

Practical Monitoring Strategies for Drinking Water and Bioaerosols in Resource-Limited Settings

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

The main goal of this dissertation was to develop practical water and bioaerosol monitoring strategies for resource limited settings. This goal was established because there are ~2.2 billion people who lack access to safe drinking water services, ~4.6 billion without access to safe sanitation services, and, in February 2021, more than 95% of the world population was susceptible to COVID-19 infection. Herein, widely available drinking water quality testing technologies were used to develop a scalable methodology to standardize water quality monitoring in cities. Passive and active aerosol sampling methods were optimized to facilitate bioaerosol monitoring near open wastewater canals in cities with poor sanitation. Stochastic mathematical models were used to estimate the risk of infection, illness, and mortality posed by bioaerosols near open wastewater canals, translating monitoring data into potential health outcomes. Lastly, a stochastic mathematical model was developed and converted into a web-application to facilitate the understanding of long-range aerosol transmission of COVID-19 indoors. It was demonstrated that sampling drinking water at the point of consumption provided a more accurate characterization of water safety than access to a type of infrastructure. Passive aerosol sampling can provide quantitative fecal coliform data in low-resource settings, and active aerosol sampling allowed the collection of pathogen-specific data to identify which pathogens may pose an exposure risk. Risk models indicated that aerosolized fecal bacterial pathogens present non-negligible risks of infection in La Paz, Bolivia, warranting future longitudinal investigations. The risk assessment application that was developed and applied for both assessment of exposure to fecal bioaerosols and SARS-CoV-2 contributed to the understanding of aerosol transmission of infectious diseases.

Dedication

This work is dedicated to:

My parents, Inés Melogno and Juan José Rocha, who sold their house to support my brother's and my undergraduate studies, gifting us the best possible inheritance.

My wife and best friend, Laura Naslund, who supported me throughout my PhD journey and taught me R, but most importantly, reminded me that faith can be an integral part of a scientist's life.

My brother, Sebastián, who taught me that there's more to life than academic or professional success, to cherish the time we have with family and friends as we never know when that time will be gone.

My scientific mentors and friends, particularly in the aerosol science and risk assessment community, with whom we worked together in 2020 to provide information about aerosol transmission of diseases, and practical tools to address the COVID-19 pandemic.

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Acknowledgements

I am thankful for the incredible support my friends and colleagues at Duke and abroad gave me during my doctoral studies. Particularly, my PhD cohort with whom we studied for the PhD preliminary examination and graduate classes. Doing a PhD is hard and lonely in most cases. Your friendship made the journey enjoyable, and I am forever in debt with you.

To my advisor, Marc Deshusses, for giving me the opportunity to study relevant water and sanitation challenges. Your empathy, guidance and rigorous training pushed me to get out of my comfort zone and become a researcher that sees opportunities instead of barriers when faced with resource constraints.

To my scientific mentors, Joe Brown, Mike Bergin and Greg Gray, for your invaluable insights and supporting my research with equipment, methodological guidance and the opportunity to investigate the aerosol transmission pathway of COVID-19. The opportunity to work in this topic pushed me to thoroughly understand aerosol science, opening windows of collaboration and contribution during a critical time for humanity.

To the US and Bolivian undergraduate students who dedicated time and effort in collecting water and bioaerosol samples with me during multiple trips to Bolivia. Your commitment to our projects showed me true passion and intellectual curiosity.

Finally, to the elementary, middle and high school teachers who dedicated their careers to educate me and many others. It is through those 12 years in school that I learned the value of perseverance, getting me through countless challenges during my PhD career.

1. Introduction

Pursuing universal access to safe drinking water and sanitation has been a continuous challenge for the nations of the world as human populations keep growing and becoming urbanized. In fact, it is estimated that by 2050 there will be 9.8 billion people living in the world with 70% living in cities (United Nations, 2017, 2014). By 2015, 71% of the world population had access to safe drinking water services which involve access to water on-site, available when needed and free of contamination. Only 39% of the global population had access to safely managed sanitation services: contained and treated excreta either on-site or elsewhere (WHO/UNICEF, 2017). In this light, the UN established the Sustainable Development Goal 6 (SDG 6): Water and Sanitation for All, to be achieved by 2030 (United Nations, 2016a). This ambitious goal was set due to the impact that access to these services has on public health (Lim et al., 2012; Prüss-Üstün et al., 2008) and its impact in other aspects of sustainable development (Hutton and Haller, 2004; Mara et al., 2010).

One of the efforts to achieve SDG 6 is the monitoring of water quality at scale. Microbial water quality, particularly, is one of the main concerns because of its immediate impact on human health as the presence of human pathogens in drinking or recreational waters and in uncontained human waste puts the population at risk of diarrheal disease (Mara et al., 2010; Prüss-Ustün et al., 2014). Because of this, different microbial indicators have been widely used and are recommended by regulatory agencies for monitoring efforts (Gruber et al., 2014; Moe et al., 1991; National Research Council, 2004; WHO, 2017a).

The possibility to monitor water quality with a scientific approach efficiently could help local authorities and utility companies prioritize their efforts based on robust data. However, previous drinking water assessment methodologies were developed prior to SDG 6, using definitions of water and sanitation access in the millennium development goals' framework that relied heavily on water and sanitation infrastructure type, not services (WHO/UNICEF, 2012). Therefore, there is need for data collection methods with updated terminologies to characterize the level of water and sanitation services in a community, and synthesized techniques to collect water safety data. The second chapter of this dissertation focuses on addressing these needs by providing a monitoring strategy to collect basic water quality data in cities.

The third and fourth chapter of this dissertation focus on fecal bioaerosol sampling optimization techniques, and risk assessment tools to understand their role in disease burden in cities with poor sanitation. Unsafe sanitation services enable the transmission on enteric diseases through direct contact with waste, contaminated food and water, fomites, and flies (Wagner and Lanoix, 1958). These pathways have been characterized and studied globally, leading to a large body of literature assessing the impact of water, sanitation and hygiene interventions in reducing the burden of diarrheal disease (Fewtrell et al., 2005). However, few studies have looked at the direct or indirect role of the aerosol transmission pathway in places with poor sanitation. Therefore, we aimed at characterizing this disease transmission pathway by combining culture and molecular methods for pathogen detection with quantitative microbial risk assessment techniques.

The fifth and final chapter of this dissertation was added in light of the COVID-19 pandemic. In the medical community, respiratory pathogen transmission is commonly categorized as contact, droplet, and airborne (Jones and Brosseau, 2015). However, the medical and aerosol science community have had a continuous scientific debate over the definition of airborne transmission, aerosols, and droplets, especially when viral pandemics occur (Tang et al., 2021). These unresolved definitions have limited effective risk mitigation strategies to overcome the COVID-19 pandemic. Therefore, a cut-off between aerosols and droplets at 100 μm in size was proposed in 2020 because it effectively distinguishes their aerodynamic behavior, exposure route (e.g. inhalation vs. deposition onto mucosa), and the impact of mitigatory measures (Prather et al., 2020a). This suggested cut-off and the occurrence of multiple COVID-19 outbreaks in indoor settings beyond 2 m of distance from an index case (i.e., the first identified infected person) (Tang et al., 2021) have warranted a revision of the definition of airborne transmission in the medical community.

While there is little direct evidence of transmission of COVID-19 through any route (contact/fomite, ballistic droplets, or aerosol inhalation), multiple reports have found molecular evidence and viable SARS-CoV-2 in aerosols (<100 μm in size) (Binder et al., 2020; Chia et al., 2020; Lednicky et al., 2021, 2020). In this light, Chapter 5 presents a practical risk assessment tool and assessment of hypothetical scenarios focusing on aerosol transmission of COVID-19 indoors. In this introductory chapter, I present the state of the art of *E. coli* testing in drinking water, followed by a review of bioaerosol science focusing primarily on enteric pathogens to lay the foundations of my work in this dissertation.

1.1 Indicators of microbial safety in water

Indicator bacteria such as total coliform and fecal coliform have been used for decades to assess microbial water safety in temperate climates (Moe et al., 1991). However, the behavior of these microorganisms depends on the environmental conditions where they are. In example, some can live in low nutrient conditions because of their slow metabolism and others require significant amounts of nutrients to thrive (Madigan and Brock, 2012). Many low and middle income countries have a tropical climate, with nutrient-rich waters at temperatures ranging 21-31°C which can have non-fecal thermotolerant microorganisms that lead to false-positive detection of fecal coliforms (Moe et al., 1991). At the same time, few of these countries have safely managed sanitation services (WHO/UNICEF, 2017), consequently releasing high concentrations of fecal microorganisms into the environment. Facing these challenges, researchers and regulatory agencies had to refine the approach to determine microbial water safety with more accuracy (WHO/UNICEF, 2017, 2012).

E. coli is a commonly used indicator of fecal contamination (WHO, 2017a). This microorganism lives in warm-blooded animals but only a few *E. coli* types are pathogenic, “such as the verotoxigenic (VTEC) (Taylor, 2008) enterohaemorrhagic (EHEC, a subclass of the VTEC class), enteroinvasive (EIEC) and uropathogenic/extraintestinal pathogenic (UPEC/ExPEC) classes” (van Elsas et al., 2011). The most common example of EHEC *E. coli* is the strain 0157:H7 which has been linked to several diarrheal disease outbreaks in food and in water (Ishii and Sadowsky, 2008). While these strains of *E. coli* cause diarrheal disease, they are not the only pathogens of concern.

1.2 Pathogens associated with fecal contamination and enteric disease

Enteric disease is the result from infection from either bacterial, viral or parasitic organisms. Some bacterial model organisms found in water besides *E. coli* include *Vibrio cholerae*, responsible for secretory diarrhea; *Clostridium difficile* and *Shigella* spp., associated with inflammatory diarrhea (Hodges and Gill, 2010). Invasive species include *Salmonella* spp. and *Campylobacter* spp. which can cause fever and abdominal cramps in addition to inflammatory diarrhea. While *Salmonella* spp. and *Campylobacter* spp. are usually regarded as foodborne pathogens, they can be found in drinking water and in raw sewage as well (Levantesi et al., 2012).

Of increasing concern, viral pathogens such as rotavirus, norovirus, adenovirus, astrovirus, hepatitis A and E viruses have been identified as the cause of diarrheal disease, being in much higher concentrations than bacteria (WHO, 2017a). These viruses have also been associated with encephalitis, meningitis, myocarditis, cancer, and hepatitis (Gall et al., 2015; WHO, 2017a). Furthermore, while bacterial populations decrease in a matter of days to weeks, enteric viruses can remain viable for months (WHO, 2017a), they are harder to detect and test their viability (Gall et al., 2015).

Two common model parasitic organisms are *Giardia* spp. and *Cryptosporidium* spp., the latter having caused the largest recognized waterborne outbreak of enteric disease in the United States with 285 laboratory-confirmed cases of cryptosporidiosis (MacKenzie et al., 1994). *Cryptosporidium* oocysts were found in water from ice for consumption during the time the city's water treatment plant was shut down, finding concentrations >100 higher than commonly detected (MacKenzie et al., 1994). *Giardia*

lamblia, on the other hand, is the most common and prevalent parasite in children from the developing world (Bryan et al., 1994), being more frequently found in urbanized populations than rural (Fraser, 1994). The lack of continuous monitoring and available practical methods of detection have limited the prevention and early warning of contamination (Marshall et al., 1997), contributing to the global burden of diarrheal disease.

While bacterial, viral and parasitic model organisms have been studied for years, emerging pathogens continue to be found and new public health concerns have appeared e.g. antibiotic resistance development (Singer et al., 2016). Because of this, frequent monitoring of these organisms is needed to evaluate if systems in place are working as designed or if optimization is needed to reduce the risk of exposure.

1.3 Why *E. coli* as model indicator of microbial water safety?

E. coli is a gram-negative chemoheterotroph from the fecal coliform group (Ishii and Sadowsky, 2008; van Elsas et al., 2011) that has lived in both warm-blooded animals and the environment continuously. Exposure to low pH in its hosts or highly variable temperature profiles in the environment made *E. coli* able to survive under stress (Ishii and Sadowsky, 2008; van Elsas et al., 2011). This biphasic lifestyle pushed the microorganism to live without a host (van Elsas et al., 2011). In fact, *E. coli* has been found in soil, sand, sediment, and algae in tropical (Byappanahalli and Fujioka, 2004, 1998; Carrillo et al., 1985), subtropical (Desmarais et al., 2002; Solo-Gabriele et al., 2000) and temperate climates (Byappanahalli et al., 2006; Ishii and Sadowsky, 2008). These characteristics made *E. coli* the focus of numerous reviews about the

organism's pathogenesis, origin and diagnostic (Kaper et al., 2004; Mainil and Daube, 2005; Nataro and Kaper, 1998; Paton and Paton, 1998).

While *E. coli* has proven to be a highly adaptive organism, other bacteria may have similar capabilities. For example, EIEC has genetic, biochemical and pathogenic similarities with *Shigella* (Ishii and Sadowsky, 2008; Nataro and Kaper, 1998; Pupo et al., 1997). Similarly, *P. aeruginosa* was found to be the closest relative to *E. coli* (Stover et al., 2000). *P. aeruginosa* is an opportunistic pathogen capable of resisting several front-line antibiotics (Hancock, 1998). Knowing that there are mechanisms of gene transfer between microorganisms, it is possible that this antibiotic resistance capabilities can be acquired by human pathogens (Berendonk et al., 2015). This is a clear limitation to the use of *E. coli* as model organism for indication of safety for other bacteria, viruses or protozoa. However, testing for *E. coli* has become simple and possible worldwide (Edberg et al., 2000) while testing for viruses and protozoa requires expensive equipment and technical skills that make it harder to include in regular monitoring efforts. As technologies become technically and financially accessible, pathogen-specific monitoring should be the standard. That said, this ideal scenario may not be possible during the SDG 6 timeline. Therefore, sticking to *E. coli* testing as part of routinely monitoring may help low and middle income countries better track their progress towards SDG 6.

1.4 State-of-the-art of practical *E. coli* detection technologies

The WHO guidelines for drinking water recommend frequent testing by simple methods compared to less frequent complex methods, as microbial contamination varies

with meteorological conditions as well as pressure continuity in piped systems (Bivins et al., 2017; U.S. EPA, 2018; WHO, 2017a). Multiple methods are available to detect *E. coli* (e.g. molecular amplification, genome-targeting, and culture-based techniques) (WHO, 2017a). However, many of these methods are not being used extensively in less developed countries because of regulatory constraints or their cost (Bain et al., 2015). Culture mediums that target specific enzymes are particularly advantageous due to their simplicity and time efficiency (Abramson et al., 2013). They are also highly specific for *E. coli* (Bain et al., 2015), making them ideal in contexts where confirmation steps through molecular methods are cost-prohibitive or not available.

Enzyme-based approved *E. coli* testing methods are currently limited to few options. The most common are manufactured by IDEXX: Colilert-18[®] and Colilert[®], and while broadly available, they have limitations, i.e., patent protected tests, confirmatory tests' incubation and work time (Bain et al., 2015; Olstadt et al., 2007). This lead Bain and his collaborators to develop a novel low-cost medium named Aquatest, used for plate counts of colony forming units (CFU) or presence/absence detection through color change in the liquid medium. They validated the medium against Colilert-18[®], having significantly higher sensitivity in temperate and sub-tropical climates (98.0% : $p < 0.0001$; 99.5%: $p = 0.0030$, respectively) and similar specificity (>95%) (Bain et al., 2015).

These results were confirmed in tropical climates in 2020, and included an additional test called Compact Dry[™]. Both mediums were tested against MI Agar as the standard test. Aquatest had a sensitivity and specificity of 97% and 96%, respectively, when incubated at 37 °C for 24 h, and 97% for both sensitivity and specificity when incubated at ambient temperature (27 °C) and incubated for 48 h. Compact Dry[™] had a

sensitivity and specificity of 99% and 97%, respectively (Brown et al., 2020). These easy to use and reliable tests may become the ideal alternative during this decade, especially for Aquatest given its open source development, being free to be manufactured anywhere (Bain et al., 2015). Extensive efforts to promote its use outside of academia are needed, and future research should look into developing similar tests for viruses and protozoa.

1.5 Bioaerosol research: studying the invisible drivers of disease

Research on the fecal-oral disease transmission from unsafe water consumption, poor sanitation and hygiene is abundant. Even so, the aeromicrobiological exposure pathway to enteric pathogens and associated risk of infection is scarce in the literature. It has been shown that concentrated sources of fecal matter can release biological aerosols (bioaerosols) through different mechanisms, e.g., wind erosion or mechanical disturbance (Delort and Amato, 2018; Farling et al., 2019; Paez-Rubio et al., 2005). These bioaerosols may be transported and pose an exposure risk and cause infectious diseases. However, most of the research has been conducted in high income countries and in relatively isolated contexts, such as wastewater treatment plants, agricultural use of biosolids or in animal farms; places where personnel work close to animal and human waste (Baertsch et al., 2007; Jahne et al., 2015; Schaeffer et al., 2017; Uhrbrand et al., 2011). However, the belief that air played a role in enteric disease transmission is not recent, going back to the 19th century. I present here a literature review of bioaerosol research to lay the base in which my work was developed, found on the third, fourth and fifth chapter of this dissertation.

The “miasma theory” was a predominant belief in the 19th century that suggested that disease transmission happened through “poor air”. This theory stated that smell from any waste could carry diseases through the air. For example, people that lived near swamps were thought to be particularly at risk of illness from the gases emitted from them (Bloom, 1965). The theory was challenged when a cholera outbreak took place in London, England in 1854.

John Snow, a physician, noticed that most disease cases had happened near a drinking water pump. He mapped out the cases and presented his findings to the authorities, who proceeded to ban the pump’s use. The cholera cases began to decrease and the outbreak was under control (Snow, 1855). Snow’s hypothesis was later confirmed through the “germ theory”. Robert Koch discovered that the bacillus *Vibrio cholerae* was the organism that caused the disease, almost 30 years after Snow passed away (Hill, 1965).

While the Miasma Theory was rightfully discredited then, the field of aerobiology was not. This field studies the aerosolization, fate and transport of bioaerosols in the environment (Pepper and Dowd, 2009). Bioaerosols range in aerodynamic diameters of 1 nm to 100 µm and originate from plants, animals (Georgakopoulos et al., 2009) and microorganisms. The latter can be viable or nonviable bacteria, fungi and viruses (nucleic acids in a protein coat), and travel long distances due to their size and light weight (Van Leuken et al., 2016).

The belief that the environment influences our health has been around since the times of Hippocrates (600 B.C), but the 20th century markedly brought light into the science of disease transmission. Alexander Langmuir primarily contributed through his

studies to the better understanding of airborne disease transmission since the 1940's (Eickhoff, 1996). In a similar way, Wells challenged the belief that air did not represent an important pathway for disease transmission (Wells, 1948). Wells then joined other researchers to look into respiratory diseases in military camps (Bourdillon, 1948; Robertson, 1947). These researchers challenged the theory that disease transmission only happened through direct contact with few exceptions such as tuberculosis, suggested by Chapin in 1910 (Riley, 1982).

Most respiratory diseases and ways to prevent them were unknown at the time Langmuir began his studies in 1943 (Willmon et al., 1948). The United States government had established the Commission on Acute Respiratory Diseases, where Langmuir worked as an epidemiologist at Fort Bragg, North Carolina (Eickhoff, 1996). His studies sought to determine the cause of acute respiratory diseases (Dingle and Langmuir, 1968). He noticed that new personnel were at higher risk of illness compared to those who had been there for a longer period of time. In the absence of evidence suggesting that water, food or vectors were the reason of these diseases, he hypothesized that sneeze droplets may have been the source of pathogens (Eickhoff, 1996).

