitals and ARGs in the environment have focused on categorical comparisons of sites, hospital impacted versus not, typically focusing on just one hospital. Furthermore, these studies have been of water or sediment samples. However, our study goes further by demonstrating that each additional hospital along a receiving surface water body can have an additive effect on *aerosolized* ARG concentrations within ~150m of that water body. This implies that the concept of WASH in healthcare facilities (HCFs) needs to be expanded to include the activities of hospitals not only within their walls, but also within their local environment.

Several recommendations follow from these public health implications and from our experience with this study. First, future spatial studies of aerosolized ARGs should include quality measurements of wind speed, relative humidity, and temperature at the sampling site. Local data on daily waste flow oscillations would also help diagnose diurnal bioaerosol concentrations, if observed in the future. Second, if this work is to be conducted again in La Paz, it would be advisable to sample at sites along covered waste flows/ rivers as well as exposed to verify the effectiveness of these measures in reducing local aerosolized ARG concentrations.

For local officials seeking to safeguard their citizens and local environment from environmental ARG transmission, we recommend that urban waste flows are covered, where feasible. Based on our analysis, we recommend that these efforts start with the most hospital-impacted sections of the river system, namely the Rios Choqueyapu and Orkojahuira. Portions of the river that are within 200 m of densely populated areas

should also be prioritized. An alternative, and possibly more cost-effective, solution to covering waste flows would be to implement programs specifically for safely managing hospital waste.

Finally, we recommend that more environmental AMR studies are conducted using spatial methods and analyses. Indeed, the spatial nature of our bioaerosol study is uncommon within the literature as a whole, not just for studies in Bolivia. A Pubmed search for “Spatial Analysis” AND “Antibiotic resistance” returned only 6 studies. This may be due to the perception that a spatial study requires significantly more resources or expertise. However, our experience demonstrates that with field-ready tools like the ACD-200 Bobcat and access to publicly available geodatabases, a given environmental study can be made ‘spatial’ with limited additional inputs. AMR transmission is a process that happens in space, likely along gradients impacted by a host of human and environmental factors, and we cannot fully grasp it using simple categorical comparisons.

Appendices

	Full Model		AM Model		PM Model	
Variable	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
River Distance (m)	-0.003	0.282	0.000	0.985	-0.009	0.075*
Network Distance (100m)	-0.002	0.673	---	---	-0.015	0.117
Hospitals Upstream	0.066	0.522	0.031	0.319	0.513	0.080*
Population Density (1000 persons per km^2)	0.014	0.412	0.044	0.061*	---	---
Minimum Daily Temperature (C)	---	---	0.165	0.462	---	---
Maximum Daily Temperature (C)	---	---	---	---	0.201	0.247
Elevation (m)	---	---	---	---	0.003	0.147
Hospital distance (10 m)	---	---	---	---	0.005	0.082*
Adjusted R^2 =	-0.020		0.083		0.073	

* = p < 0.10 | ** = p < 0.05

Table 5: Model results with zero values dropped

References

[1] World Health Organization, "Ten threats to global health in 2019," *WHO bulletin https://www.who.int/emergencies/ten-threats-to-global-health-in-2019*, 2019. .

[2] A. Resistance and Interagency Coordination Group on, "No time to wait: Securing the future from drug-resistant infections. Report to the Secretary-General of the United Nations," 2019.

[3] H. K. Allen, J. Donato, H. H. Wang, K. A. Cloud-Hansen, J. Davies, and J. Handelsman, "Call of the wild: antibiotic resistance genes in natural environments.," *Nat. Rev. Microbiol.*, vol. 8, no. 4, pp. 251–259, Apr. 2010.

[4] J. Guzman-Otazo *et al.*, "Diarrheal bacterial pathogens and multi-resistant enterobacteria in the Choqueyapu River in La Paz, Bolivia," *PLoS One*, vol. 14, no. 1, Jan. 2019.

[5] V. Poma, N. Mamani, and V. Iñiguez, "Impact of urban contamination of the La Paz River basin on thermotolerant coliform density and occurrence of multiple antibiotic resistant enteric pathogens in river water, irrigated soil and fresh vegetables," *Springerplus*, 2016.

[6] A. Ohno *et al.*, "Enteropathogenic bacteria in the La Paz River of Bolivia," *Am. J. Trop. Med. Hyg.*, vol. 57, no. 4, pp. 438–444, 1997.

[7] E. G. WAGNER and J. N. LANOIX, "Excreta disposal for rural areas and small communities.," *Monogr. Ser. World Health Organ.*, vol. 39, p. 12, 1958.

[8] J. O'Neill, "Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations," *Rev. Antimicrob. Resist.*, 2016.

[9] WHO, "Antimicrobial resistance. Global report on surveillance," *World Heal. Organ.*, 2014.

[10] M. E. A. de Kraker, A. J. Stewardson, and S. Harbarth, "Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050?," *PLoS Med.*, 2016.

[11] M. Woolhouse, C. Waugh, M. R. Perry, and H. Nair, "Global disease burden due to antibiotic resistance - State of the evidence," *J. Glob. Health*, 2016.

[12] CDC, "Antibiotic resistance threats in the United States, 2013," *Current*, 2013.

[13] "Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017-2018.," Geneva, 2018.

[14] G. M. Eliopoulos, S. E. Cosgrove, and Y. Carmeli, "The Impact of Antimicrobial Resistance on Health and Economic Outcomes," *Clin. Infect. Dis.*, 2003.

[15] J. Davies and D. Davies, "Microbiology and Molecular Biology Reviews Microbiology and Molecular Biology Reviews Origins and Evolution of Antibiotic Resistance," *Mol. Biol. Rev. Microbiol. Mol. Biol. Rev*, 2010.

[16] T. P. Van Boeckel *et al.*, "Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data," *Lancet Infect. Dis.*, vol. 14, no. 8, pp. 742–750, 2014.

[17] K. E. Fleming-Dutra *et al.*, "Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010-2011.," *JAMA*, vol. 315, no. 17, pp. 1864–73, May 2016.

[18] T. P. Van Boeckel *et al.*, "Global trends in antimicrobial use in food animals," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, no. 18, pp. 5649–5654, May 2015.

[19] T. P. Van Boeckel *et al.*, "Reducing antimicrobial use in food animals," *Science*, vol. 357, no. 6358. American Association for the Advancement of Science, pp. 1350–1352, 29-Sep-2017.

[20] T. F. Landers, B. Cohen, T. E. Wittum, and E. L. Larson, "A review of antibiotic use in food animals: Perspective, policy, and potential," *Public Health Reports*, vol. 127, no. 1. Association of Schools of Public Health, pp. 4–22, 2012.

[21] P. M. C. Huijbers, H. Blaak, M. C. M. De Jong, E. A. M. Graat, C. M. J. E. Vandenbroucke-Grauls, and A. M. De Roda Husman, "Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review," *Environ. Sci. Technol.*, 2015.

[22] D. G. J. Larsson *et al.*, "Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance," *Environment International*, vol. 117. Elsevier Ltd, pp. 132–138, 01-Aug-2018.

[23] R. Cantón, "Antibiotic resistance genes from the environment: A perspective through newly identified antibiotic resistance mechanisms in the clinical setting," *Clinical Microbiology and Infection*, vol. 15, no. SUPPL. 1. Blackwell Publishing Ltd, pp. 20–25, 2009.

[24] R. Cantón, J. M. González-Alba, and J. C. Galán, "CTX-M enzymes: Origin and diffusion," *Frontiers in Microbiology*, vol. 3, no. APR. Frontiers Research Foundation, 2012.

[25] R. Cantón and T. M. Coque, "The CTX-M β-lactamase pandemic," *Current Opinion in Microbiology*, vol. 9, no. 5. pp. 466–475, Oct-2006.

[26] A. Lupo, S. Coyne, and T. U. Berendonk, "Origin and evolution of antibiotic resistance: The common mechanisms of emergence and spread in water bodies," *Frontiers in Microbiology*, vol. 3, no. JAN. Frontiers Research Foundation, 2012.

[27] P. T. Biyela, J. Lin, and C. C. Bezuidenhout, "The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes," *Water Sci. Technol.*, 2004.

[28] P. T. Biyela, J. Lin, and C. C. Bezuidenhout, “The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes,” *Water Sci. Technol.*, vol. 50, no. 1, pp. 45–50, Jul. 2004.

[29] A. Pruden, R. Pei, H. Storteboom, and K. H. Carlson, “Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado,” *Environ. Sci. Technol.*, 2006.