Langmuir conducted a series of intervention studies to test this hypothesis. One study looked at double bunking the barracks and increase the space between beds to reduce close contact (Commission on Acute Respiratory Diseases, 1946). The study found a decrease in the incidence rate compared to the control barracks. Another study looked at the impact of UV radiation and dust suppressive measures (Willmon et al.,

1948) but had inconclusive results that led the authors to suggest that contact remained the main cause of respiratory diseases.

Not discarding the idea that disease could be spread through the air, Langmuir began directing the institution known today as the Center for Disease Control and Prevention in 1949 (Eickhoff, 1996). He pushed for laboratory studies of aerosolized pathogens' fate and transport, pulmonary immune responses to deposited aerosols in the respiratory system, animal and human infection trials (Riley et al., 1959) and further epidemiological studies. For the following four decades, the United States spearheaded the discoveries of multiple airborne pathogens causing infectious diseases i.e., measles, anthrax and pulmonary tuberculosis (Eickhoff, 1996). Langmuir and others had shown that airborne disease transmission was indeed possible (Langmuir, 1980).

The field of bioaerosol science and technology has grown rapidly since then, with the availability of new molecular techniques as well as devices that can measure bioaerosol concentrations in real-time at high volume samples and off the grid. Events such as the 2003 severe acute respiratory syndrome (SARS) outbreak, the 2009 H1N1 flu pandemic, the 2019 Middle East respiratory syndrome coronavirus (MERS-CoV) and the most recent COVID-19 pandemic have raised concern globally, calling for stronger monitoring efforts to prevent and react effectively to these events. With the ongoing global sanitation crisis, the threat of antibiotic resistance and the emergence of new zoonotic diseases, the study of bioaerosols plays a key role in identifying and assessing these global health threats.

1.6 Evidence for aerosolized enteric microorganisms from wastewater

While the scientific evidence for respiratory disease-causing pathogens is large, aerosolized enteric pathogens have only been studied in recent decades. Researchers have suggested that infection happens from bioaerosol deposition in the upper respiratory tract (>10 µm in size), passing to the digestive tract by ciliary action through the pharynx (Peccia et al., 2008). A large portion of published studies have shown that concentrated fecal matter can release bioaerosols that remain viable and potentially infectious (Gerba and Smith, 2005).

A higher incidence of enteric diseases in WWTP workers has been found in a series of WWTPs, compared to control working environments (Khuder et al., 1998; Rylander, 1999; Thorn et al., 2002). Rylander suggested that the higher incidence of enteric diseases was associated with exposure to endotoxins while others have suggested the potential for aerosolized bacteria and viruses being the cause of the disease cases (Clark, 1987; Lundholm and Rylander, 1983). Based on these findings, Uhrbrand et al. evaluated exposure to airborne noroviruses and other bioaerosols in a wastewater treatment plant in Denmark (Uhrbrand et al., 2011) and found aerosolized Noroviruses, Adenoviruses, endotoxins, molds and bacteria as the potential infectious agents. Their study included personal exposure active sampling (taking air samples using pumps and filters carried by the personnel) and taking stationary dust samples over the course of one day. They found that bacteria and mold concentrations were below occupational exposure limits. However, the endotoxin exposure concentrations for 1 out of 16 workers were at levels of concern and norovirus concentrations were above

infectious dose levels (Uhrbrand et al., 2011). While this was an original investigation at the time, the small sample size and short time-frame during which the sampling event happened raise concern regarding their findings' generalizability.

In a similar study, others sampled for bioaerosols at a wastewater irrigated site in Baja California, México (Paez-Rubio et al., 2005). Irrigation with untreated wastewater is a common practice in low and middle income countries, with an estimated domestic wastewater reuse of 80% (Cooper, 1991). Paez-Rubio and her collaborators sampled bioaerosols during low and high-speed wind events at an agricultural piece of land growing Bermuda grass that was irrigated with the effluent of an anaerobic lagoon treating domestic wastewater. The water had fecal coliform concentrations >1000 Colony Forming Units (CFU)/mL (100 times higher than the WHO guideline of 10 CFU/mL for the use of wastewater in agriculture and aquaculture (WHO, 1989)). Their objective was to assess the impact from environmental conditions on the concentration and culturability of bioaerosols. They took stationary air samples using glass liquid impingers throughout the day over the course of three days, facing the irrigated field. They also used a bioaerosol concentrator to develop a phylogenetic library which would allow her to match the air samples with the water samples and assess if the bioaerosols did in fact originated from the water. Their viability and molecular analyses suggested that high-speed events can aerosolize waterborne microorganisms. However, they did not find a relationship between CFU concentrations and solar radiation, which has been associated to bioaerosol inactivation (Tong and Lighthart, 1997). Nevertheless, their findings suggest that bioaerosols could be emitted from standing water bodies in high wind speed events, even in absence of mechanical aeration.

Fast-forward a decade, Perring et al. published a study where they used a zeppelin to sample fluorescent aerosols (as a surrogate for bioaerosols) across the United States. They found higher fluorescent particle concentrations in warm marine environments, having similar observations to Paez-Rubio from aerosols near standing water bodies (Perring et al., 2015). Their model was able to predict concentrations in the eastern United States accurately but underestimated those observed in the west. While their study did not focus on public health, it provided evidence that bioaerosols represent a significant portion of airborne particles in arid and humid environments alike. However, their presence in multiple environments cannot be assumed to be the case for all harmful bioaerosols, as pathogens have unique responses to different environmental stressors and sampling mechanisms (Haig et al., 2016).

Courault most recently combined field measurements, atmospheric dispersion modelling and Quantitative Microbial Risk Assessment (QMRA) to provide a fuller picture of how enteric viruses are emitted from wastewater being reused for irrigation (Courault et al., 2017). The sampling events took place over the course of one day on different days in 2014 and 2015 for a total of seven sampling events. Although the authors recognized not having enough samples for statistical analyses, dispersion models using this limited data could lead to wrong estimations of the fate and transport of bioaerosols. This limitation was addressed in their risk assessment by using a Bayesian approach where they incorporated the variability and uncertainty of bioaerosol atmospheric dispersion. That said, current technologies cannot assess the infectivity of non-culturable viruses which could also lead to the overestimation of risk posed by viruses measured through current molecular methods. Despite these limitations, such interdisciplinary

approach is likely to yield more useful information for policy-makers and environmental regulators than pathogen detection or quantification alone.

1.7 Evidence on aerosolized enteric microorganisms from animal waste

Brooks assessed bioaerosol emission from land application of biosolids in his doctoral dissertation from 2004. Through an experimentally-derived transport model, he estimated low viral infection risk estimates ($<7 \times 10^{-6}$, seven infections per million people at exposure) at the households closest to land applications of biosolids (Brooks et al., 2005a). In that same study, they noticed that gram-negative bacteria were inactivated faster than viruses, as bacterial concentrations were below detection limits at >20 m from the source. Later, they conducted a larger study sampling bioaerosols in 10 sites across the United States (Brooks et al., 2005b). While the annual probability of infection of e.g., coxsackievirus A21 was of 1×10^{-4} infections/personal exposure events at 30.5 m from the source, he estimated that the risk of infection of the operators could be as high as 34×10^{-2} .

Schaeffer et al. conducted a thorough characterization of aerosols emitted from dairy farms in Colorado, US in 2017. They sampled at three dairies with more than 1000 lactating cows for 12 days, throughout the four seasons of the year. Their objective was to assess the physical, chemical and biological characteristics of the emitted airborne particles in the range of 0-100 μm in size. They noticed a bimodal distribution in bioaerosols sizes at 3 μm and at > 30 μm in diameter and found correlations with multiple microbial markers, e.g., endotoxins and muramic acid. Their molecular analysis identified the genera *Staphylococcus*, *Pseudomonas*, and *Streptococcus*, all of which

have pathogenic strains and proinflammatory characteristics (Schaeffer et al., 2017). That said, the authors acknowledge that they did not test for bacterial viability, which is an indicator of potential infectivity. While this was a limitation of their study, their findings suggest that enteric disease-causing pathogens can become airborne and travel in aerosol particles with sizes $>10\ \mu\text{m}$. This is of particular concern regarding enteric pathogens as these particles have a high likelihood to deposit in the upper respiratory tract.

Jahne and collaborators investigated the emission and dispersion of bioaerosols in places where manure is applied in agricultural land (Jahne et al., 2015). Their objective was to use field bioaerosol measurements in combination with QMRA to estimate the risk of infection downwind of land-applied manure sites. Jahne et al. used field data and molecular analyses in a dispersion model to estimate fecal bacteria gene copies' transport in the air, using *Enterococcus* spp. as their indicator organism. Their study found that viable bacterial organisms were associated with coarse particles with diameters $>2.1\ \mu\text{m}$, which can deposit in the respiratory tract (Jahne et al., 2015) but also reach the deep lungs (Wark et al., 1998). They also found *Campylobacter* and *E. coli* strains through qPCR but did not detect *E. coli* O157:H7 or *Salmonella*, known for their pathogenic capabilities. However, their QMRA estimated an infection risk of 4×10^{-4} infections/personal exposure events at 1000 m downwind in an 8-h exposure period. These findings strengthen the argument that aerosolized enteric pathogens represent a public health concern but seem to disagree with Brooks' observations (Brooks et al., 2005a).

1.8 Evidence on aerosolized enteric microorganisms in poor sanitation contexts

The concept of bioaerosols being emitted from human waste is not recent. Gerba et al. demonstrated in 1975 that high concentrations of bacteria and viruses remained in toilets after numerous flushing events. In addition, droplets emitted from flushing were found to carry these organisms suggesting that they remain airborne and settle onto surfaces in bathrooms, posing a risk of infection (Gerba et al., 1975). While the obvious solution would be to “just put down the seat”, it is a solution that only applies to the individuals that have access to western-style toilets.

Farling and collaborators published the first study looking into bioaerosol emissions associated to pit latrine emptying operations (Farling et al., 2019). Pit latrines are one of the on-site sanitation alternatives to the typical flush toilet and the most used worldwide (Berendes et al., 2017). Their objective was to assess bioaerosol emission during pit emptying in Blantyre, Malawi – a peri-urban informal settlement. They sampled bioaerosols for a week in December of 2017, before, during and after pit emptying operations and at different distances from the latrines. They used an active sampling device with a flow-rate of 100 L/min, and a highly specific growth medium (MI Agar) to detect viable aerosolized *E. coli*. They also took sludge samples for further molecular analysis back in the US, finding total coliforms in all of the collected samples and airborne *E. coli* in background samples and during pit emptying activities.

Molecular analyses were positive for enterotoxigenic *E. coli* in 8 out of 46 air samples. While they did not detect other enteric pathogens, their findings suggested that enteric pathogens can be aerosolized in poor sanitation settings and related activities

(Farling et al., 2019). This was the only study published prior to our work looking at bioaerosols in poor sanitation settings.

1.9 Knowledge gaps and future concerns

There is a growing body of literature showing that human pathogens can become aerosolized from concentrated animal and human waste, yet our understanding of their fate and transport in urban environments remains on early stages. Emerging public health concerns such as the COVID-19 pandemic, and antibiotic resistance development have caught the attention of researchers and epidemiologists (Ventola, 2015) and led to hypothesize that air may be playing a bigger role that is currently overlooked e.g., by the emphasis of surface disinfection over the improvement of ventilation systems to reduce respiratory disease transmission (Pitol and Julian, 2021; Tang et al., 2021). The recent COVID-19 pandemic and the efforts to harmonize our current understanding of respiratory disease transmission (Prather et al., 2020a) support the position that the aerobiological pathway is a relevant exposure route to health-threatening bioaerosols in both low- and high-income countries. While Snow's findings boosted the now abundant theory of how pathogens infect people through water (Cabral, 2010), the literature regarding the aerobiological exposure to pathogens in poor sanitation settings remains in early stages (Clasen and Kirk, 2019).

Considering that the citizens in cities with poor sanitation are broadly exposed to a variety of fecal pathogens through multiple pathways, e.g., fluids, fingers, flies, floors or food (Wagner and Lanoix, 1958), it is paramount to understand fecal pathogen transport and exposure routes in order to minimize disease transmission. Moreover,

cities with rapid urban growth will face unprecedented challenges in waste management, potentially causing an increase in diarrheal diseases that could create lifetime health deficits (Neiderud, 2015). At the same time, while unsafe sanitation is not directly related to lower respiratory infections - the leading cause of mortality in low-income countries (WHO, 2018) – it may lead to diarrheal events which weaken the subject and may make them susceptible to lower respiratory infections, as observed before (Schmidt et al., 2009). In light of the COVID-19 pandemic, it is not known if SARS-CoV-2 could spread through fecal bioaerosols and potentially contribute to the burden of disease in places with poor sanitation. These knowledge gaps and the most recent estimates of animal and human waste's global generation reaching 4.6×10^{12} kg per year in 2030 (Berendes et al., 2017) call for a One Health approach (AVMA, 2008) to address sanitation-related and zoonotic diseases in a multidisciplinary way. The development of practical monitoring methods as well as risk assessment frameworks could help prevent outbreaks of infectious diseases, contributing to the scientific literature and becoming routinely tools for realistic and comparable evaluations.

1.10 Dissertation goals, leading hypotheses and justification

The main goal of this dissertation was to develop practical water and bioaerosol monitoring strategies for deployment in resource limited settings. Four aims were established to achieve this goal:

Aim 1. Pilot a scalable method for rapid drinking water quality estimation (Ch. 2)

Hypothesis: If drinking water testing techniques are widely available, then a standardized data collection methodology at city-scale will be technically and logistically feasible.

Justification: Robust and comparable data on drinking water quality is not currently available at city-scale around the world. Thus, a standardized, representative, and rapid urban water quality assessment methodology is needed to develop evidence-based solutions to achieve the Sustainable Development Goal (SDG) 6: “safe water for all”.

Aim 2. Optimize a bioaerosol sampling strategy, to assess the exposure to enteric and respiratory pathogens in cities with poor sanitation (Ch. 3)

Hypothesis: If poor sanitation is linked to the presence of enteric pathogens, then bioaerosols will play a role in the exposure to human pathogens in cities with poor sanitation and need to be monitored.

Justification: Studies on the aeromicrobiological exposure pathway to enteric pathogens and associated risk of infection are scarce in the literature. It has been shown that concentrated sources of fecal matter release bioaerosols through different mechanisms, resulting in infectious diseases. Characterizing bioaerosols' fate and transport combining culture and molecular methods would provide further insights on this exposure pathway in places without safe sanitation services. The optimization of bioaerosol sampling strategies in such context would contribute to better monitoring efforts.

Aim 3. Develop a Quantitative Microbial Risk Assessment (QMRA) framework for aerosolized pathogens in cities with poor sanitation (Ch. 4)

Hypothesis: If aerosolized pathogens can be detected near open waste canals, then they will contribute to the burden of disease from unsafe human waste management.

Justification: There is need for robust tools to assess the risk of infectious diseases caused by poor sanitation. While monitoring methodologies and technologies have emerged in recent decades to prevent outbreaks, the interpretation of their findings is

limited to reporting pathogen's presence/absence or concentrations without providing details on what that could mean in terms of public health outcomes. QMRA puts monitoring data into context by 1) identifying the hazards, 2) assessing the exposure routes 3) choosing the right dose-response models and 4) characterizing the risk at which the population is exposed. QMRA frameworks for bioaerosols can improve monitoring efforts to prevent outbreaks and support data-driven decisions.

Aim 4. Develop a stochastic risk assessment web-application to estimate the risk of long-range aerosol transmission of COVID-19 indoors (Ch. 5)

Hypothesis: If aerosolized SARS-CoV-2 poses a risk of infection in indoor settings, then a risk assessment tool will contribute to the better understanding of this risk in retrospective and prospective evaluations.

Justification: The COVID-19 pandemic demonstrated a communication deficiency between aerosol scientists, epidemiologists and infectious disease physicians.

Bioaerosol science in 2020 indicated that respiratory pathogens, including SARS-CoV-2, mainly infect through inhalation exposure. A user-friendly web application can serve as a practical tool to facilitate the communication of this risk, and the optimization of mitigatory measures to reduce the transmission of COVID-19 indoors.

2. Rapid drinking water safety estimation in cities: piloting a globally scalable method in Cochabamba, Bolivia

This chapter presents a comprehensive methodology to monitor urban drinking water quality worldwide, with an emphasis on microbial water quality. This work was published in the journal *Science of the Total Environment* (Rocha-Melogno et al., 2019).

2.1 Introduction

2.1.1 Background and objectives

Systematically collected, scientifically credible, and comparable data regarding drinking water safety are not currently available at city-scale around the world. Thus, a standardized, representative, and rapid urban water quality assessment methodology is needed for decision makers such as government officials and commercial water suppliers to implement solutions based on robust and current data. In order to achieve the United Nations (UN) Sustainable Development Goal 6 (SDG 6) – “safe water for all” - by 2030 (United Nations, 2016a), progress in cities is key to meeting global targets, especially because estimates indicate that 70% of the world’s population (6.4 billion) will live in cities by 2050 (United Nations, 2014). Cities will face challenges in providing safe drinking water to nearly 3 billion new urban citizens due to development pressures, resource constraints and climate change impacts on local and regional hydrology, among other factors. Already, an estimated 150 million people live in cities with perennial water shortages, signifying limited water availability and access to safe water sources. This number is estimated to reach 1 billion by 2050, affecting primarily low and middle-income countries, including Bolivia (McDonald et al., 2011).

Bolivia has been the focus of multiple studies regarding access to water and sanitation (German Corporation for International Cooperation (GIZ) GmbH, 2013; Nickson and Vargas, 2002; Quick et al., 1999; Rufener et al., 2010; Sobsey et al., 2003). This country has a population of over 11 million people (World Bank, 2018), of which 93% have access to *basic drinking water services* and 53% have access to *basic sanitation services*, as defined by WHO and SDG 6 (United Nations, 2014; WHO/UNICEF, 2017). Most Bolivians currently have access to *improved water sources*, “which are those which by nature of their design and construction have the potential to deliver safe water” (WHO/UNICEF, 2017). However, the microbial and chemical safety of water from *improved water sources* vary (Bain et al., 2014a, 2014b; Onda et al., 2012; Shaheed et al., 2014; WHO/UNICEF, 2017). Furthermore, future access to clean drinking water is limited in part due to contamination from inadequate sanitation coverage and/or functionality (German Corporation for International Cooperation (GIZ) GmbH, 2013). In conjunction with SDG 6, Bolivian President Evo Morales set normative goals of meeting universal access to safe drinking water and sanitation by 2025 (Ministry of Autonomies of the Plurinational State of Bolivia, 2014). With the help of various aid projects, access to improved facilities are increasingly becoming available. However, apart from estimates on access to *improved water sources* by the United Nations International Children’s Emergency Fund (UNICEF) and the WHO Joint Monitoring Programme (JMP) for Water Supply, Sanitation and Hygiene (WHO/UNICEF, 2017), there are no current estimates on the safety of drinking water in urban Bolivia, including Cochabamba.

This pilot study is part of a broader collaboration between the World Resources Institute (WRI), the New York University (NYU) Urban Expansion Program (UEP), and

other academic partners. It intends to monitor water quality across a global representative sample of 200 cities. The UEP developed this globally representative sample of cities, which contains 4,231 free-standing urban areas in 172 countries or territories that had 100,000 or more people in 2010 (Angel et al., 2016). The 200-city sample is stratified according to world regions, city population size, and the number of cities in a country. A current goal of the UEP is to include measures of environmental quality and environmental health, including water and sanitation infrastructure and water safety in the selected cities. This will enable comparisons between cities in lesser and more developed countries, world regions, and cities of different populations (Angel et al., 2016).

Our objective was to design and implement a rapid and representative urban drinking water quality assessment of a city by analyzing microbial, physical, and chemical parameters of drinking water at point-of-consumption at a point in time. This effort was a pilot study conducted in Cochabamba, intended to demonstrate a scalable, representative, city-scale water quality assessment for use in the 200 Cities Project (Atlas of Urban Expansion, 2016). This paper describes the assessment methodology used and suggests that it is likely to provide representative data for inter-city comparisons.

2.1.2 Previous studies

As part of the JMP, UNICEF and WHO released a handbook for Rapid Assessment of Drinking Water Quality (RADWQ) in 2012 (WHO/UNICEF, 2012). RADWQ presents a broad method of collecting and analyzing drinking water quality data from *improved sources* to provide baseline water quality information useful for policy change. The method includes a microbial, physical, and chemical water quality

assessment, as well as a sanitary inspection. The water quality assessment method lists possible indicators of water quality and testing methods of each indicator. All assessments in RADWQ discuss contaminants' association with disease, drinking water treatment and distribution systems and costs. The RADWQ also outlines the logistics of planning, training, and data management. Accordingly, by following the RADWQ guidelines, stakeholders can develop appropriate interventions or investment strategies that are appropriate for each location or water quality risk (e.g., type of contaminant or behavioral patterns). This RADWQ framework has been implemented in India, Bangladesh (WHO/UNICEF, 2012), Bolivia (Rufener et al., 2010), and Kenya (Blanton et al., 2015).

Two previous RADWQ studies focused on determining the level of fecal contamination by systematically measuring total coliforms, *E. coli*, and free residual chlorine. The first study performed a RADWQ of 398 households in the Korogocho and Mukuru informal settlements – those with inadequate or non-existing infrastructure – located in Nairobi, Kenya (Blanton et al., 2015). Blanton et al. (2015) found that the total coliforms in drinking water were greater in stored water than in the water supplied to the household. Eleven percent (11 out of 96) and 32% (32 out of 97) of the household drinking water samples were found to be contaminated with *E. coli* in the Korogocho and Mukuru settlements, respectively. Even so, the authors state that the poor infrastructure of Nairobi's informal settlements was not necessarily associated with increased cholera risk, which has been reported in Kenya annually since 1971 (Blanton et al., 2015). Unfortunately, Blanton et al. (2015) do not report the technical and non-technical logistics of their study. While this study reported meaningful drinking water results, the sample size was limited and therefore was not confirmed to be representative of the

entire population. This is recognized in their paper by warning about the interpretation of their findings about the informal settlements in Nairobi (Blanton et al., 2015).

The second study analyzed 5 peri-urban (rural-urban transition zone) villages in Bolivia, three of which were located in southern Cochabamba (Rufener et al., 2010). This study analyzed 81 households and used *E. coli* as the primary indicator for fecal contamination of drinking water. Rufener et al. (2010) found that water storage and hygiene behaviors typically resulted in recontamination of household drinking water that had been treated by boiling or solar radiation (Rufener et al., 2010). Accordingly, these authors found that 35% (28 out of 81) of the households had recontamination at point-of-consumption and that 65% (34 out of 52) of the drinking vessels were contaminated with *E. coli*. Both of these studies suggest that without hygiene education, physical intervention may not be sufficient to reduce the consumption of contaminated drinking water (Blanton et al., 2015; Rufener et al., 2010).

Building on studies (Blanton et al., 2015; Rufener et al., 2010; WHO/UNICEF, 2012) following the RADWQ handbook (World Health Organization (WHO) and United Nations Children's Fund (UNICEF), 2012), we sought to develop and fully document a standardized water quality evaluation methodology for cities encompassing samples from all sources. Whereas RADWQ provides a flexible method that can be tailored to the level of depth desired and can achieve detailed results, our study proposes a standardized method producing results that are comparable across cities. Although many cities have existing data that can be used for inter-city comparisons, most cities do not, revealing the need for a standard evaluation methodology with common indicators, testing techniques, and surveys. RADWQ also aims to evaluate the water quality of *improved* sources only, in order to shed light on the different conclusions that can be

made from characterizing clean water access by water quality as opposed to source type. While our method also aims to characterize clean water access through water quality analyses, it includes samples bounded by urban limits rather than by source type, as the aim was to characterize the water quality at the city level. Our proposed standard methodology further includes details on the logistics as part of the methodology, as logistics introduce key challenges for replicability and scalability of locational evaluations. As a result, this method provides a replicable and scalable methodology that can be used as a standard for all cities in the next phase of the 200 Cities Project (Atlas of Urban Expansion, 2016).

2.2 Methods

2.2.1 Study site description

Of the 1.9 million people who live in the Department of Cochabamba, 69% live in the City of Cochabamba, which is located in central Bolivia (National Institute of Statistics of the Plurinational State of Bolivia, 2016). With an annual population growth rate of 3.6%, Cochabamba represents one of the world's most populated cities in terms of growth (United Nations, 2016b). The annual gross domestic product (GDP) per capita in Cochabamba is US \$2,645 in 2015 compared to an average of US \$4,367 per country in low- and middle-income countries (LMICs), which suggests that Cochabamba is representative of low-income cities (National Institute of Statistics of the Plurinational State of Bolivia, 2017; World Bank, 2015). Cochabamba has a high level of socioeconomic diversity, with income levels generally higher in the north and lower towards the south of the city. Similar geographical stratification by socioeconomic status is common across cities (Hu and Kaplan, 2001). Cochabamba is environmentally

representative of many tropical and subtropical cities as well, with its strongly seasonal rainfall pattern creating water quality challenges that exist in many tropical and subtropical cities worldwide (Zhang and Wang, 2008).

We first defined the area of study as the urban extent of Cochabamba, that included all contiguous built-up areas of the city circa 2013. Within this area, we selected 80 locales, each consisting of a 10-hectare circular area. The locations of these locales were determined by combining a quasi-random series of numbers known as a Halton Sequence with the XY (latitude and longitude) origins of a bounding box that encompassed the urban extent of Cochabamba (Angel et al., 2016). The main advantage associated with this spatial sampling method is that the generated set of quasi-randomly distributed points cover the area of the study more evenly than a set of points generated at random. In contrast, a true spatially random sampling process will always generate clusters of points.

The pre-selected household to be surveyed was chosen by selecting the nearest household to each locale's center point. Defining exact locations in Cochabamba by traditional methods proved difficult due to non-systematic house numbering and street naming. Therefore, the pre-selected household was identified using coordinates. Any household that fell within a 10-hectare buffer zone of the pre-selected coordinates was eligible to be surveyed if the pre-selected household could not participate. If a pre-selected household was not available or could not respond, the next household to the left of the pre-selected household was selected as the household to be sampled. If the pre-selected household resided within a multi-level building, only the household at the ground floor was approached. We surveyed the adults living in the household and their answers were assumed representative of the people living in the household.

The rationale for adopting this spatial sampling, rather than household sampling, has to do with the scope and objectives of the study. The total number of 80 locales was determined as a minimum sample size given the urban extent of Cochabamba, and the fact that this pilot study is to be replicated across a global sample of 200 cities. Similarly, the spatial sampling based on the Halton sequence provides more data on peri-urban areas, where in general, there is less information about water quality. Finally, the study intends to provide a rapid monitoring assessment, rather than a detailed view of the water quality in each city. Understandably, due to its scope and objective, there are limitations with the methodology as the sample size is relatively small and does not account for important factors such as the population density in its locale. This would require recent census data which unfortunately is not available at this level for all 200 cities. However, one of the advantages of this methodology is that it is feasible to add additional samples when budgeting and resources allow, while maintaining the even spatial distribution by simply adding additional Halton points in their sequential order. It is recommended that a minimum of 200 samples is taken for more robust data analyses in rapid assessments of water quality and to confirm the adequate sample size (WHO/UNICEF, 2012).

2.2.2 Logistics

We obtained all necessary materials such as water quality tests and surveys, as well as a review and confirmation of exclusion by the Institutional Review Board at the Georgia Institute of Technology prior to sampling. Team coordination including colleagues from the Bolivian Catholic University (Universidad Católica Boliviana or UCB) in Cochabamba began 2 months prior to field work. UCB had expertise in water quality analysis, a fully equipped laboratory for sample processing and a team of students who

were selected through an interview process on the basis of several criteria including proficiency in English and 3 or 4 years of college education. Communication between local and non-local researchers occurred bi-weekly through email and conference calls to discuss team organization and distribution of tasks. Laboratory and sampling techniques were taught to non-local researchers a month in advance of the study. A 1-day training in laboratory and sampling techniques was held on-site for both local and non-local researchers after being organized in mixed teams of 5, followed by a group discussion on how to conduct the survey.

2.2.3 Surveying and sampling

We conducted a survey of household water and sanitation access, behaviors, and infrastructure characteristics in parallel with sampling drinking water. The questionnaire was initially based on the 2015 Demographic and Health Survey (DHS) (DHS, 2015) in English, then was translated into Spanish and Quechua by native speakers. This survey was then back-translated by other bilinguals to ensure the accuracy and comprehensibility of questions. The ethical implications of surveying and sampling methods were discussed with local collaborators. We made changes to the surveying and sampling procedures together with local collaborators in an effort to reach the most ethical conduction of data collection as possible.

Surveys were conducted such that responses were unprompted to reduce the possibility of bias. Our local collaborators' familiarity of the city helped cluster the sampling points such that 5-person groups could travel in small buses to their designated cluster. We informed residents about the purpose of the project and stated that participation was both anonymous and voluntary. The survey consisted of questions on source, availability, storage, treatment, and location of drinking water. We also

inquired hygiene and sanitation characteristics such as location and sharing practices of sanitation facilities. Where the resident responded with more than one answer on a given survey question, we recorded all mentioned answers without noting a primary answer. The survey did not include questions on the demographic makeup of the respondent. We recommend including a survey question on the respondent's gender in future rapid assessments of urban water quality.

We requested glasses of drinking water which the household was using for drinking at the time of the visit to have a representation of the water residents would consume. We sought 300 mL from each household to meet testing requirements and poured each sample into a sterile sample bag. We offered residents complimentary bottled water before leaving the household and communicated it as a gift of gratitude for participating with the survey. We labelled each sample according to a predetermined labeling system which included identifiers such as the site number, initials of the sample collector, and the date and time of sample collection. A detailed description of the survey methodology and the survey are included as Appendix A and B, respectively.

2.2.4 Water quality testing

Water quality was characterized according to the WHO guidelines for drinking water (WHO, 2017a), focusing primarily on microbial water quality due to its immediate effect on human health. We tested key physical, chemical, and microbial parameters of each water sample. The physical parameters measured in the UCB laboratory in Cochabamba included conductivity, total dissolved solids (TDS), and turbidity. We measured conductivity and TDS using the Hach HQ14D portable conductivity and TDS meter (HACH, 2017a). We measured turbidity using the Hach 2100Q Portable Turbidimeter (HACH, 2017b). Furthermore, we measured free and total chlorine in the

field immediately after the sample was collected using a Hach Pocket Colorimeter II (HACH, 2014). This instrument has a detection limit of 0.1 mg/L of free or total chlorine (HACH, 2018).

We also sent approximately 100 mL of each sample to the Vermont's Department of Health Laboratory (which is accredited by the National Environmental Laboratory Accreditation Program, NELAP) to test for selected metals (Mills G., Inorganic Program Chief and Laboratory Certification Officer, Vermont Department of Health Laboratory. george.mills@vermont.gov. Mailing Address: PO Box 1125, Burlington, VT 05402. Physical Address: 359 South Park Drive, Colchester, VT 05446). Inductively coupled plasma mass spectrometry (ICP-MS) was used according to the United States Environmental Protection Agency (US EPA) Method 200.8 to measure the concentrations of aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), thallium (Tl), uranium (U), vanadium (V), and zinc (Zn). The concentrations of iron (Fe) were measured by flame atomic absorption spectroscopy using Standard Method 3111B (American Public Health Association et al., 2012) and fluoride was measured by ion chromatography using LACHAT method 10-109-12-2-A (LACHAT Instruments, 2014).

Primary water safety tests were microbial assays specific to *E. coli* and total coliform in 100 mL samples, using selective chromogenic and fluorogenic media. Specific candidate tests for the rapid method were identified by an expert steering committee and included Colilert (presence-absence, using 10 mL and 100 mL samples) (Vergine et al., 2017), AquaTest medium (presence-absence, using 10 mL and 100 mL samples) (Bain et al., 2015), Compact Dry (quantitative, using 1 mL samples) (Hardy

Diagnostics, n.d.) and Petrifilm (quantitative, using 1 mL samples) (Vail et al., 2003). These methods were selected due to their simplicity, ease of use, and rapid availability of results. Detailed step-by-step instructions for these tests can be found in Appendix C. For future rapid assessments of urban water quality using microbial testing, we recommend Compact Dry for its ease of use while we recommend Colilert 10 mL and Colilert 100 mL for both their ease of use and wide acceptance as standard methods (Bain et al., 2012). In order to categorize the samples into four risk levels, each sample must be tested by three microbial characterization methods which indicate the presence of microbial contamination in a 1 mL, 10 mL-, and 100 mL-volume test. We recommend conducting each of the three methods in duplicate for each sample.

Based on the fecal indicator bacteria (FIB) *E. coli*, we classified each sample of drinking water as *safe* (non-detect of *E. coli* in 100 mL of sample), “low risk” (1 - 10 *E. coli* Colony-Forming Unit (CFU) in 100 mL of sample), “medium risk” (11 - 100 *E. coli* CFU in 100 mL of sample), or “high risk” (101+ *E. coli* CFU in 100 mL of sample) according to a commonly used scale (Bain et al., 2014a; Gruber et al., 2014; Moe et al., 1991; WHO, 2017a).

We collected eight blank control samples and eight duplicate samples in order to produce blank control samples and duplicate samples for 10% of all samples. We reported the most protective estimate of risk for each sample in cases where results from different testing methods conflicted. Note that because we sampled at point-of-consumption, there are multiple ways (e.g., storage or hygiene practices) through which water could have been contaminated after being collected. In addition, the samples were collected in a narrow time interval, meaning that the results discussed here only represent the conditions of Cochabamba’s drinking water quality at that interval of time.

2.3 Results and discussion

2.3.1 Feasibility of the study

We analyzed the time and resources required to complete the pilot study on a per-site basis. To estimate the time spent per site, we recorded the total hours spent working each day, multiplied by the total number of workers to obtain an estimate of person-hours, and divided by the total number of sites in the study to estimate person-hours per site (Equation 1). An average of 6.4 person-hours was required per site, totaling 512 person-hours for all 80 sites. Therefore, we recommend at least a team of 6 researchers for future assessments to sample 200 locales in less than three weeks. Sampling and surveying consumed 55% (282 out of 512 hours) of the time, followed by 32% (163 out of 512 hours) in analysis and 13% (67 out of 512 hours) in planning and training on-site. To calculate the total cost per site, we totaled all expenses and divided by the number of sites in the study. The total cost per site excluding travel, visas, food and vaccinations was US \$171, which consisted of US \$120 for reusable equipment and US \$51 for consumable materials. For a study sampling 200 locales, we estimated a cost of \$25 for consumable materials per locale.

$$\text{Eq. 1: Time per site} = (\text{daily work hours} \times \text{number of workers}) / (\text{total number of sites})$$

This resource consumption calculation is crucial to develop a global monitoring framework that aims to affordably monitor drinking water quality and survey households in cities in progress towards SDG 6. Such surveys provide important reference data for the international community to gauge progress on global water safety while providing robust data that is scientifically credible.

Our local collaborators were crucial in overcoming cultural barriers and increasing the efficiency of our time in Bolivia. Without the representation of local

partners on the research team, replication would be challenging as cultural understanding is key for successful surveying (Narayan, 1994; Pfadenhauer and Rehfuess, 2015). Another non-quantifiable challenge was the logistics of travelling with scientific equipment. Airports were suspicious of our equipment and requested researchers to deplane for further inspection of luggage. Documentation, including safety data sheets (SDSs), bilingual descriptions of all supplies and equipment and letters from our respective institutions allayed concerns.

2.3.2 Water, sanitation and hygiene (WASH) characterization

WASH access, behavior, and infrastructure in Cochabamba were characterized by survey respondents' types and use of water source, sanitation and hygiene facilities. This data determined their level of service to drinking water, sanitation, and hygiene. All levels of service, such as *safely managed*, *limited*, and *basic* are shown in Table 1 according to the SDG 6 framework (WHO/UNICEF, 2017). *Improved drinking water sources* are defined as those that conceptually, can deliver safe water (WHO/UNICEF, 2017). The raw data used in this classification is available in the supplementary material.

Table 2 provides a summary of respondents' access to drinking water, sanitation, and hygiene facilities. Only 18% (14 out of 80) of respondents were categorized as having *safely managed drinking water service*. Bottled water was considered an *improved source*, but as accessibility and availability to bottled water was not confirmed, they did not contribute towards *safely managed service*. If bottled water were assumed to be accessible, available, and clean, the percentage of respondents with *safely managed drinking water service* would rise to 34% (27 out of 80).

Table 1: Definitions of Water, Sanitation and Hygiene (WASH) service levels according to the Joint Monitoring Programme (WHO/UNICEF, 2017).

| Drinking Water | |
|--|---|
| Safely managed service | Drinking water from an <i>improved water source</i> that is (1) located on premises, (2) available when needed and (3) free from faecal and priority chemical contamination. |
| Basic service | Drinking water from an <i>improved source</i> , provided collection time is not more than 30 minutes for a round trip, including queuing. |
| Limited service | Drinking water from an <i>improved source</i> for which collection time exceeds 30 minutes for a round trip, including queuing. |
| Unimproved service | Drinking water from an unprotected dug well or unprotected spring. |
| Surface water | Drinking water directly from a river, dam, lake, pond, stream, canal or irrigation canal. |
| Sanitation Services | |
| Safely managed service | People should use improved Sanitation facilities (SF) designed to hygienically separate excreta from human contact that are not shared with other households. In addition, the excreta produced should either be: (1) treated and disposed of in situ; (2) stored temporarily and then emptied, transported and treated off-site, or (3) transported through a sewer with wastewater and then treated off-site. |
| Basic service | Has improved SF, but the excreta are not safely managed. |
| Limited service | Has improved SF, but they are shared with other households. |
| Unimproved service | Use of pit latrines without a slab or platform, hanging latrines or bucket latrines. |
| Open defecation | Disposal of human faeces in fields, forests, bushes, open bodies of water, beaches or other open spaces, or with solid waste. |
| Hygiene Facilities | |
| Basic service | Households that have handwashing facility with soap and water available on premises. |
| Limited service | Households have a handwashing facility but lack soap and/or water. |
| No facility | Households do not have a handwashing facility on premises. |
| <p>Note: <i>Improved water sources</i> include: piped water, boreholes or tubewells, protected dug wells, protected springs, rainwater, and packaged or delivered water. <i>Improved SF</i> include: flush/pour flush to piped sewer systems, septic tanks or pit latrines; ventilated improved pit latrines, composting toilets or pit latrines with slabs. Handwashing facilities may be fixed or mobile and include a sink with tap water, buckets with taps, tippy-taps, and jugs or basins designated for handwashing. Soap includes bar soap, liquid soap, powder detergent, and soapy water but does not include ash, soil, sand or other handwashing agents. The Joint Monitoring Program recommends testing the water at the point of delivery/collection. The Multiple Indicator Cluster Programme from UNICEF recommends testing the water at point of consumption. The choice will depend on the goal of the assessment.</p> | |

Of the survey respondents, 75% (60 out of 80) had *basic service* or *limited service* to drinking water (WHO/UNICEF, 2017). If bottled water were assumed to be accessible and available, the percentage of respondents with *basic service* or *limited service* to drinking water would fall to 59% (47 out of 80). We were not able to distinguish between *basic service* and *limited service* to drinking water because we did not inquire about the round-trip collection times (e.g., purchasing bottled water in less than or greater than 30 minutes). Accordingly, we suggest that any future rapid assessment of urban water quality inquire the round-trip collection time of respondents' drinking water.