[30] Y. Jiang, Y. Chen, and Y. He, “Study on antibiotic resistance genes contamination in a reservoir located at Yangtze River downstream,” *Huanjing Kexue Xuebao/Acta Sci. Circumstantiae*, vol. 38, no. 3, pp. 884–891, Mar. 2018.

[31] T. R. Walsh, J. Weeks, D. M. Livermore, and M. A. Toleman, “Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study,” *Lancet Infect. Dis.*, 2011.

[32] F. F. Reinthaler *et al.*, “Antibiotic resistance of E. Coli in sewage and sludge,” *Water Res.*, vol. 37, no. 8, pp. 1685–1690, 2003.

[33] Y. Zhang, C. F. Marrs, C. Simon, and C. Xi, “Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp.,” *Sci. Total Environ.*, vol. 407, no. 12, pp. 3702–3706, Jun. 2009.

[34] W. Eduard, D. Heederik, C. Duchaine, and B. J. Green, “Bioaerosol exposure assessment in the workplace: The past, present and recent advances,” *Journal of Environmental Monitoring*, vol. 14, no. 2. NIH Public Access, pp. 334–339, Feb-2012.

[35] D. Haas, M. Unteregger, J. Habib, H. Galler, E. Marth, and F. F. Reinthaler, “Exposure to bioaerosol from sewage systems,” *Water. Air. Soil Pollut.*, vol. 207, no. 1–4, pp. 49–56, Mar. 2010.

[36] M. K. Owen, D. S. Ensor, and L. E. Sparks, “Airborne particle sizes and sources found in indoor air,” *Atmos. Environ. Part A, Gen. Top.*, vol. 26, no. 12, pp. 2149–2162, 1992.

[37] A. Montero, M. E. Dueker, and G. D. O’Mullan, “Culturable bioaerosols along an urban waterfront are primarily associated with coarse particles,” *PeerJ*, vol. 2016, no. 12, 2016.

[38] S. Dee, S. Otake, S. Oliveira, and J. Deen, “Evidence of long distance airborne transport of porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae,” *Vet. Res.*, vol. 40, no. 4, Jul. 2009.

[39] M. Gao, R. Jia, T. Qiu, M. Han, and X. Wang, “Size-related bacterial diversity and tetracycline resistance gene abundance in the air of concentrated poultry feeding operations.,” *Environ. Pollut.*, vol. 220, no. Pt B, pp. 1342–1348, Jan. 2017.

[40] J. V Teixeira, P. Cecilio, D. Goncalves, V. J. P. Vilar, E. Pinto, and H. N. Ferreira, “Multidrug-resistant Enterobacteriaceae from indoor air of an urban wastewater treatment plant.,” *Environ. Monit. Assess.*, vol. 188, no. 7, p. 388, Jul. 2016.

[41] E. Korzeniewska and M. Harnisz, “Extended-spectrum beta-lactamase (ESBL)-positive Enterobacteriaceae in municipal sewage and their emission to the environment,” *J. Environ. Manage.*, vol. 128, pp. 904–911, Oct. 2013.

[42] A. Gaviria-Figueroa, E. C. Preisner, S. Hoque, C. E. Feigley, and R. S. Norman, “Emission and dispersal of antibiotic resistance genes through bioaerosols generated during the treatment of municipal sewage.,” *Sci. Total Environ.*, vol. 686, pp. 402–412, Oct. 2019.

[43] J. Gao *et al.*, “Antibiotic resistance and OXA-type carbapenemases-encoding genes in airborne Acinetobacter baumannii isolated from burn wards.,” *Burns*, vol. 40, no. 2, pp. 295–299, Mar. 2014.

[44] M. Jiang, Y. Mu, N. Li, Z. Zhang, and S. Han, “Carbapenem-resistant Acinetobacter baumannii from Air and Patients of Intensive Care Units.,” *Polish J. Microbiol.*, vol. 67, no. 3, pp. 333–338, 2018.

[45] S. H. Mirhoseini, M. Nikaeen, Z. Shamsizadeh, and H. Khanahmad, “Hospital air: A potential route for transmission of infections caused by beta-lactam-resistant bacteria.,” *Am. J. Infect. Control*, vol. 44, no. 8, pp. 898–904, Aug. 2016.

[46] K. J. Wooten, G. D. Mayer, and P. N. Smith, “Persistence of elevated concentrations of PM, affiliated pharmaceuticals, and tetracycline resistance genes downwind of feedyards.,” *Environ. Pollut.*, vol. 247, pp. 467–473, Apr. 2019.