The most common drinking water source was bottled water (39%, 31 out of 80), followed by piped tap water (28%, 22 out of 80) and protected wells (21%, 17 out of 80). Additionally, 26% (21 out of 80) of the respondents indicated having multiple sources of drinking water. Dependence on various water sources may indicate the lack of a single reliable source of drinking water. Furthermore, many respondents did not have continuously piped water service, and suffered from outages ranging from hours to days per week. As a result, 38% (8 out of 22) of respondents with piped tap water did not meet the *safely managed service* criteria, which requires continuously available clean water (WHO/UNICEF, 2017). Additionally, 76% (61 out of 80) of the respondents who met the accessibility and availability criteria treated the water on-site, indicating respondents' habit of treating water before use and consumption.

Our results show that 95% of respondents (76 out of 80) had access to an *improved water source* according to the SDG definition which includes bottled and sachet water (WHO/UNICEF, 2017) (Table 2). We compared the water source data to a JMP survey of urban Bolivia where it was found that 97% of Bolivians have access to an

improved water source, as formerly defined by the Millennium Development Goals (MDG) (WHO/UNICEF, 2015). In contrast, we found that only 18% (25 out of 80) of respondents had *safely managed service* of drinking water due to lack of accessibility, availability, and water quality. This exemplifies the difference between access to infrastructure versus access to reliable and *safe* drinking water.

A majority of respondents (90%, 55 out of 61) who reported treating their drinking water did so by boiling. Of the boiled samples, 69% (38 out of 55) were deemed *safe* in terms of microbial contamination (<1 *E. coli* CFU/100 mL). For the 24% (19 out of 80) untreated samples, which included untreated bottled water, 79% (15 out of 19) were considered *safe*. This suggests that inadequate treatment, storage or hygiene behaviors could result in recontamination of drinking water (Rufener et al., 2010).

In regard to sanitation services, none of the survey respondents qualified for the *safely managed service* category. The City of Cochabamba transports only 19% of the excreta through a sewer with wastewater to be treated off-site and the treated effluent further does not comply with effluent discharge quality parameters (e.g., Biochemical Oxygen Demand (BOD) > 80 mg/L, fecal coliforms >1000 Most Probable Number (MPN)/100 mL) (Ministry of Water and Environment of the Plurinational State of Bolivia, 2014). The remaining 81% of excreta is collected from piped sewers and other *improved facilities* (e.g., latrines or septic tanks) and is typically disposed to surface waters (e.g. open channels or rivers) without treatment (Ministry of Water and Environment of the Plurinational State of Bolivia, 2014). According to a 2013 environmental management case study by the World Bank, none of the generated wastewater received adequate treatment and 70% received no treatment at all (World Bank, 2013). Accordingly, 81% (65 out of 80) of the respondents were classified as having a *basic service* level of

sanitation facilities. Of the 80 respondents, 14% (11 out of 80) were classified as having *limited service* because they share sanitary facilities with other households.

Table 2: Summary of survey responses and categorization from 80 households. Refer to Table 1 for term definitions.

| Drinking Water Sources | n | (%) | Categorized Services and Facilities | n | (%) |
|----------------------------------|-----------|------------|--|----------|------------|
| Improved Water Source | 76 | 95 | Drinking Water Service | | |
| Bottled water | 31 | 39 | Safely managed | 14 | 18 |
| Piped water tap | 22 | 28 | Basic or limited | 60 | 75 |
| Protected well | 17 | 21 | Unimproved | 4 | 5 |
| Tube well/borehole | 13 | 16 | Surface water | 2 | 2 |
| Tanker-truck | 5 | 6 | | | |
| Protected spring | 4 | 5 | Sanitation Service | | |
| Cart with small tank | 1 | 1 | Safely managed | - | - |
| Rainwater | 1 | 1 | Basic | 65 | 81 |
| Sachet Water | - | - | Limited | 11 | 14 |
| Unimproved Sources | 6 | 8 | Unimproved | 4 | 5 |
| Unprotected well | 2 | 2 | Open defecation | - | - |
| Unprotected spring | 2 | 2 | Hygiene Facility | | |
| Surface water | 2 | 2 | Basic service | 62 | 78 |
| Multiple Sources | 21 | 26 | Limited service | 6 | 8 |
| Multiple Improved Sources | 18 | 23 | No facility | 12 | 15 |

Note: More than one response was acceptable per survey question. As a result, the sum of responses per category may sum to greater than 100%.

Only four respondents were classified as having *unimproved sanitation service* because they did not know what type of facilities they had available on-site; none of the four shared sanitary facilities. Finally, 78% (62 out of 80) of the respondents had access to *basic hygiene services* (e.g., handwashing station with soap and water).

2.3.3 Water quality and associated risk

2.3.3.1 Microbial risk

Microbial risk of drinking water was characterized per commonly used microbial safety guidelines (Bain et al., 2014b; Gruber et al., 2014; Moe et al., 1991; WHO, 2017a), focusing primarily on microbial water quality due to its immediate effect on human health. Of all samples, 29% (23 out of 80) were determined as *unsafe* for drinking (≥ 1 *E. coli* CFU/100 mL) while 71% (57 out of 80) were considered *safe* (<1 *E. coli* CFU/100 mL). Of the samples classified as *unsafe* (23 out of 80) for drinking, 30% (7 out of 23) were in the *high risk* level (>100 *E. coli* CFU/100 mL). The samples with *high risk* levels resulted in an average concentration of 343 *E. coli* CFU/100 mL. All samples categorized as *safely managed water service* (14 out of 80) were *safe* while all *surface water* samples (2 out of 80) were *unsafe*. Of samples categorized as *basic service* or *limited service*, 32% (19 out of 60) were considered *unsafe*. If bottled water were not included in the *basic service* or *limited service* category, the percentage of *unsafe* samples would rise to 40% (19 out of 47). Half of *unimproved service* samples (2 out of 4) were considered *unsafe*.

Figure 1 shows the risk level at each site location across Cochabamba. All reported sources had at least 1 sample with confirmed *E. coli* contamination including bottled water, suggesting the importance of sampling the water at point-of-consumption rather than at the source. Given the wide range of brands of bottled water and few

number of households reporting bottled water brands, we did not statistically compare the different risk levels of the brands due to sample size constraints. There was no noticeable difference in the risk levels between reported drinking water sources of the samples, supporting previous studies which suggest that *improved sources* have negligible relation with health risk (Lim et al., 2012; Shaheed et al., 2014). A larger sample size, as would be expected across several cities, will allow a multivariate analysis to assess how different households, communities, and environmental variables affect water safety.

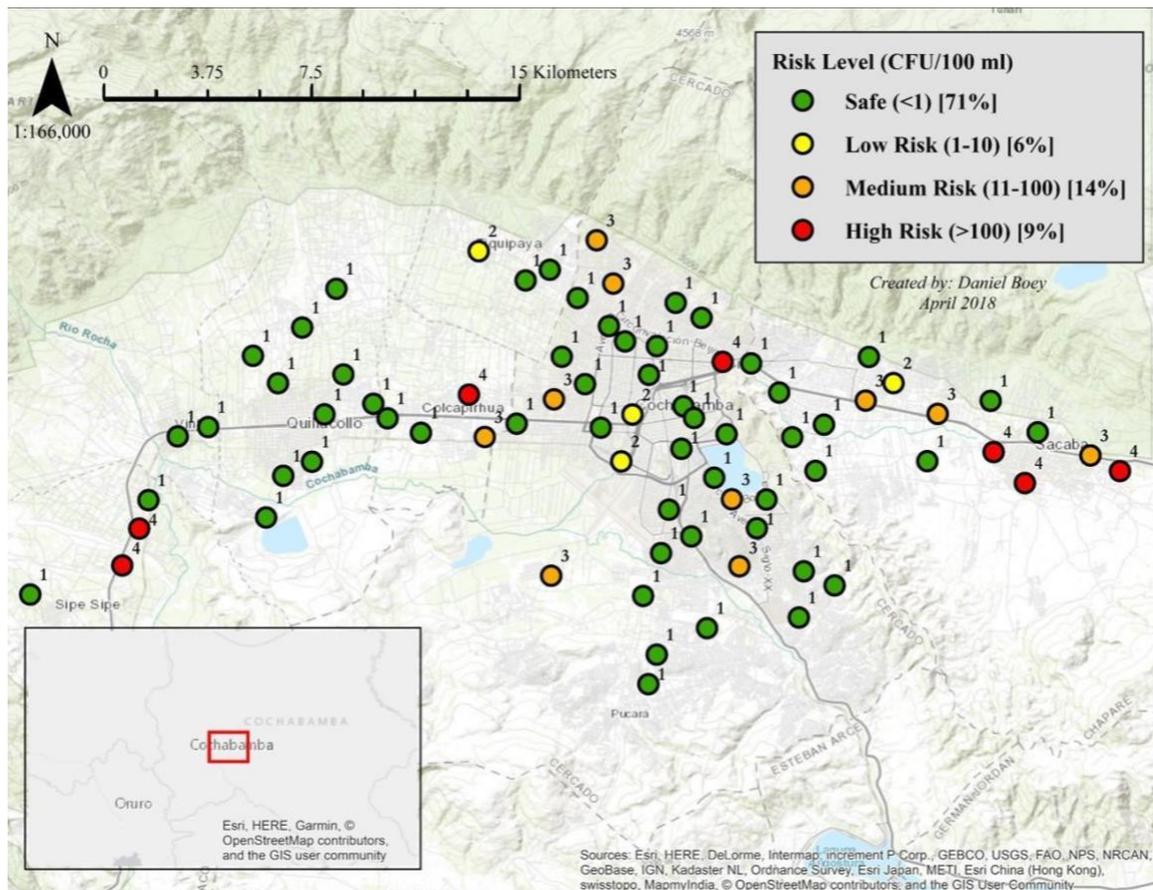


Figure 1: Distribution of microbial risk levels (*E. coli* CFU/100 mL) of water samples collected across Cochabamba. Numbers have been included for redundancy adjacent to each sampling point, indicating the risk level, where 1 indicates safe and 4 indicates *high risk*.

No negative control samples, which were sterile laboratory water samples filled at the point of sampling, indicated the presence of *E. coli*. One duplicate sample was lost during testing. Five (71%, 5 out of 7) of the remaining seven duplicates produced the same risk levels as their complementing samples. Of the two duplicates that were inconsistent with their associated samples, one was categorized one risk level higher (*low risk level*) than its associated sample, which was categorized as *safe*. The second inconsistent duplicate was categorized three risk levels lower (*safe*) than its associated sample, which was categorized as *high risk level*. The inconsistency in duplicates may be attributed to both the small sample size and the inherent variability in microbial testing. A sample size of 200 with 10% duplicates would allow for 20 duplicate samples, which may yield more meaningful conclusions on the reliability of results. Additionally, duplicate microbial tests are known to vary due to imperfect mixing and particle association of target microbes (WHO, 2017a) as no 2 aliquots are identical. Duplicate samples taken from the same time and place can yield potentially very different results due to the inherent variability in microbial measures and should be taken into account when interpreting results of duplicates (WHO, 2017a).

Maintenance of positive pressure in the distribution system is required to safeguard microbial water quality (U.S. Environmental Protection Agency, 2007). The 2014 International Benchmarking Network (IBNET) for Water and Sanitation Utilities Databook reported that water supply in Bolivia (all cities reporting data to IBNET, n = 2453) was available for an average of 20 hours per day in 2006 (Danilenko et al., 2014). However, the 2014 metropolitan master plan for water and sanitation in Cochabamba states that water availability dropped as low as 3 hours per day and did not surpass 15 hours per day (Ministry of Water and Environment of the Plurinational State of Bolivia,

2014). In our sample, 48% (38 out of 80) of households reported lacking tap water availability on a daily basis, reflecting high risk of contamination from outside sources. Though formal hypothesis testing was limited by sample size, we noticed that 24% (9 out of 38) of samples taken from households without daily access to water were determined to be medium or high risk, versus 21% (9 out of 42) of samples with more consistent access.

2.3.3.2 Physical risk

The results for turbidity and TDS showed that the drinking water in Cochabamba met both WHO recommendations in low-income settings of <5 NTU (WHO, 2017b) and <600 mg of TDS/L (WHO, 2017a) and Bolivia's own standards (<5 NTU and <1000 mg of TDS/L) (Ministry of Water and Environment of the Plurinational State of Bolivia, 2014). However, the average concentrations for free and total chlorine were near the 0.1 mg/L detection limit of our instrument (HACH, 2018). The lack of any disinfectant residual in drinking water samples suggests that centralized disinfection may have been inadequate. Added chlorine may have been consumed during distribution due to high chlorine demand of treated water, intrusion of environmental waters during low-pressure events, or extended hydraulic residence times - all risk factors for microbial contamination (Bivins et al., 2017; WHO, 2017a). Odds ratio analysis at CI: 95% demonstrated no significant bivariate association between physical parameters (turbidity $p=0.50$; conductivity $p=0.47$; TDS $p=0.83$) and microbial risk level. This indicates that physical parameters cannot be reliably used as a sole indicator of microbial safety of drinking water.

2.3.3.3 Chemical risk

The chemical testing showed that 4% (3 out of 80) of the samples exceeded the WHO provisional drinking water guideline for arsenic (>0.01 mg/L) (WHO, 2017a). Note that this guideline is not based on human health effects but rather on the detection limits that are achievable in routine drinking water testing laboratories (WHO, 1996).

In 1993, the WHO set a provisional guideline of 0.01 mg/L for arsenic in drinking water (WHO, 1993). More specifically, at this time, a skin cancer risk in humans from exposure to arsenic in drinking water was well established (WHO, 1996, 1993). A lifetime of drinking water with arsenic at this provisional WHO 0.01 mg/L guideline was estimated to cause 6 extra skin cancer deaths per 10,000 people (WHO, 1996, 1993). This cancer risk is 600 times higher than the 1 extra cancer death per 1,000,000 people factor that is typically used to protect public health (Brown, 2007; CalEPA, 2004). In 2004, a risk assessment by the California Environmental Protection Agency (CalEPA) included risk of death from skin and other cancers, and estimated that the cancer risk from 0.01 mg/L of arsenic in drinking water is 1 excess cancer death per 400 people (Brown, 2007; CalEPA, 2004). This 1 extra death from all cancers per 400 people estimated by the CalEPA is a greater risk than the 6 extra deaths from just skin cancer per 10,000 people estimated by the WHO (Brown, 2007; CalEPA, 2004; WHO, 1996, 1993). The results from our screening survey in Cochabamba and the relative toxicity of arsenic in drinking water suggests that a detailed exposure assessment for arsenic in the drinking water of this city is highly recommended.

2.3.4 Limitations

This study and its conclusions should be interpreted in light of important limitations and constraints. First, our study had a limited sample size of 80 households.

Consequently, we were only able to make univariate comparisons across survey results, limiting the analysis to descriptive statistics rather than hypothesis testing. With a larger sample size, such as is expected across several cities, a multivariable analysis will be possible, potentially allowing for a fuller analysis of how different household, community, and environmental variables affect water safety. Also, the sensitivity and specificity of various water quality tests were not reported due to sample size limitations. Though widely accepted and recommended by regulatory agencies for identification of *E. coli* in water as an indicator of safety, the culture-based assays used in this study could benefit from additional molecular methods to confirm presence of presumptive *E. coli*, to provide source-tracking information, or to directly measure pathogens that may be present in the sample. Second, the drinking water samples were assumed to be characteristic of all drinking water sources the respondent mentioned. As a result, the drinking water sources may have been inaccurately associated with the sampled drinking water where more than one source was reported. As data on contamination from source to point-of-consumption were not observed, information on the difference in contamination from the drinking water source versus source to point-of-consumption is lacking. Third, the survey data was self-reported. It is possible that people did not give truthful answers for the survey questions, or that they were misunderstood, introducing bias (Clasen et al., 2012; Jenkins et al., 2014). Fourth, we had no control over the influence created by the estimated 30% (24 out of 80) of pre-selected households that did not participate. Those who refused to participate may have used unconventional methods of obtaining potable water and may have felt uncomfortable to participate. Lastly, this study was a cross-sectional study, representing a narrow time interval. All data were collected over the course of 2 days. This provides a snapshot of the conditions of water in Cochabamba at

that point in time, but may not be indicative of the long-term safety of water in Cochabamba. The time of year and weather conditions, among other factors, could have an impact on water quality. Frequent and periodic repetition of this experiment over a longer course of time would provide a more accurate estimate of water safety.

2.4 Conclusions

2.4.1 Key insights from the rapid urban water quality assessment of Cochabamba

We determined several key findings from the data we collected. First, 71% (57 out of 80) of drinking water at the time of the survey was classified as *safe* according to WHO microbial safety recommendations (WHO, 2017a), indicating the overall safety of the water being consumed. Second, sites with daily water supply had safer water than sites with intermittent water supply according to commonly used microbial safety guidelines (Bain et al., 2014a; Gruber et al., 2014; Moe et al., 1991; WHO, 2017a). Sample points with water continuously flowing through pipes yielded noticeably lower microbial risk levels. This result suggests that Cochabamba can significantly improve drinking water safety by focusing on increasing access to continuously piped water supplies (Bivins et al., 2017). Third, given the concentration of arsenic found in the tested drinking water, we recommend a detailed exposure assessment for Cochabamba as a follow-on study based on our results. Though our study estimated bacteriological, physical, and chemical water quality at only one point in time, a significant number of samples (29%, 23 out of 80) were outside the current recommended microbial guidelines for drinking water safety (WHO, 2017a), highlighting the need for investment into safe water access and distribution to meet SDG 6 (United Nations, 2016a).

2.4.2 Essentials of the methodology and recommendations for future studies

- We recommend a minimum sample size of 200 for future studies in order to allow for more in-depth statistical analyses. Potential benefits of this increased sample size include identifying key relationships between variables such as water quality, access, source, sanitation, and hygiene.
- This assessment demonstrates that access to *improved drinking water sources* does not necessarily equate with access to *safe* drinking water. This exemplifies the importance of sampling drinking water at point-of-consumption rather than at the point of collection or source.
- We recommend that future studies inquire about the round-trip collection time of respondents' drinking water to categorize their level of service according to the UN SDG 6 definitions.
- This proposed methodology shows potential to be used for monitoring purposes particularly in areas where water quality testing does not occur often or at all. With an average time requirement of 6.4 person-hours and an estimated consumable cost of US \$25 per site if scaled to a 200-site study, this methodology shows scalability and replicability for other cities. The logistical considerations of this methodology further address many challenges such as cultural, technical, financial, time, and spatial limitations. We highly recommend ensuring local partner collaborators to help design and implement future urban water quality studies.
- We suggest taking at least three evenly-distanced samples per locale in cities with higher population density or in need of an in-depth, intra-city analysis of the water quality. While this would increase the cost and time spent in the assessment,

representability should not be compromised. In addition, at least one microbial method should be used for each of the three testing volumes. We recommend conducting chemical testing in a local, certified laboratory instead of abroad in order to reduce time, cost, and logistical complications.

In summary, this pilot study achieved a meaningful point-in-time estimate of drinking water safety in Cochabamba while assessing key logistical, cost, and time considerations that represent important constraints for scalable global water safety monitoring. Future scaled assessments can be designed and conducted to deliver more in-depth information and contribute to more rigorous assessments of water safety monitoring at scale. Such monitoring will be important to gauge progress against UN SDG 6, intended to realize access to water and sanitation for all.

3. Bioaerosol sampling optimization for community exposure assessment in cities with poor sanitation: a one health cross-sectional study

This chapter presents a field investigation of bioaerosols near open waste canals, using microbial culture techniques and molecular analyses to screen for human pathogens. This work was published in the journal *Science of the Total Environment* (Rocha-Melogno et al., 2020).

3.1 Introduction

Current estimates suggest that 70% of the world population will be urbanized by 2050 (United Nations, 2014). Many of these growing cities are in low- and middle-income countries, which, according to the World Health Organization (WHO), do not have broad access to safely managed sanitation services (WHO/UNICEF, 2017). The lack of safe water, sanitation and hygiene (WASH) is linked to gastro-intestinal infectious diseases, which caused 1.38 million deaths in 2016 (with 60% attributed to WASH) (Prüss-Ustün et al., 2019) and being one of the leading causes of mortality in children under five years of age (Troeger et al., 2019). In addition, while poor sanitation is not directly related to acute respiratory infections, a 2009 study found that 26% of acute lower respiratory infections in children under five years old were linked with recent diarrheal disease events (Schmidt et al., 2009). These results suggest that reducing the incidence of diarrheal disease by increasing access to safely managed sanitation services, could reduce acute lower respiratory infections (Mara et al., 2010). These, and the recent coronavirus pandemic and evidence of SARS-CoV-2 viral excretion through the gastrointestinal tract (Xu et al., 2020) suggests that a better understanding of the

aeromicrobiological route of exposure and the above mentioned possible linkages are warranted (Clasen and Kirk, 2019). Our study helps with bridging this knowledge gap.

Plenty of research has been done on the fecal-oral infection route from unsafe water consumption, poor sanitation and hygiene. Even so, the exposure to enteric pathogens through the air and associated risk of infection is scarce in the literature outside of narrowly defined settings. It has been shown that concentrated sources of fecal matter can release bioaerosols through different mechanisms, e.g., wind erosion or mechanical disturbance (Delort and Amato, 2018; Farling et al., 2019; Paez-Rubio et al., 2005). These bioaerosols may be transported and pose an exposure risk and cause infectious diseases. However, most of the research has been conducted in developed nations and in relatively isolated contexts, such as wastewater treatment plants, agricultural use of biosolids or in animal farms, e.g., places where personnel work close to animal and human waste (Baertsch et al., 2007; Jahne et al., 2015; Schaeffer et al., 2017; Uhrbrand et al., 2011). Recent research has focused among others on developing real-time bioaerosol sensors using fluorescence spectra to monitor bioaerosol emissions (Tian et al., 2020), characterizing the effect of aeration on bioaerosol generation during wastewater treatment (Wang et al., 2019) and chemical and molecular fingerprinting of outdoor bioaerosols to track their source and transport in different meteorological conditions (Garcia-Alcega et al., 2020). As these novel methods and findings keep arising, it is important to use existing methods and instruments to understand bioaerosol dynamics and exposure risks in low-resource settings.

Considering people living in cities with poor sanitation are broadly exposed to a variety of fecal pathogens through multiple pathways, e.g., fluids, fingers, flies, floors or food (Wagner and Lanoix, 1958), it is paramount to better understand fecal pathogen

transport and exposure routes in order to minimize disease transmission. Moreover, cities with rapid urban growth will face unprecedented challenges in waste management, potentially causing an increase in diarrheal diseases that could create lifetime health deficits (Neiderud, 2015). Recent modelling efforts have found high risks of infection and illness from airborne Rotavirus and Norovirus emitted from wastewater treatment plants in Iran (Pasalari et al., 2019) as well as identification of key areas in such facilities that increase the risk of illness from bioaerosol exposure (Carducci et al., 2018). Hence, we are conducting a series of studies looking at sanitation-related bioaerosols in developing countries to better understand their potential health impacts. The main goals of our study were 1) to optimize a robust and practical personal exposure bioaerosol surveillance strategy in outdoor environments with poor sanitation and 2) explore if these bioaerosols are at personal exposure levels ($\sim 3\text{-}13 L_{\text{air}}/\text{min}$ (EPA, 2011)) in such contexts. We conducted this study testing different sampling methods to characterize the fate and transport of sanitation-related bioaerosols during the rainy and dry seasons in La Paz, Bolivia in 2019, as seasonal effects on aerosolized pathogenic microorganisms have been observed (Fan et al., 2019; Lu et al., 2019). Thus, we evaluated multiple environmental factors to explore their impact upon bioaerosols in real-world scenarios using low-cost instrumentation, identifying the key parameters to be included in future context-specific risk assessments and surveillance efforts.

3.2 Methods

3.2.1 Study site

La Paz is located at 3600 m above sea level in the Andean region of Bolivia. It is a rapidly growing city with 800,000 people (1.9 million in the metropolitan area) (Bolivian

National Institute of Statistics, 2020). With a unique geography, the city lies in a canyon with poor urban planning. Industrial wastewater, hospital sewage and domestic sewage are discharged into the Choqueyapu River that crosses downtown La Paz and is fed by several tributaries also serving as sewers. Traversing steep slopes, the Choqueyapu River forms several waterfalls, creating an environment conducive to waste aerosolization.

3.2.2 Bioaerosol sampling

We sampled bioaerosols during the rainy (September-April) and dry (May-August) seasons of 2019 during a 4-week field campaign split in two visits in March and June, respectively. Five spatially distributed sites (~1.6 km from each other) adjacent to the Choqueyapu River were selected by proximity to the river and waterfalls and five additional sampling locations were selected at 100-1000 m away from each site in the rainy season (n=10, 6 replicates). Only three sites adjacent to the river were selected in the dry season, to increase our sample size (beginning of open-sewer, mid-way point and city exit; sites a, b and c in Appendix D, Figure 6) and two concurrent transect samples were taken at 10-100 m downwind (n=21, 3 replicates). Three to six 100 mm settle Petri dishes (replicates) were set for 2 hours at 1 m from the ground and 1 m from any obstacle, based on published methods for passive sampling (Haig et al., 2016; Pasquarella et al., 2000). We calculated the fluxes (CFU/(m²*h)) by dividing the CFU counts by the area of the Petri dish (7.854*10⁻³ m²) and the time at each site (2 h). We used the open-source Aquatest (AT) (Bain et al., 2015) selective growth medium, Difco™ MI Agar (BD Biosciences, San Diego, CA, USA) and Compact-Dry-EC plates (Hardy Diagnostics, Santa Maria, CA, USA) for sampling and enumeration of viable fecal coliforms and *E. coli*.

We conducted active sampling using the National Institute for Occupational Safety and Health (NIOSH) BC 251 Personal Aerosol Samplers (Cao et al., 2011) in parallel to passive sampling, for two hours at each sampling event (n=10 in rainy season, n=25 in dry season). The NIOSH sampler was selected because it had been used for personal exposure studies (Bailey et al., 2018; Choi et al., 2018; Coleman et al., 2018). It uses a sampling rate of 3.5 L/min which simulates human breathing and thus is relevant to personal exposure. For the experiments reported herein, the sampler was located ≥ 2 m next to the passive sampling setup. The sampling flow-rate was calibrated at 3.5 L/min before each sampling event. The NIOSH device sorts organisms by size in three compartments: greater than 4 μm , 1-4 μm , and < 1 μm . These particles were collected in a 15 mL falcon tube, a 1.5 mL centrifuge tube, and a polytetrafluoroethylene (PTFE) back-up filter (0.03 μm pore, 37 mm), respectively (Choi et al., 2018). All samples were taken between 8:00 am and 6:00 pm.

3.2.3 Sample processing and molecular assays

We took the samples collected on Petri dishes to the Universidad Católica Boliviana's (UCB) laboratories within three hours of collection and incubated them at 37 °C for 20-24 h. Samples collected with the NIOSH device were rinsed with 1-1.5 mL PBS (0.5% BSA), combining the filter eluent with the 1-4 μm compartment's eluent. The two resulting eluents were mixed with DNA/RNA Shield™ reagent (Zymo Research, Irvine, CA, USA) for molecular analyses back in the USA. Viral nucleic acids were extracted using the Quick-DNA/RNA Viral Kit (Zymo Research, Irvine, CA, USA). We screened for 10 viruses (Influenza virus A/B/C/D, Coronavirus NL63/OC43/HKU1/229E, Human Adenovirus and Human Enterovirus) using a RT-PCR method previously described

(Bailey et al., 2018). Given the sampling methodology, the results for those viruses are expressed in presence/absence, with our limit of detection being approximately 7 copies per m³ air.

3.2.4 Environmental conditions monitoring

We used low-cost sensors on-site to collect minute-interval measurements of PM_{2.5}, temperature and relative humidity (RH). These sensors have been described and tested previously (Barkjohn et al., n.d.; Zheng et al., 2018). We also collected wind speed data on-site (Vernier Software and Technology, Beaverton, OR, USA). We obtained solar UV irradiance (UVB, 280-320 nm) data from a stationary radiometer (Yankee Environmental Systems, Turners Falls, MA, USA) at Universidad Mayor de San Andrés.

3.3 Results and discussion

3.3.1 Passive and active sampling findings

The median flux of total coliforms in the rainy season was 71 CFU/(m²*h) [range: 0-5411] while the median flux in the dry season was 64 CFU/(m²*h) [range: 0-3374] with 38% of the dry season samples being positive for *E. coli*. Unfortunately, the percentage of positive samples for *E. coli* during the rainy season is unknown due to growth medium being damaged by sunlight, making it difficult to reliably differentiate *E. coli* from total coliforms. The sampling site located at the beginning of the open sewer (location a on SI2 map) had the highest fluxes, with a mean flux of 4064±1184 CFU/(m²*h) in the rainy season and 2706±388 CFU/(m²*h) in the dry season. We note here that this site is at the starting point of the open sewer, and has a 2-3 m waterfall, coming off a tunnel with 1-2 m of headspace. We observed higher fluxes next to the river in both seasons and our

concurrent transect samples taken at 10-100 m downwind of the open sewer at two different locations showed a reduction in fluxes as the distance from the river increased (Figure 2). The deposition of aerosolized pathogens on food, water or fomites is known to be a potential source for exposure (de Man et al., 2014). For example, it was the suspected cause of an *E. coli* O157:H7 outbreak at a county fair in Oregon, USA (Keene et al., 2004). Continuous passive sampling with a highly selective medium such as AT could allow rapid and low-cost monitoring of bioaerosols to better understand the prevalence of such events, without needing highly trained personnel or high-tech equipment.

We conducted molecular analyses (RT-PCR) of actively sampled aerosols (using the NIOSH sampler) for influenza viruses, adenoviruses, coronaviruses, and enteroviruses to test for potential presence of respiratory pathogens with aerosolized enteric bacteria. One sample (10%) was positive for adenoviruses (positive hit on >4 µm compartment and in the <4 µm combined eluent) and one sample (10%) was positive for influenza A virus during the rainy season (positive hit on >4 µm compartment only). Both adenovirus positive samples were from the site with the highest bacterial flux. Four samples (8%) were positive for influenza A virus in the dry season (all in the <4 µm compartment). The detection of these viruses at low-flow rates (3.5 L/min) is of concern, as viruses cause ~60% of infection cases, and to date, we have limited vaccines or antiviral medications (Boone and Gerba, 2007). The recent coronavirus pandemic highlights the susceptibility of our society to viral infection.

Our efforts to sequence aerosolized viruses to identify the sub-types were unsuccessful because the concentration of DNA was too low. However, our detection of viruses at sites with high enteric bacterial fluxes indicates that open sewers may be

associated with enteric virus detection through aerosolization of contaminated water and sewage. Enteric viruses are known to remain viable for weeks to months, while respiratory viruses can do so for hours to multiple days (Boone and Gerba, 2007). Nevertheless, the contamination of non-porous fomites, such as the several playgrounds of La Paz located near our sampling sites, could harbor enteric viruses, potentially remaining infectious. At minimum, fomite sampling at those location is needed to begin understanding personal exposure.

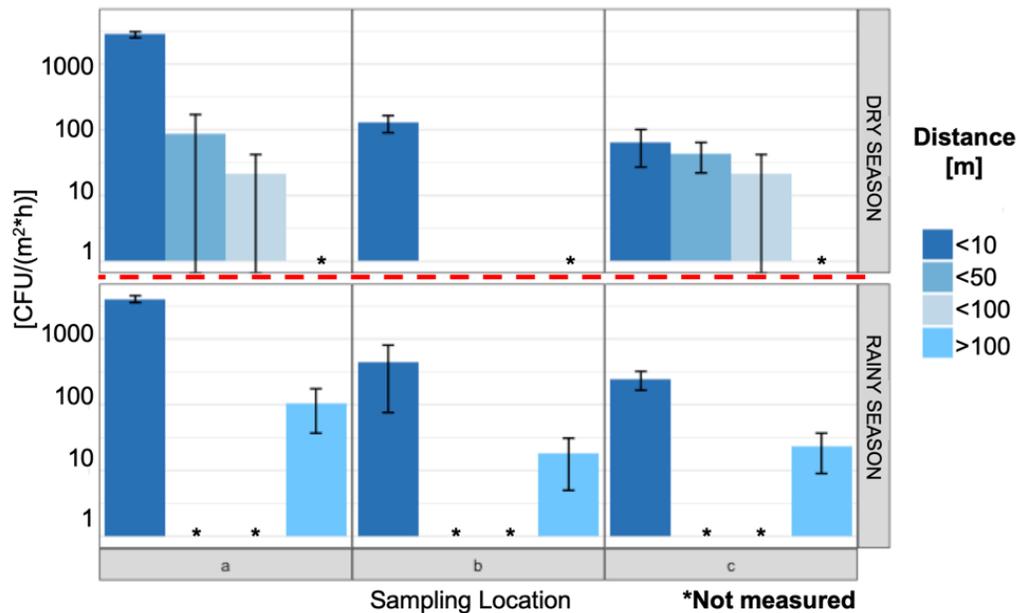


Figure 2: Fluxes at three sampling site during the rainy season and dry season.

3.3.2 Context-specific environmental co-variables identification

The effects of co-variables were observed by using a zero-inflated mixed effects regression model to assess the impact of the monitored environmental conditions on bacterial fluxes. This regression model addressed the fact that 35% and 33% of our passive samples were below detection limit (<1 CFU per plate) in the rainy and dry seasons, respectively. We also fitted a negative binomial distribution to these data to

account for its overdispersion (residual variance \gg predicted variance). We found that wind speed had a significant positive, and UVB irradiance a significant negative effect on fluxes ($p < 0.05$ and $p < 0.001$, respectively) in the rainy season while positive effects from RH and UVB ($p < 0.05$ for both) were found in the dry season. While the trends during the rainy season are as expected, the positive effect of UV during the dry season is puzzling. We suspect that it could be due to i) higher bacterial flux rates compared to UVB induced death rates during the study (Tong and Lighthart, 1997). ii) Light shielding effects by large particles attached to the bacteria (Tong and Lighthart, 1998), or an effect of low RH on UVB-induced inactivation of bacteria (Peccia et al., 2001). Overall, our results suggest that sanitation-related bioaerosols' viability and transport are likely to be most affected by UV radiation and wind speed, in agreement with previous bioaerosol studies (Tong and Lighthart, 1997; Van Leuken et al., 2016). Further detailed studies are needed to better quantify these effects.

3.3.3 Bioaerosol transport estimation model

As a proof of concept, we applied a Gaussian plume model to estimate how far the bioaerosols emitted from an open sewer could travel (Figure 3). We experimentally cross-validated the deposition velocity by dividing our mean flux [$155 \text{ CFU}/(\text{m}^2 \cdot \text{h})$] by the mean concentration from the rainy season measured by Ginn et al. 2019 [$54 \text{ CFU}/\text{m}^3$] (not published) during the rainy season, as sampling events coincided in time and location. The experimental deposition velocity was in the same order of magnitude of the theoretical deposition velocity (10^{-4} m/s), calculated using the Stokes settling velocity equation corrected by the Cunningham factor (Seinfeld and Pandis, 2016) (Equation 2). A summary of the collected data can be found in Appendix D, Table 9. The concentration at 10 m downwind from the river was found using the Gaussian plume

model (Equation 3) and with the following assumptions: i) Bioaerosols only traveled in the wind direction and estimates were for ground level concentrations only ($z=0, y=0$) ii) Concentrations were back-calculated using bacterial fluxes and theoretical particle deposition velocities. iii) The stack height was fixed at 4.68 m above the sewer, incorporating the height (3.68 m) from the water level to the ground and adding one meter above ground at which the measurements were made. iv) Wind speed was constant for each site. v) One outlier data point was removed for the model. vi) The theoretical deposition velocity was estimated to be 1.37 m/h for spherical particles with a diameter range of 2-5 μm and a density of 1000 kg/m^3 .

$$\text{Eq. 2: Concentration (CFU m}^{-3}\text{)} = [\text{Flux (CFU m}^{-2}\text{h}^{-1}\text{)}] \times [\text{Deposition velocity (m h}^{-1}\text{)}]^{-1}$$

$$\text{Eq. 3: } C_{x,0,0} = \frac{\frac{Q}{e^{0.5}} \times \left(\frac{H}{\sigma_z}\right)^2}{W_s \times \sigma_y \times \sigma_z \times \pi}$$

Where Q is the rate of bacteria emission per time [CFU/h]; H is the effective stack height [m]; W_s is the wind speed [m/h]; σ_y and σ_z are the standard deviation coefficients of dispersion [m] using Briggs formulas for Pasquill's atmospheric stability category A-B, C and D (Wark et al., 1998); and π is 3.14. These stability categories are semiquantitative; A and B are characterized for having wind speeds <2 m/s and slight, moderate or strong solar radiation (Wark et al., 1998).

This simple model shows that bioaerosol surveillance could reasonably predict concentrations downwind. This is useful to public health officials as it could be used to establish a threshold distance at which playgrounds or food stands should be located to reduce exposure to potential hazards. The variability observed highlights the importance of using this transport model with discretion when using passive sampling data. Due to the inherent variability of the sampling method, we recommend a minimum of 30

samples (180 replicates) per site before drawing any conclusions. This would also allow the incorporation of environmental co-variates and their effect on the detected bioaerosols in the transport models. We did not include these here to avoid increasing the model complexity in an already limited data set.