[47] D. Liu *et al.*, “Formation and transmission of Staphylococcus aureus (including MRSA) aerosols carrying antibiotic-resistant genes in a poultry farming environment.,” *Sci. Total Environ.*, vol. 426, pp. 139–145, Jun. 2012.

[48] J. L. Rinsky *et al.*, “Livestock-Associated Methicillin and Multidrug Resistant Staphylococcus aureus Is Present among Industrial, Not Antibiotic-Free Livestock Operation Workers in North Carolina,” *PLoS One*, vol. 8, no. 7, Jul. 2013.

[49] S. M. Hatcher *et al.*, “The Prevalence of Antibiotic-Resistant *Staphylococcus aureus* Nasal Carriage among Industrial Hog Operation Workers, Community Residents, and Children Living in Their Households: North Carolina, USA,” *Environ. Health Perspect.*, vol. 125, no. 4, pp. 560–569, Apr. 2017.

[50] Y. Wang, C. Wang, and L. Song, “Distribution of antibiotic resistance genes and bacteria from six atmospheric environments: Exposure risk to human,” *Sci. Total Environ.*, 2019.

[51] J. Li *et al.*, “Global Survey of Antibiotic Resistance Genes in Air.,” *Environ. Sci. Technol.*, vol. 52, no. 19, pp. 10975–10984, Oct. 2018.

[52] A. K. Haritash, S. Gaur, and S. Garg, “Assessment of water quality and suitability analysis of River Ganga in Rishikesh, India,” *Appl. Water Sci.*, vol. 6, no. 4, pp. 383–392, Nov. 2016.

[53] A. Bartoloni *et al.*, “Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in Escherichia coli: 20years of surveillance in

resource-limited settings from Latin America," *Clin. Microbiol. Infect.*, vol. 19, no. 4, pp. 356–361, 2013.

[54] A. Bartoloni *et al.*, "Antimicrobial susceptibility and emerging resistance determinants (blaCTX-M, rmtB, fosA3) in clinical isolates from urinary tract infections in the Bolivian Chaco," *Int. J. Infect. Dis.*, vol. 43, pp. 1–6, Feb. 2016.

[55] A. Bartoloni *et al.*, "Multidrug-resistant commensal Escherichia coli in children, Peru and Bolivia," *Emerg. Infect. Dis.*, vol. 12, no. 6, pp. 907–913, 2006.

[56] P. A. H. Organization, "Latin American Network for Antimicrobial Resistance Surveillance - ReLAVRA," 2015. [Online]. Available: https://www.paho.org/hq/index.php?option=com_content&view=article&id=13682:relavra-home&Itemid=42427&lang=en. [Accessed: 07-Oct-2019].

[57] A. Bartoloni *et al.*, "Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in Escherichia coli: 20years of surveillance in resource-limited settings from Latin America," *Clin. Microbiol. Infect.*, 2013.

[58] L. Pallecchi *et al.*, "Detection of CTX-M-type β-lactamase genes in fecal Escherichia coli isolates from healthy children in Bolivia and Peru," *Antimicrobial Agents and Chemotherapy*. 2004.

[59] L. Pallecchi *et al.*, "High prevalence of qnr genes in commensal enterobacteria from healthy children in Peru and Bolivia," *Antimicrob. Agents Chemother.*, 2009.

[60] T. Giani *et al.*, "High prevalence of carriage of mcr-1-positive enteric bacteria among healthy children from rural communities in the Chaco region, Bolivia, september to october 2016," *Eurosurveillance*, vol. 23, no. 45, Nov. 2018.

[61] O. . Ginn *et al.*, "Airborn Antimicrobial Resistance Genes in Urban India and Bolivia," vol. Manuscript, 2020.

[62] AlburtyLab, "Summary Technical Report InnovaPrep LLC Dry Filter Collection / Wet Elution Aspiration and Recovery Efficiency Testing For : Andrew Page , President & CTO."

[63] V. Hill, J. Narayanan, R. Gallen, K. Ferdinand, T. Cromeans, and J. Vinjé, "Development of a Nucleic Acid Extraction Procedure for Simultaneous Recovery of DNA and RNA from Diverse Microbes in Water," *Pathogens*, vol. 4, no. 2, pp. 335–354, May 2015.

[64] S. Jyothi, S. Anand, and K. B. R. Varma, "Digitizing the Forest Resource Map Using ArcGIS." 2010.