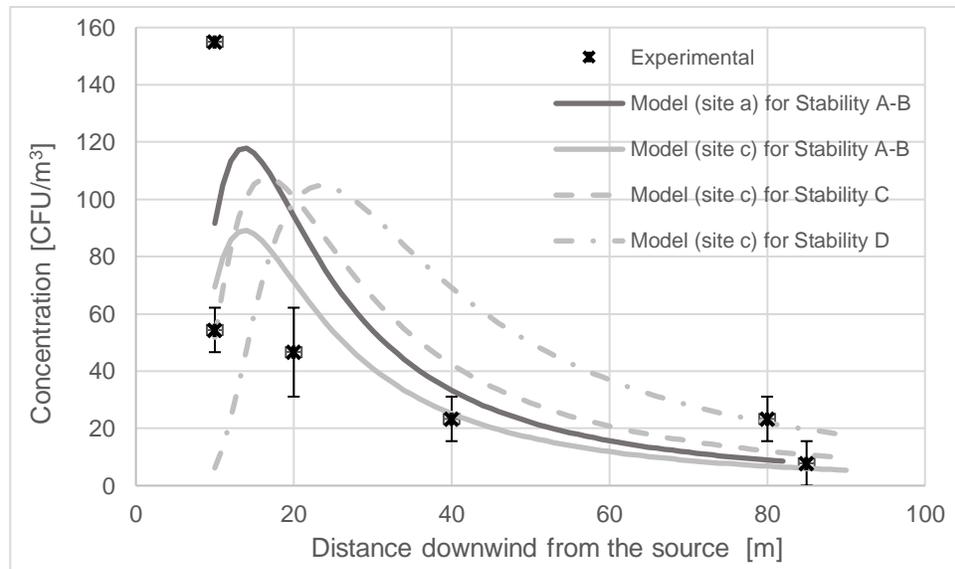


Figure 3: Gaussian plume model fitted to experimental measurements taken during the dry season. The atmospheric stability category during the sampling campaigns was A-B.

3.3.4 Optimization of passive sampling

We found that only Aquatest (AT) medium could withstand the field conditions for passive sampling, compared to MI agar and Compact Dry plates (Appendix E). The *E. coli* staining chromogen was damaged after extended sunlight exposure, preventing its identification and quantification vs. total coliforms. The dry environmental conditions also affected MI agar, resulting in dehydration of the medium and loss of surface area coverage (Appendix E – Figure 7). Finally, we followed a published method to enhance AT's use for bioaerosol passive sampling (Xu et al., 2013), and spread 0.1 mL mineral oil onto AT plates and tested them against regular AT plates during the rainy season, in

triplicates. We did not find a significant difference in the CFU fluxes observed after 24 h of incubation between plates with or without mineral oil ($p=0.9055$, Wilcoxon signed-rank test, $n=60$). Our findings suggest that mineral oil does not increase CFU recoverability in outdoor bioaerosol passive sampling.

3.3.5 Study limitations

We optimized a simple, yet effective strategy of sampling bioaerosols in low-income settings for surveillance efforts, but as any study, it had its limitations. First, our sample size was limited due to time constraints for both sampling events (2 h at each site per sample) and the campaign (10 days for sample collection in each season). Continuous surveillance efforts would allow further hypothesis testing and improvement of bioaerosol transport modelling. Second, the damage that happened to the growth media may have resulted in viable but non culturable organisms, leading to an underestimation of fluxes or misidentification of *E. coli*. Third, the low volumes sampled for molecular analysis, while realistic from an exposure perspective, resulted in a low number of positive hits. Increased sampling flow-rates or longer sampling periods would permit better quantification of pathogens as well as the use of the data for microbial source tracking.

3.4. Conclusions

Our findings suggest that aerosols play a role in the exposure to enteric microorganisms in cities with poor sanitation, at personal exposure levels. The use of passive sampling, despite its limitations, can provide quantitative data on microorganisms' viability within realistic timeframes of personal exposure. Parallel active sampling at higher flowrates combined with current molecular methods could further

identify and quantify pathogens of interest. Our future work will involve additional sampling and the development of Quantitative Microbial Risk Assessment (QMRA) frameworks to better understand the risk associated with the aeromicrobiological route of pathogen exposure of populations living in poor sanitation conditions. This will enable a better characterization of pathogen's fate and transport and the estimation of disease risks posed by these organisms, providing both technical and analytical surveillance tools.

4. Quantitative microbial risk assessment of outdoor aerosolized pathogens in cities with poor sanitation

This chapter focuses on characterizing the risk of infection, and illness attributable to bioaerosols near open wastewater canals. The work presented here was reviewed by the collaborating authors listed below with their roles.

K. Crank: Web-application co-development, experimental design, manuscript review, final approval for submission

O. Ginn: Data collection, manuscript review, final approval for submission

M.H. Bergin: Manuscript review, final approval for submission

J. Brown: Manuscript review, final approval for submission

G.C. Gray: Manuscript review, final approval for submission

K. Hamilton: Experimental design, manuscript review, final approval for submission

K. Bibby: Manuscript review, final approval for submission

M.A. Deshusses: Experimental design, manuscript review, final approval for submission

4.1 Introduction

Access to safe drinking water, sanitation and hygiene continues to be limited in many areas of the world, accounting for 60% of the global diarrheal deaths in 2016 (800,000) (Prüss-Ustün et al., 2019). While many studies have been conducted on the fecal-oral route for transmission of enteric diseases, studies on aerosol transmission of enteric pathogens in places with poor sanitation and associated risk of infection are limited in the literature. Numerous studies found enteric microorganisms in aerosols emitted from land application of biosolids (Dungan, 2014; Viau et al., 2011), concentrated animal feeding operations (Jahne et al., 2015; Millner, 2009), and toilet

flushing (Johnson et al., 2013; Wilson et al., 2020). Our recent work detected multiple enteric pathogens in aerosols sampled near open wastewater canals (OWCs) (Rocha-Melogno et al., 2020). In the present analysis we sought to quantify the risk of disease that these aerosolized pathogens near OWCs may pose through food, fomite, and inhalation routes using Quantitative Microbial Risk Assessment (QMRA). The steps involved in this risk assessment were: hazard identification, exposure assessment, dose-response model selection, and risk estimation.

Biological aerosols (bioaerosols) result from wastewater aerosolization, bubble bursting, human waste handling, droplet impaction and multiple other processes (Farling et al., 2019; Kim et al., 2019; Rocha-Melogno et al., 2020; Schmale and Ross, 2015; Wéry et al., 2017). Some studies at e.g., wastewater treatment plants (WWTP) workers' symptoms, found positive associations between working with sewage and flu-like symptoms (Douwes et al., 2001), and gastrointestinal symptoms (Thorn and Kerekes, 2001). However, similar studies have pointed out a common limitation involving recall bias and over-reporting illness when odors were present (Viau et al., 2011). These limitations led to risk assessment studies sampling bioaerosols at WWTPs and characterizing the risk they pose on the plant's workers, i.e., estimating a risk of illness of 14×10^{-2} after a three minute exposure to aerosolized human adenovirus (HadV) (Carducci et al., 2018). Similarly, annualized risk of illness from exposure to aerosolized rotavirus and norovirus at WWTPs have been estimated to be of $5.25 \times 10^{-3} - 5 \times 10^{-1}$ and $1.77 \times 10^{-1} - 5 \times 10^{-1}$, respectively (Pasalari et al., 2019). A study in France used urinary biomarkers and aerosol samples at WWTPs and found that sewage workers were exposed to airborne genotoxicants. These workers had higher genotoxicity in urine samples than their office counterparts who were exposed to lower concentrations of

genotoxigants (Al Zabadi et al., 2011). Others have looked at the impact of aeration systems as sources of bioaerosols in Spain, finding that mechanical agitation aerosolized 10 to 100 times more coliform bacteria at WWTPs compared to air diffusers (measured with a single stage impactor), potentially increasing exposure risks (Sánchez-Monedero et al., 2008). However, a 2016 literature review of atmospheric dispersion modelling of pathogenic bioaerosols identified the lack of QMRAs being conducted, limiting studies to qualitative observations about the risk that pathogenic bioaerosols present (Van Leuken et al., 2016).

Open wastewater canals such as the Choqueyapu River in La Paz, Bolivia, were channelized to collect the city's wastewater (Vega et al., 2017). This OWC has cascades throughout its course given the city's steep slopes, and flowrates averaging 14400 m³/h during the rainy season (Medina et al., 2021). This causes sewage aerosolization, potentially contaminating nearby food stands, playgrounds, and households. Indeed, we recently found fecal bioaerosols near these OWCs (Rocha-Melogno et al., 2020), including pathogens and antibiotic resistant coliforms (Medina et al., 2021),(Salazar et al., 2020). These bioaerosols may pose an exposure risk to populations which live near heavily polluted streams found in cities from Africa, Latin America as well as South and East Asia (Peal et al., 2014). We hypothesized that aerosolized enteric pathogens from the Choqueyapu River pose an exposure risk for the population of La Paz. Therefore, the goal of this report was to assess the potential public health impact from bioaerosols near OWCs.

We used QMRA and data we have acquired in Bolivia as a case study. We previously collected fecal indicator bacterial fluxes through passive bioaerosol sampling, and detected human pathogens through active bioaerosol sampling and RT-PCR (Ginn

et al., 2021; Rocha-Melogno et al., 2020). This work identified the need for tools to characterize the risk of exposure to bioaerosols from OWCs. For this reason, we added the current food and fomite QMRA to a web-based application we developed, called Aerosol-Mediated Infectious Disease Risk Assessments, or AMIDRA. The website is a collection of aerosol QMRAs that facilitates their dissemination, allowing interested parties such as public health professionals to conduct QMRAs for their specific application. The web application uses the methodology presented in this paper with user inputted data, fitting the data to gamma or lognormal distributions and runs 10,000 stochastic simulations (using the Monte Carlo method) to estimate the risk of infection, illness, and mortality for different exposure scenarios. See Appendix G for examples of the application's user interface.

4.2 Methods

We used QMRA (Haas et al., 2014) to estimate the risk of infection, illness, and mortality from three relevant scenarios: contaminated surfaces (fomites) in playgrounds, contamination of food sold in food stands, and inhalation near the OWCs. This selection was made after observing colocation of human activity with a high concentration of viable *E. coli* and total coliforms immediately next to OWCs in La Paz, Bolivia (Rocha-Melogno et al., 2020). Aerosol samples analyzed through real time PCR using a TaqMan Array Card (TAC) (Liu et al., 2013) indicated the presence of *Giardia* spp., *Cryptosporidium parvum*, *Yersinia* spp., *Salmonella* spp., *Enterococcus faecium*, enterotoxigenic *Escherichia coli* (ETEC, heat-stable enterotoxin), *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC, ipaH gene), enteroviruses (all serotypes with the enterovirus genus), adenoviruses (serotypes 40/41), norovirus GII, and astroviruses

(all human serotypes) (Ginn et al., 2021). See Tables S1 and S2 in Ginn. et al. 2021 for further details on the TAC and ddPCR targets (Ginn et al., 2021). Upon subsequent droplet-digital PCR (ddPCR) confirmatory and quantitative tests, we decided to develop the QMRA presented here using ETEC and *Shigella flexneri* only. We chose these bacterial pathogens because we detected them in 12% and 42% of our samples, respectively, and because of their genetic similarity to the *E. coli* spp.(Vieira et al., 2007) we enumerated through culture methods (Ginn et al., 2021; Rocha-Melogno et al., 2020). In addition, we used *Campylobacter jejuni* as a reference pathogen (Bivins et al., 2017) given its low median infectious dose (N50) (Black et al., 1988), and yielding more conservative risk estimates.

4.2.1 Pathogen's viability estimation

In our previous study we used a selective growth medium, Aquatest (Bain et al., 2015), to enumerate culturable fecal coliforms and *E. coli* that deposited onto three to six 100 mm Petri dishes over the course of 2 hours (Rocha-Melogno et al., 2020). We enumerated these bacterial targets as colony forming units (CFUs). We assumed that the reference pathogens used in this QMRA were culturable as well as viable. We acknowledge that not all viable pathogens are culturable (Oliver, 2005), and even those that are culturable may not be viable at the time of exposure (Haas, 2020). It is known that aerosols are a hostile micro-environment for bacteria which are subject to multiple killing stresses, e.g., desiccation, ultraviolet radiation (UV) exposure, and nutrient deprivation (Chang et al., 2017). Newer techniques are available and in development to assess a pathogen's viability i.e., assays with ethidium monoazide, propidium monoazide (Elizaquível et al., 2014) or azide intercalators (Leifels et al., 2019). However, it is not known which of these assays would provide the most accurate data for

risk assessments (Haas, 2020). Considering the low resource context of our work, culture methods that are widely available may allow the advancement of our understanding of the risk that bioaerosols near OWCs pose despite methodological limitations.

4.2.2 Hazard identification

4.2.2.1 Selection of reference pathogens and deposition flux

For this QMRA we selected ETEC, *Shigella flexneri* and *Campylobacter jejuni* as reference pathogens. ETEC is the most common bacterial cause of traveler's diarrhea, commonly found in places with unsafe access to water and sanitation (Black, 1990). It is an organism that produces heat labile or stable enterotoxins and colonizes the small intestine (Wolf, 1997). It is frequently detected in children with diarrhea; symptoms in response to infection by ETEC vary from mild to severe (Qadri et al., 2005). ETEC's median incubation period is estimated to be 42 h, with a median duration of illness of 3 days (Dalton et al., 1999) and a rate of 0-20% of asymptomatic cases in children (Qadri et al., 2005).

Shigella spp. are also fecal-oral transmitted pathogens, being able to easily adapt and reproduce in the colonic epithelial cells (Thomas and Keusch, 1996), causing ~165 million cases of shigellosis disease yearly, mostly in low and middle income countries (LMICs) (99%) and in children (69%) (Kotloff et al., 1999). They are considered endemic in places with poor sanitation (Thomas and Keusch, 1996) and have an incubation period of 1 to 4 days, and a duration of illness of 5-7 days (Dekker and Frank, 2015). *Shigella flexneri* is the most abundant serogroup, identified in ~60% of *Shigella* isolates in LMICs (Kotloff et al., 1999). It has been shown that *Shigella* spp. (including *Shigella flexneri*) have low median infectious doses compared to pathogenic *E. coli* spp.

(Enger, 2015; Haas et al., 1999), having low dose-responses in people exposed to *Shigella* spp. (DuPont et al., 1989). However, *Shigella* spp. are genetically very similar to EIEC, making them hard to differentiate experimentally (Vieira et al., 2007). This is the reason why we previously reported detections of both *Shigella* spp./EIEC, finding *Shigella* spp./EIEC genes in 11 out of 26 (42%) of our aerosol samples collected in La Paz during the rainy and dry seasons (Ginn et al., 2021).

Campylobacter spp. infections have risen in the last decade, affecting developed and developing countries and being commonly transmitted through the fecal-oral route, raw meat and water (Kaakoush et al., 2015). This organism is known to cause gastroenteritis in humans, with an incubation period of 2 to 5 days (Horn and Lake, 2013), though symptoms appear 24-72 h after ingestion, and last 6 days on average (Kaakoush et al., 2015). Infections by *Campylobacter jejuni* can also lead to autoimmune disorders (Guillain-Barré syndrome and Miller Fisher syndrome) (Man, 2011). *Campylobacter jejuni* also has a low median infectious dose compared to *E. coli* spp. (Black et al., 1988; Enger, 2015; Haas et al., 1999) and several studies have reported its growing resistance to antibiotics, increasing the risk of prolonged disease and mortality (Kaakoush et al., 2015), making it an important pathogen in settings with limited sanitation services.

Experimentally, we were not able to differentiate with certainty *E. coli* from total coliforms in our previous study, given the damage to the growth medium caused by sunlight exposure when conducting passive sampling on site (Rocha-Melogno et al., 2020). Therefore, we assumed that our deposition flux (expressed in CFU $m^{-2}h^{-1}$) reported previously was composed solely of *E. coli* spp. CFUs as a conservative approach. We used averaged pathogen genome copies (GC) to *E. coli* GC ratios (Table

3) to determine the fraction of pathogenic organisms present in our samples, an approach previously used in drinking water QMRAs (Bivins et al., 2017; van Lieverloo et al., 2007). We highlight that the bacterial flux data were the same for all scenarios, having the same starting point for the models.

4.2.3 Exposure assessment

We considered three exposure pathways: a) deposition on food followed by ingestion, b) deposition on surfaces followed by hand to mouth contact and ingestion, and c) inhalation of bioaerosols followed by ingestion, all near OWCs. Each scenario was modelled independently. Lacking behavioral data sets, our models combine assumptions and exposure parameters from published literature listed in Table 3. Our analyses do not consider bacterial regrowth, inactivation or acquired host immunity. We calculated the doses for the scenarios using eq. 1, eq. 2 and eq. 3.

$$\text{Eq. 1: Dose}_{\text{food}} = F_{[\text{CFU m}^{-2} \text{ h}^{-1}]} \times \text{PA}_{[\text{m}^2]} \times T_{[\text{h}]} \times \text{PF}_{[\%]}$$

$$\text{Eq. 2: Dose}_{\text{fomite}} = F_{[\text{CFU m}^{-2} \text{ h}^{-1}]} \times \text{HA}_{[\text{m}^2]} \times \text{TE}_{[\%]} \times T_{[\text{h}]} \times \text{TEb}_{[\%]} \times \text{Co}_{[\text{contacts h}^{-1}]} \times \text{Tb}_{[\text{h}]} \times \text{PF}_{[\%]}$$

$$\text{Eq. 3: Dose}_{\text{inhalation}} = C_{[\text{CFU m}^{-3}]} \times \text{IR}_{[\text{m}^3 \text{ h}^{-1}]} \times \text{Ti}_{[\text{h}]} \times \text{DF}_{[\%]} \times \text{PF}_{[\%]}$$

Where F = flux, PA = plate area, T = food or fomite contamination time, PF = pathogen fraction, HA = hand area, TE = fomite-to-hand transfer efficiency, TEb = hand-to-mouth transfer efficiency, Co = number of hand-to-mouth contacts, Tb = time spent at the playground, C = concentration, IR = inhalation rate, Ti = time spent walking near an OWC, DF = deposited fraction in upper respiratory airways followed by ingestion.

We back-calculated the CFU airborne concentrations converting our flux data set by dividing the fluxes ($\text{CFU} \times \text{m}^{-2} \times \text{h}^{-1}$) by the deposition velocity of a 6 μm diameter aerosol in still air ($3.6 \text{ m} \times \text{h}^{-1}$). We chose this particle size to model the worst case

inhalation followed by ingestion scenario, as previous research showed that these aerosols can deposit in the extrathoracic airways with a maximum of 65% deposition efficiency (Heyder, 2004). We assumed inhalation rates for light and moderate intensity activities for children and adults (EPA, 2011).

We then fit our left-censored flux and concentration data using recommended maximum likelihood estimation (MLE) methods (Canales et al., 2018), available in the 'fitdistrplus' package (Delignette-Muller and Dutang, 2015) in RStudio. This allowed the replacement of values below detection limit (<1 CFU) with values obtained from the cumulative distribution function. A gamma distribution was chosen instead of a lognormal distribution after assessing goodness of fit with Akaike's Information Criterion (Δ AIC: 22.564). Considering that the rainy season had higher CFU fluxes compared to the dry season, we used the rainy season culture-based data in this QMRA to evaluate the worst-case scenario.

4.2.4 Dose-response and risk characterization

We estimated the daily probability of infection upon ingestion using pathogen-specific dose-response functions, assuming the events happened once per day. We used a Beta-Poisson function for all reference pathogens (Bivins et al., 2017; Haas et al., 1999; Rose et al., n.d.), shown in equation 4 where N_{50} is the median infectious dose and α is a dimensionless infectivity constant (Deepnarain et al., 2020), included in Table 3 for each microorganism. The health endpoint (response) for ETEC is diarrheal disease (Haas et al., 1999), and positive detection and isolation in stool for *Shigella flexneri* (Rose et al., n.d.) and *Campylobacter jejuni* (Bivins et al., 2017; Black et al., 1988). We assumed a morbidity rate of 22-58% (uniform distribution) for *Shigella flexneri* (Dupont et

al., 1972), and a 30% morbidity rate for *Campylobacter jejuni* (Bivins et al., 2017). We also considered a probability of mortality of 1 in 10,000 cases for the three reference pathogens (Bivins et al., 2017)(Anderson et al., 2019).

$$Eq. 4: P = 1 - [1 + (\text{dose}/N_{50}) (2^{1/\alpha} - 1)]^\alpha$$

We calculated the daily probability of infection, illness and mortality by conducting stochastic Monte Carlo simulations in RStudio, drawing 10,000 values at random from the variables considered in the exposure pathways. This resulted in a distribution of probability of infection, illness and mortality for each of the reference pathogens. We conducted a sensitivity analysis of our models using Spearman's rank correlations (Hamilton et al., 2018) between the input variables and calculated risks to identify the most relevant inputs and uncertainty sources in our models. We also estimated the annual risk of infection using eq. 5 and assuming daily exposure.

$$Eq. 5: P_{\text{ann}} = 1 - (1 - P)^{365}$$

Table 3: Model parameters included in the QMRA.

| Model | Input parameter | Units | Distribution and/or value | Source |
|--------------------|-----------------------------|--------------------------------------|---|------------------------------|
| Food contamination | Bacterial flux (F) | CFU×m ⁻² ×h ⁻¹ | Gamma Shape: 1.55×10 ⁻¹ Rate: 2.83×10 ⁻⁴ | (Rocha-Melogno et al., 2020) |
| | Food contamination time (T) | h | Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17 - 2 | |
| | Plate area (PA) | m ² | 6×10 ⁻² | (Sharp and Sobal, 2012) |

| | | | | |
|---|--|---------------------------------|---|--|
| Fomites in playgrounds (includes flux, contamination time) | Children's hand area (HA) | m ² | Truncated normal Mean: 8×10^{-3} Std. deviation: 2×10^{-3} Range: 4×10^{-4} - 13×10^{-3} | (Agarwal and Sahu, 2010) |
| | Fomite contamination time (T) | h | Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17 - 2 | (Rocha-Melogno et al., 2020) |
| | Transfer efficiency: fomite to hand (TE) | % | Uniform distribution Range: 6-33 | (Mattioli et al., 2015) |
| | Transfer efficiency: hand to mouth (TEb) | % | Truncated normal Mean: 41 Std. deviation: 25 Range: 0.01 – 0.9 | |
| | Hand to mouth contacts (Co) | Number of contacts/h | Weibull Shape: 0.75 Scale: 12.59 | |
| | Time spent at playground (Tb) | h | Truncated normal Mean: 1 Std. deviation: 0.5 Range: 0.17 - 2 | (Rocha-Melogno et al., 2020) |
| Inhalation followed by ingestion | Bacterial concentration (C) | CFU×m ⁻³ | Gamma Shape: 1.78×10^{-1} Rate: 1.18×10^{-3} | (Rocha-Melogno et al., 2020) (Heyder, 2004) |
| | Inhalation rate (IR) | m ³ ×h ⁻¹ | Uniform Range: 0.65 – 1.74 | (EPA, 2011) |
| | Time spent walking near OWC (Ti) | h | Truncated normal Mean: 1 Std. deviation: 0.5 | (Rocha-Melogno et al., 2020) |

| | | | | |
|---|--|----------|--|--|
| | | | Range: 0.17 - 2 | |
| | Aerosol deposition fraction that will be ingested (DF) | % | 65 | (Heyder, 2004) |
| Food, Fomites and Inhalation followed by ingestion | Morbidity rate | % | (<i>S. flexneri</i>) Uniform Range: 22 – 58 | (Dupont et al., 1972) |
| | | | (<i>C. jejuni</i>) 30 | (Bivins et al., 2017) |
| | Probability of mortality | % | 0.01 | (Anderson et al., 2019; Bivins et al., 2017) |
| | Pathogen to <i>E. coli</i> ratio (PF) | ratio | 4.20×10 ⁻⁴ (<i>ETEC</i>) 3.90×10 ⁻⁴ (<i>Shigella flexneri</i>) 4.05×10 ⁻⁴ (<i>Campylobacter jejuni</i>) | (Ginn et al., 2021)(Bivins et al., 2017) |
| | Dose-response parameters (<i>ETEC</i> , <i>S. flexneri</i> , and <i>C. jejuni</i>) | multiple | $\alpha = 1.78 \times 10^{-1}$ $N_{50} = 8.60 \times 10^7$ | (Haas et al., 1999) |
| $\alpha = 2.65 \times 10^{-1}$ $N_{50} = 1.48 \times 10^3$ | | | (Dupont et al., 1972; Rose et al., n.d.) | |
| $\alpha = 1.51 \times 10^{-1}$ $N_{50} = 1.69 \times 10^3$ | | | (Bivins et al., 2017) | |

4.3 Results and discussion

Fecal bacterial aerosols from OWCs present non-negligible risks of infection. We estimated the daily risk of infection, illness and mortality for each reference pathogen for the aforementioned scenarios: food contamination from bioaerosol deposition, transmission from fomites in playgrounds and bioaerosol inhalation followed by ingestion. We caveat that the health endpoint (response) for ETEC was illness in the

following results. The highest median risk of infection for food contamination was linked to *Campylobacter jejuni*, followed by *Shigella flexneri* and ETEC (Table 4). We observed a similar trend in the estimated risk of infection through fomites in playgrounds near OWCs, and this scenario presented a lower daily risk of infection overall. Inhalation followed by ingestion had the highest median infection risk compared to contaminated food and fomites. Table 4 summarizes our daily and annual risk estimates by scenario.

Table 4: Summary of daily and annual median infection risk estimates by scenario with 90% confidence intervals in parentheses.

| Scenario | Pathogen | Daily probability of infection | Annual probability of infection | Greater than EPA's annual risk threshold? |
|-----------------------------------|--------------------|---|--|---|
| Food contamination | <i>C. jejuni</i> | 5×10^{-6} (2×10^{-10} – 4×10^{-4}) | 2×10^{-3} (7×10^{-8} – 14×10^{-2}) | Yes |
| | <i>S. flexneri</i> | 1×10^{-6} (5×10^{-11} – 9×10^{-5}) | 4×10^{-4} (2×10^{-8} – 3×10^{-2}) | Yes |
| | ETEC | 6×10^{-11} (2×10^{-15} – 4×10^{-9}) | 2×10^{-8} (7×10^{-13} – 1×10^{-6}) | No |
| Fomite transmission at playground | <i>C. jejuni</i> | 2×10^{-7} (5×10^{-12} – 3×10^{-5}) | 7×10^{-5} (2×10^{-9} – 1×10^{-2}) | No |
| | <i>S. flexneri</i> | 4×10^{-8} (1×10^{-12} – 8×10^{-6}) | 1×10^{-5} (4×10^{-10} – 3×10^{-3}) | No |
| | ETEC | 2×10^{-12} (1×10^{-16} – 4×10^{-10}) | 7×10^{-10} (4×10^{-14} – 1×10^{-7}) | No |
| Inhalation followed by ingestion | <i>C. jejuni</i> | 3×10^{-5} (4×10^{-9} – 1×10^{-3}) | 1×10^{-2} (1×10^{-6} – 3×10^{-1}) | Yes |
| | <i>S. flexneri</i> | 6×10^{-6} (8×10^{-10} – 3×10^{-4}) | 2×10^{-3} (3×10^{-7} – 4×10^{-6}) | Yes |
| | ETEC | 3×10^{-10} (4×10^{-14} – 1×10^{-8}) | 1×10^{-7} (1×10^{-11} – 4×10^{-6}) | No |

A 10^{-4} annual probability of infection (1 infection for every 10,000 personal exposure events), or 2.7×10^{-7} daily probability of infection (27 infections for every 100,000,000 personal exposure events) are commonly used as benchmark for tolerable risk of infection from drinking water (Fewtrell et al., 2001; Hamilton et al., 2019; Schoen

et al., 2017). *Campylobacter jejuni* and *Shigella flexneri* exceeded this threshold through food contamination and inhalation followed by ingestion, but did not through fomites. ETEC did not exceed the aforementioned risk threshold in any of the assessed exposure scenarios.

We include the estimated probability of illness per day for all scenarios in Figure 4. The median risk of illness was 6×10^{-11} (food), 2×10^{-12} (fomites), and 3×10^{-10} (inhalation) for ETEC; 5×10^{-7} (food), 2×10^{-8} (fomites), and 2×10^{-6} (inhalation) for *Shigella flexneri*; and 2×10^{-6} (food), 5×10^{-8} (fomites) and 8×10^{-6} (inhalation) for *Campylobacter jejuni*. Mortality risk was four orders of magnitude lower for all pathogens in all scenarios. These estimates suggest that the number of affected individuals would be below the limit of detection of epidemiological studies given their sample size limitations.

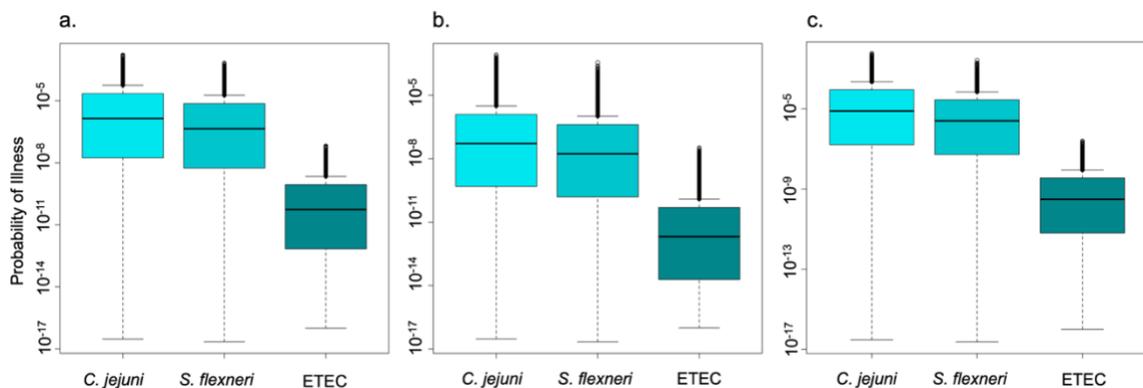


Figure 4: Probability of illness from aerosolized enteric pathogens a) contaminating street food, b) contaminating surfaces in playgrounds near OWCs, and c) being inhaled and then ingested.

Variability in risks by different pathogens is attributable to their specific dose-response functions. The average genome copies (GC) ratio to *E. coli* was of 4.20×10^{-4} for ETEC and 3.90×10^{-4} for *Shigella* spp., which we assumed to be for *Shigella flexneri*. Lacking GC data for *Campylobacter jejuni*, we used the average ratio (4.05×10^{-4}) of the

other reference pathogens for *Campylobacter jejuni*. These ratios are highly variable in faeces, wastewater and raw water but e.g., the ratio of *Campylobacter* spp. to faecal coliforms (*E. coli* and *Klebsiella* spp.) is typically of 1×10^{-4} in the aforementioned matrices (WHO, 2017a). Despite these ratios being in the same order of magnitude in our samples, the estimated risks of infection differed between *Campylobacter jejuni* and ETEC by five orders of magnitude, and so did for *Shigella flexneri* and ETEC in the food contamination scenario. We found a similar trend in the other scenarios. Therefore, the cause of this difference is the dose-response function, specific to each reference pathogen.

4.3.1 Sensitivity analysis

We assessed the sensitivity of our Monte Carlo models to their input variables using Spearman's rank correlation coefficients. These coefficients are a measure of the relationship of two variables (where 1 is equivalent to a perfect positive correlation, 0 indicates no correlation and -1 is a perfect negative correlation). The most important predictive factors were the bacterial fluxes (coefficient of 0.99 in the food scenario and inhalation scenario, 0.90 in the playground scenario). The number of hand-to-mouth contacts had the second highest coefficient (0.35), included only in the playground scenario. Exposure time was second in relevance in the inhalation model (0.11), and third in relevance in the playground model (0.18) and the food model (0.11). Inhalation rates had the lowest correlation coefficient (0.07). Our results agree with previous QMRAs that found pathogen measurements through culture and molecular methods were one of the largest contributors to risk estimation variability. (Canales et al., 2018; Crank et al., 2019; Julian et al., 2009). These results underline the importance of

continuous and spatially resolved monitoring of microbial indicators and human pathogens through active or passive aerosol sampling to improve future QMRAs accuracy.

4.3.2 Disease burden in La Paz using risk estimates

Lacking observational data regarding population behavior near OWCs, we estimated the number of people eating or commuting by foot near OWCs in La Paz using available reports online (Cooperación Suiza en Bolivia, 2015; El Deber, 2019). It is estimated that 25% of the urban population of Bolivia commute by foot (Cooperación Suiza en Bolivia, 2015), and 36.5% of the population of La Paz eat street food daily (El Deber, 2019). Considering the population of La Paz city (800,000) (Rocha-Melogno et al., 2020) there are 200,000 commuters by foot and 292,000 people eating street food every day.

Assuming that 1 out of 10 citizens commute or eat near OWCs, this yields 20,000 daily commuters and 29,200 people eating street food near OWCs. We assessed each exposure pathway independently, without adding exposures through inhalation and contaminated food. We assumed that commuters were the population at exposure risk through inhalation only, and people that eat street food as the population at exposure risk through food contamination only. Using our highest median annual risk estimates, we estimated 200 annual infection cases from inhaling and ingesting *Campylobacter jejuni*, and 58 annual infection cases from food contamination. These numbers suggest that bioaerosols near OWCs present a non-negligible annual risk of infection for the current population of La Paz that commutes by foot or consumes street food. Around 100,000 cases of diarrheal disease were registered in La Paz in 2016 (Página Siete, 2017). Assuming a similar prevalence in 2020, the cases attributable to bioaerosols near

OWCs would represent 0.26% of the total. These risks could increase in the future as the city's population continues to grow and more fecal waste is disposed in OWCs.

For the playground scenario, we considered children under 10 years old, who are 19% of the total population in La Paz (Bolivian National Institute of Statistics, 2020), or 152,000 children in 2020. Assuming 1 out of 10 children uses a playground near an OWC, this amounts to 15,200 children at risk of exposure to fecal bioaerosols. Using our highest median annual risk estimates from *Campylobacter jejuni* exposure through fomites, we estimated 1 annual infection case despite the assumed number of children at exposure. These numbers suggest that fomites contaminated with bioaerosols present a low risk of infection for the children of La Paz that use the playgrounds near OWCs, compared to the 10^{-4} annual risk threshold aforementioned (Fewtrell et al., 2001; Hamilton et al., 2019; Schoen et al., 2017). The Bolivian National Institute of Statistics estimated a diarrheal disease prevalence of 13.5% in children under 5 in La Paz in 2016 (Bolivian National Institute of Statistics, 2018). Assuming a similar prevalence in 2020 for children under 10 years old, the cases attributable to bioaerosols in playgrounds near OWCs would represent 0.005% of the total diarrheal disease prevalence. The Bolivian Ministry of Health and Sports estimates that 70% of diarrheal diseases are caused by contaminated food in Bolivia (Bolivian Ministry of Health and Sports, 2015). In this context, our findings suggest that bioaerosols near OWCs are not a major cause of diarrheal disease in La Paz. However, we recommend not placing playgrounds near OWCs to prevent exposure to enteric pathogens given previous overflows of the OWCs in La Paz (Hardy, 2009; Página Siete, 2020). Alternatively, we recommend implementing containment measures to prevent aerosol release from OWCs.

Given uncertainty in select model parameters, we can rapidly evaluate alternative exposure conditions using our AMIDRA web application. For example, let's assume a person in La Paz eats street food that has been exposed to the environment for 1 h near an OWC, and a ratio between *Campylobacter jejuni* and *E. coli* of 1×10^{-3} . The median risk of infection for that event would be of 2×10^{-5} (one order of magnitude higher than our previous estimate using a ratio of *Campylobacter jejuni* to *E. coli* of 4.05×10^{-4}). In the context of La Paz, this would result in 212 infection cases per year – 4 times greater than our previous estimate and 70 times greater than the EPA's annual risk standard for drinking water (1×10^{-4}). This highlights the importance of pathogen detection and quantification, as slight changes in the pathogen to *E. coli* ratios can have a noticeable effect on risk estimates.

4.3.3 Study limitations and recommendations

Our current QMRA models have the following limitations that should be considered to avoid oversimplification of their results. First, the models depend heavily on sampling data which have a direct impact on risk variability and thus are a major source of uncertainty. Large or longitudinal data sets of bacterial fluxes or bioaerosol concentrations are currently scarce in 2020, but would serve to reduce model uncertainty. Second, the lack of pathogen-specific viability data increased model uncertainty. Because of this, we used pathogen to *E. coli* GC ratios to estimate potential viable pathogens present in the samples. We acknowledge that the correlation between indicator organisms and pathogens varies greatly (Wu et al., 2011). However, we justify the use of indicator organisms given the low cost, wide availability and low technical skill requirement for their monitoring in resource-constrained settings (Rocha-Melogno et al.,

2019). Third, our data sets were collected in cross-sectional studies with small sample sizes. Therefore we qualify our risk estimates being at the screening level, requiring validation using longitudinal data. Fourth, microorganisms could be viable but non-culturable (Oliver, 2005), and our culture-based sampling methodology will have missed these organisms. This can result in risk underestimation, highlighting the importance of continuous monitoring to have larger data sets to work with. Fifth, we had to make multiple assumptions for the exposure assessment, adding uncertainty to our risk estimates. Further observational studies characterizing behaviors that result in exposure will reduce such uncertainties and the need for assumptions. In addition, further experimental studies are needed to better understand the inhalation exposure to pathogens and their consequent deposition in the upper respiratory airways followed by ingestion. Previous studies have estimated a 50% retention and deposition of aerosolized *Legionella* bacteria in lower respiratory airways of guinea pig models (Hamilton and Haas, 2016). This deposition rate may not vary widely by pathogens, as it is dictated by the size of the aerosol in which pathogens were transported into the respiratory tract (Thomas, 2013). However, the infection site will be pathogen-dependent due to their infection mechanism, sensitivity to immunological responses, or resistance to stomachal acidity (Thomas, 2013). Sixth, the dose-response functions we used were derived from human feeding studies in high income countries, not being specific to the population of Bolivia. The risk of infection could be lower or higher in a population that may have acquired immunity through chronic exposure or have compromised immune systems, respectively (Korpe and Petri, 2012), or when we consider secondary transmission of infections. Our estimates could improve in accuracy with more aerosol and fomite measurements and epidemiological studies that incorporate the aerosol

transmission pathway, secondary transmission, and dose-response models specific to the inhalation mode of exposure. This would allow model (re)calibration, particularly in the dose-response and exposure assessment. To our knowledge, there are no epidemiological studies characterizing the aerosol exposure pathway to fecal or enteric pathogens in cities with poor sanitation. Although our study provides an initial insight into the likely low health risks associated to fecal aerosols from OWCs, we recommend aerosol sampling as part of monitoring efforts of sanitation technology implementation and city infrastructure development. Seventh, a significant limitation was not including viruses in our assessment, estimated to cause ~60% of human respiratory and enteric infections (Boone and Gerba, 2007). We did not test viral viability and did not extrapolate the observed viability for the viruses we detected through PCR because bacteria and viruses respond differently to environmental and sampling conditions (Tang, 2009). This limited our ability to characterize the risk of viral infections near OWCs.