[65] K. Malarvizhi, S. V. Kumar, and P. Porchelvan, "Use of High Resolution Google Earth Satellite Imagery in Landuse Map Preparation for Urban Related Applications," *Procedia Technol.*, vol. 24, pp. 1835–1842, Jan. 2016.

[66] V. Osorio, A. Larrañaga, J. Aceña, S. Pérez, and D. Barceló, "Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers," *Sci. Total Environ.*, vol. 540, pp.

267–277, Jan. 2016.

[67] Y. S. Ham, H. Kobori, and M. Takasago, “Effects of combined sewer overflow and stormwater on indicator bacteria concentrations in the tama river due to the high population density of Tokyo metropolitan area,” *Environ. Monit. Assess.*, vol. 152, no. 1–4, pp. 459–468, May 2009.

[68] G. Singh, P. Vajpayee, N. Rani, I. D. Amoah, T. A. Stenström, and R. Shanker, “Exploring the potential reservoirs of non specific TEM beta lactamase (blaTEM) gene in the Indo-Gangetic region: A risk assessment approach to predict health hazards,” *J. Hazard. Mater.*, vol. 314, pp. 121–128, Aug. 2016.

[69] C. C. I, S. N. Ibe, and G. C. Okpokwasili, “Detection of bla SHV and bla TEM beta-lactamase genes in multi-resistant Pseudomonas isolates from environmental sources,” *African J. Microbiol. Res.*, vol. 5, no. 15, 2067.

[70] C. Cátia *et al.*, “Presence of BlaCTX-M and BlaTEM among environmental Enterobacteriaceae isolated from the São Domingos River, Peniche, Portugal,” *Front. Mar. Sci.*, vol. 3, 2016.

[71] B. S. Lopes, L. Gallego, and S. G. B. Amyes, “Multi-drug resistance profiles and the genetic features of Acinetobacter baumannii isolates from Bolivia,” *J. Infect. Dev. Ctries.*, vol. 7, no. 4, pp. 323–328, Apr. 2013.

[72] C. Narciso-Da-Rocha, A. R. Varela, T. Schwartz, O. C. Nunes, and C. M. Manaia, “BlaTEM and vanA as indicator genes of antibiotic resistance contamination in a hospital-urban wastewater treatment plant system,” *J. Glob. Antimicrob. Resist.*, vol. 2, no. 4, pp. 309–315, Dec. 2014.

[73] N. Devarajan *et al.*, “Occurrence of antibiotic resistance genes and bacterial markers in a tropical river receiving hospital and urban wastewaters,” *PLoS One*, vol. 11, no. 2, Feb. 2016.

[74] S. Walia, C. Murleedharn, J. Band, M. Kanwar, and A. Kumar, “Quantitation of antibiotic resistance genes pollution in hospital waste water effluent and Urban Clinton River Water, Michigan, USA,” *Curr. Med. Res. Pract.*, vol. 6, no. 4, pp. 149–151, Jul. 2016.

[75] L. . Ruzer and N. Harley, Eds., *Aerosols Handbook: Measurement, Dosimetry, and Health Effects, Second Edition*. CRC Press, 2013.

[76] R. Mutzner, S. V. Weijs, P. Tarolli, M. Calaf, H. J. Oldroyd, and M. B. Parlange, “Controls on the diurnal streamflow cycles in two subbasins of an alpine headwater catchment,” *Water Resour. Res.*, vol. 51, no. 5, pp. 3403–3418, May 2015.

[77] U. E. P. A. (EPA), “Project Summary: Hourly Diurnal Flow Variations in Publicly-Owned Wastewater Treatment Facilities,” 1981.

[78] A. M. Jones and R. M. Harrison, “The effects of meteorological factors on atmospheric bioaerosol concentrations - A review,” *Science of the Total Environment*, vol. 326, no. 1–3. Elsevier, pp. 151–180, 29-Jun-2004.

[79] F. Gassmann, “Investigation of Meteorological Elements around La Paz and Oruro Sustainable Energy Technology A Project of the Investigation of Meteorological Elements around La Paz and Oruro,” 2000.

[80] A. Wiedensohler *et al.*, “Black carbon emission and transport mechanisms to the free troposphere at the La Paz/El Alto (Bolivia) metropolitan area based on the Day of Census (2012),” *Atmos. Environ.*, vol. 194, pp. 158–169, Dec. 2018.