Finally, despite a noticeable increase in QMRA scientific literature (Haas et al., 2014; Owens et al., 2020), practical tools for its larger implementation are scarce. With our web application (AMIDRA), researchers and public health practitioners can translate bioaerosol monitoring data into potential health outcomes without having to develop QMRAs in statistical software. Our simple models can also serve as a starting point for professional risk assessors, with the caveat that QMRA's complexity and inclusion of multiple variables has not been found to be related with increased certainty of risk estimation (Owens et al., 2020). The results of our sensitivity analysis suggest that certainty of risk estimates would benefit more from continuous monitoring, providing larger pathogen's datasets of the most relevant input and uncertainty source. The correct handling of fluxes or concentrations data when having values below detection

limits could also improve risk estimation certainty (Canales et al., 2018). Including PCR or pathogen-specific culture data to estimate pathogen to indicator ratios or concentrations in the environment could further reduce uncertainty in risk estimates, at higher costs and technical skill requirements.

5. Quantitative risk assessment of covid-19 aerosol transmission indoors: a web application

This chapter discusses the development of a web-based application to estimate the risk of infection of COVID-19 through aerosols indoors, assuming physical distancing is kept between people, i.e., neglecting short range transmission. Three modelled scenarios are presented and compared to epidemiological studies looking at similar settings: classrooms, weddings, and heavy exercise sessions.

5.1 Introduction

The Coronavirus Disease 19 (COVID-19) pandemic has clearly shown the deficiencies of our global health systems, highlighting the lack of sufficient interdisciplinary collaboration to establish effective transmission prevention strategies. One of the key areas affected by this lack of collaboration is the understanding of aerosol transmission of pathogens. It took the WHO and CDC several months to recognize the role of aerosol transmission in the spread of COVID-19, leading to slow implementation of transmission controls like increased ventilation with outdoor air and indoor air filtration.

There is a pressing need to use tools and terminologies that bring together the scientific community to understand respiratory disease transmission, beyond traditionally accepted terminologies in independent fields. Fortunately, attempts to reconcile the

terminology used in the medical and epidemiological field with aerosol science are ongoing (Milton, 2020; Morawska and Cao, 2020; Morawska and Milton, 2020). In parallel, mechanistic tools have been developed to understand fate and transport of respiratory pathogens since the 1950s (Noakes and Sleight, 2009). Here we present a mechanistic stochastic model focused on SARS-CoV-2, the respiratory virus that causes COVID-19.

5.1.1 Theoretical framework

SARS-CoV-2 is the third known coronavirus to cause deadly pneumonia in humans in the last 20 years (Walls et al., 2020). It is a single-stranded RNA betacoronavirus of zoonotic origin that infects cells using its spike glycoprotein to hijack the angiotensin converting enzyme 2 (ACE2) receptors (Romano et al., 2020) abundantly present in the surface of the human lung and small intestine (Hamming et al., 2004). Viable SARS-CoV-2 and its genetic material have been detected in oral and nasal secretions (Jeong et al., 2020; Wölfel et al., 2020), and it is known that these secretions can be aerosolized when a person exhales, talks, coughs, sneezes or undergoes a medical procedure e.g., endotracheal intubation (Tang et al., 2020). In fact, oral viral loads in symptomatic and asymptomatic patients have been found in the range of 10^8 - 10^9 RNA copies/mL (Buonanno et al., 2020b; Pan et al., 2020) and multiple studies have detected SARS-CoV-2 RNA in the air with concentrations in the range of 1.8×10^3 – 3.4×10^3 RNA copies/ m^3_{air} (Binder et al., 2020; Chia et al., 2020; Ong et al., 2020; Santarpia et al., 2020). Lednicky et al. were able to culture the aerosolized virus both in clinical and non-clinical settings, finding 2×10^3 – 74×10^3 TCID₅₀ units/ m^3_{air} (Lednicky et al., 2021, 2020). Considering the aforementioned field findings, the

hypothesis that COVID-19 could be transmitted through aerosols that began with laboratory studies (van Doremalen et al., 2020), was further strengthened by retrospective outbreak analyses (de Man et al., 2020; Tang et al., 2020). This increasing evidence in support of aerosol transmission of COVID-19 warranted the need of risk assessment tools to establish mitigatory measures in addition to physical distancing, masking and hygiene. Therefore, the goal of this work was to develop a practical web-based tool for academics and non-academics to quantify and help understand the risk of aerosol transmission of COVID-19.

Quantitative microbial risk assessments (QMRAs) have been conducted for decades now, typically following four steps: hazard identification, exposure assessment, dose-response model selection, and risk characterization (Haas et al., 2014). This structured approach allows us to determine the human health risk from exposure to pathogens by incorporating environmental measurements, epidemiological and laboratory data. However, pathogen dose-response functions are not readily available when new pathogens emerge. This common lag leads to the use of the classic Wells-Riley model (Nicas et al., 2005; Noakes and Sleight, 2009) and modifications (Gammaitoni and Nucci, 1997) of airborne transmission. The Wells-Riley model uses a similar approach to QMRA, without including pathogen-specific dose-response functions. To do this, the model invokes the use of *quantum* (or *quanta* if plural) – the equivalent to an unknown dose of infectious pathogen with a 63% probability of infecting a susceptible person (Sze To and Chao, 2010).

Miller et al. used the amended Wells-Riley model (Gammaitoni and Nucci, 1997) to characterize the Skagit Valley Chorale COVID-19 outbreak in high detail and retrospectively estimated the quanta emission rate by one index case (Miller et al.,

2020). In a similar way, Buonanno et al. built upon the Wells-Riley model to use SARS-CoV-2 RNA copies to estimate quanta emission rates in retrospective and prospective applications (Buonanno et al., 2020a). These models are particularly useful to understand the mechanistic effect of certain risk mitigation interventions to prioritize e.g. ventilation over sanitization when faced with a respiratory disease pandemic. Critics of the Wells-Riley model do not support the use of *quanta* as it is a hypothetical parameter that implicitly assumes that every pathogen has the same dose-response. QMRA, in contrast, uses pathogen-specific dose-response models, employing quantitative measurements of the pathogen (Sze To and Chao, 2010), including e.g., results of Median Tissue Culture Infectious Dose (TCID₅₀) or plaque forming units (PFU) assays.

In this paper, we built on the model by Miller et al. (Miller et al., 2020) to develop a user friendly risk assessment web application as there was a pressing need for educational and assessment tools like ours during the pandemic.

5.2 Methods

5.2.1 Key definitions in use

We define aerosol transmission as the exposure to a pathogen through inhalable aerosol particles <100 µm in size (Milton, 2020), regardless of time and distance, leading to infection. We also highlight that these aerosols are emitted by a contagious person when breathing, speaking, sneezing or coughing (Chen et al., 2020; Leung et al., 2020), not only during “aerosol-generating procedures” (Tran et al., 2012). We do not include modelling of particles >100 µm in size, which we refer to as droplets, too big to be inhaled (Milton, 2020) and only relevant for short range transmission.

Current studies have shown the importance of distance from a contagious individual shedding respiratory pathogens as a way to reduce exposure to large respiratory droplets and short-range aerosols (Chen et al., 2020). In fact, Chen et al. found that aerosol transmission through inhalation dominates at most distances when an infected individual is talking or coughing. Large droplet deposition onto mucosa only dominates exposure at 0.2 m from the contagious person talking and 0.5 m when coughing (Chen et al., 2020). These larger droplets will settle down due to gravity before they evaporate, potentially contaminating surfaces and typically travelling less than 2 m before settling down (Xie et al., 2007). On the other hand, aerosols particles (<100 μm in size) will evaporate within seconds and remain suspended in the air, potentially leading to short- and long-range aerosol transmission (beyond 2 m) (Prather et al., 2020b). These aerosols can accumulate in indoor settings, and travel long distances following airflow patterns. In this work, we present modelling results of long range aerosol transmission in indoor settings.

5.2.2 Mathematical model assumptions

Following the methodology used by Miller et al., the scenarios presented here assume that: i) there is one contagious individual emitting SARS-CoV-2 at a constant rate of quanta over time, ii) the emitted quanta by the contagious individual is immediately and uniformly dispersed in the room upon emission, iii) there is no previous presence of quanta in the room, and iv) quanta are removed from the room by filtration through masks and first-order processes including ventilation with outdoor air, aerosol deposition, and viral inactivation (Miller et al., 2020). In the Skagit Valley Chorale outbreak investigated by Miller et al., susceptible individuals became infected regardless

of their distance from the index case (i.e., the first infected person identified). This supports the assumption of an ideally mixed indoor environment where quanta are uniformly distributed in the air, used in previous modelling efforts (Buonanno et al., 2020b; Rudnick and Milton, 2003). This approach is considered appropriate when the overall goal of the exercise is to identify ways in which aerosol transmission can be mitigated regardless of the specific quanta emission temporal and spatial patterns, room geometries or airflow dynamics (Miller et al., 2020; Sze To and Chao, 2010).

5.2.3 Hazard identification

COVID-19 symptoms begin after a median incubation time of 5.2 days in a distribution with a 95th percentile of 12.5 days (Li et al., 2020). However, viral loads peak early on the course of the disease (before symptom onset), with a median of 9 days from infection to viral clearance (Pan et al., 2020; Park et al., 2020). Considering these findings and their own, Binder et al. suggested that aerosol transmission may be most important early in the course of the disease, when people are more contagious (Binder et al., 2020). Therefore, in our model, we assumed that the infected individual was shedding infectious virus at peak viral load. This assumption leads to conservative risk of infection estimates.

5.2.4 Exposure assessment

Lacking observational data, we combined environmental and exposure characteristics described in the literature for our model. The parameters used in this risk assessment are found in Table 5. These parameters are expressed as statistical distributions that allow us to draw random values from them using the Monte Carlo method (Haas et al., 2014). This approach allows us to estimate a range of possible risk

estimates, instead of single values. To calculate the inhaled dose, we used Equations 1, 2, and 3, adapted from Miller et al. 2020 by solving for the ideally-mixed quanta concentration in the room at steady state.

Eq.1:

$$C = \left\{ \left(\frac{E}{L \times V} \right) \times Fa \right\} \times \left\{ 1 - \left[\left(\frac{1}{L \times t} \right) \times (1 - e^{-L \times t}) \right] \right\}$$

Eq.2: $VI = T \times B$

Eq.3: $D = C \times VI \times Fb$

Where C = quanta concentration in the room assuming a well-mixed environment [quanta m⁻³], E = quanta emission rate [quanta h⁻¹], L= sum of first-order quanta losses due to ventilation, deposition and viral decay [h⁻¹], Fa = mask filtration inefficiency for the contagious person [%], and t = time spent by all subjects in the room [h]. VI = volume of air inhaled [m³], B = breathing rate [m³ h⁻¹], D = dose of inhaled quanta [quanta], and Fb = mask filtration inefficiency for the susceptible population [%]. We considered three exposure scenarios in indoor settings: a) a classroom with a contagious student, b) a social gathering with a contagious attendee, and c) a heavy exercise class with a contagious instructor.

5.2.5 Risk characterization

We estimated the risk of infection using eq. 4, known as the Wells-Riley model of infection amended by Gammaitoni and Nucci (Gammaitoni and Nucci, 1997; Sze To and Chao, 2010).

$$Eq.4: P_i = 1 - e^{-D}$$

Where P_i = probability of infection for the event [infections/personal exposure events], and D = dose of inhaled quanta [quanta]. We highlight that the estimated

probability of infection assumes that there is only one contagious person in each exposure scenario. The absolute probability of infection can be estimated by incorporating the prevalence of infections in the location where the event is happening e.g., multiplying the conditional probability from Eq.4 by the percentage of cases in a community. However, incorporating the aforementioned parameter introduces greater uncertainty to the model and could underestimate the risk of disease transmission because estimating the overall prevalence in the community would require large samples that are geographically and sociologically distributed (Ward, 2013). Because the main goal of our work was to provide an educational tool to understand the relative risk of COVID-19 transmission through aerosols indoors, we did not include the calculation of absolute risk of infection. In this study, we used eq.5 to calculate the risk of infection considering the frequency of events in addition to assessing independent events.

$$Eq.5: P = 1 - (1 - P_1)^n$$

Where P is the probability of infection considering the frequency of events. The inclusion of this latter calculation allows us to understand how risk is compounded and increases with the repetition of events.

Table 5: Model parameters included in the COVID-19 aerosol transmission model.

| Input parameter | Units | Distribution, range and value | Note | Source |
|--|-------------------|--------------------------------------|---|--|
| <i>Emission of virus</i> | quanta/h | triangular, 2-60.5, AV: 9.4 | classroom | (Buonanno 2020a,b), interpreted by (Jimenez, 2020) and adjusted by authors |
| | | triangular, 5.6-170, AV: 11.4 | wedding | |
| | | triangular, 13.5-408, AV: 63.1 | heavy exercise | |
| <i>Mask filtration efficiency</i> | % | 50 | for contagious person | (Jimenez, 2020) |
| | | uniform, 30-50 | for susceptible population | |
| <i>Ventilation (ACH)</i> | 1/h | uniform, 1 - 3 | all scenarios | (ANSI/ASHRAE, 2019) |
| <i>Virus deposition</i> | 1/h | 0.240 | conservative point estimate | (Chatoutsidou and Lazaridis, 2019), interpreted by Buonanno 2020a |
| <i>Virus inactivation</i> | 1/h | 1.621 | conservative point estimate | (DHS, 2020), interpreted by (Jimenez, 2020) |
| <i>Exposure time</i> | h | uniform, 1 - 3 | classroom | Assumptions |
| | | uniform, 3 - 4 | wedding | |
| | | uniform, 0.5 - 1.5 | heavy exercise | |
| <i>People in the room</i> | - | 30 | university classroom | Assumptions |
| | | 200 | wedding | |
| | | 30 | heavy exercise | |
| <i>Room's free area</i> | m ² | 330 | classroom and heavy exercise | Assumptions |
| | | 2200 | wedding | |
| <i>Room's height</i> | m | 2.75 | all scenarios | (GSA, n.d.) - Space planning |
| <i>Inhalation rates for susceptible population</i> | m ³ /h | 0.25 - 0.32 | classroom, 16-31 years old students in sedentary activity | (EPA, 2011) - Exposure Handbook Chapter 6 |
| | | 0.66 - 1.74 | wedding, min. for 3-6 year old child in sedentary activity, max. for 51-61 adult in moderate intensity activity | |
| | | 3.00 - 3.18 | heavy exercise, 21-61 years old in high intensity activity | |

5.2.6 Web application development

The web application was developed using RShiny version 1.5.0 and is available at https://rapidqmra.shinyapps.io/Rapid_QMRA/. The COVID-19 aerosol transmission model includes a user interface with basic and advanced modifiable parameters. Basic parameters include exposure time, free area and height of the indoor setting, the number of people in the room and the ventilation rate (air changes per hour or ACH). The application restricts the number of people in the room by requiring an area of 11 m² per person. This area assumes that people inside the room keep a distance of about 1.8 m at all times, preventing close-range aerosol or droplet transmission.

Advanced parameters include mask efficiencies for the susceptible population, loss of quanta by deposition, viral inactivation, inhalation rates, and quanta emission rates. The application runs 10,000 stochastic Monte Carlo simulations, drawing values at random from the inputted parameters. Every change re-starts the calculations and updates: 1) a histogram displaying the distribution of estimated quanta doses in the 10,000 simulations, 2) a boxplot showing the distribution of probability of infection estimates, and 3) a percentiles' table presenting the doses that result in a given probability of infection, and a potential number of cases arising from the event.

The user interface of the web application also includes a risk comparison chart. The table includes common risks that people are faced with on a daily basis, e.g., choking from inhalation and ingestion of food, falling, or car accidents to place the risk of COVID-19 in perspective. The table includes the WHO estimate of infection fatality rate for COVID-19 (1%) (WHO, 2020).

5.3 Results and discussion

The web application was launched on September 15th of 2020. We were granted a professional subscription free of charge by R Shiny to support the application during peak traffic in late 2020, and the application remains free to use. The application was made public through Twitter, reaching an audience of 76,000 people as of February 8th of 2021. Using Google Analytics, we found that more than 750 people from 37 countries around the globe have used the application. It had a peak of 56 users per day by the end of October, and the number of users decreased over time, as expected. Most users (77%) accessed the application through a desktop computer and 29% were returning visitors.

5.3.1 Risk comparison between the assessed scenarios

We estimated the risk of transmission of COVID-19 for the aforementioned scenarios: a classroom with a contagious student, a wedding with a contagious attendee, and a heavy exercise class with a contagious instructor. The median risk of infection [infections/personal exposure events] was highest in the heavy exercise scenario (2.6×10^{-2}), followed by the wedding (2.9×10^{-3}) and classroom (9×10^{-4}) scenarios. Next we present our observations in each specific modelled scenario, testing different conditions e.g., risk of infection when attendees are not wearing a mask, or rooms having poor ventilation.

5.3.1.1 Risk in classrooms with one contagious student

We found that no cases would arise in a single class with one contagious student, where the estimated median probability of infection was 9×10^{-4} infections/personal exposure events. In fact, no cases would arise in the worst-case

scenario that assumed that none of the 30 students wore a mask and the room was poorly ventilated (Table 6). However, when we consider the frequency of events (e.g., 10 classes without masks and with poor ventilation), one contagious individual could infect one person (90% CI: 0.3-3.2). If all the students wear a mask and the room ventilation ranges 1-3 ACH, no person would be infected in those 10 classes (90% CI: 0.1-0.7). The low number of estimated cases arising can be primarily attributable to 1) the low quanta emission rate of the contagious student, and 2) the low inhalation rates of the susceptible students because of their primarily resting condition. The first condition limits the concentration of infectious virus in the room, and the second condition limits the amount of infectious virus that the students inhale.

A previous report by Public Health England found that 5 out of 55 COVID-19 outbreaks (9%) were linked to student-student transmission, and 16 out of 55 outbreaks (7.6%) were linked to student-to-staff transmission (Ismail et al., 2021). The authors also found that student-driven outbreaks resulted in a maximum of 6 secondary cases with a median of 1 case (Ismail et al., 2021). Using our model, we can observe a similar number of cases arising (median of 1, 90% CI: 0.3-3.2) after one contagious individual attends 10 classes with 29 other classmates, if the rooms were poorly ventilated and the students were not wearing a mask. It is likely that the students in England were taking precautions, e.g., physical distancing and wearing masks, yet there were small outbreaks occurring in the summer of 2020. These observations suggest that physical distancing and mask-wearing are not sufficient risk mitigation strategies to avoid COVID-19 outbreaks in educational settings.

Table 6: Risk summary of the assessed scenarios (in percentage). See Table 5 for parameter details.

| Scenario | Percentile | Risk of infection (base model) | Risk without masks | Risk without masks and poor ventilation (0.2-1 ACH) | Risk with masks and excellent ventilation (6-8 ACH) | Risk of multiple events with masks & excellent ventilation (10 classes, 3 weddings) |
|--------------------------------------|---------------|--------------------------------|--------------------|---|---|---|
| Classroom (30 attendees) | 5th | 0.02 | 0.07 | 0.10 | 0.01 | 0.11 |
| | 25th | 0.05 | 0.16 | 0.23 | 0.02 | 0.24 |
| | median | 0.09 | 0.28 | 0.40 | 0.04 | 0.40 |
| | 75th | 0.14 | 0.46 | 0.65 | 0.07 | 0.65 |
| | 95th | 0.25 | 0.80 | 1.14 | 0.11 | 1.12 |
| Wedding (200 attendees) | 5th | 0.08 | 0.26 | 0.41 | 0.04 | 0.11 |
| | 25th | 0.17 | 0.58 | 0.85 | 0.08 | 0.23 |
| | median | 0.29 | 0.97 | 1.43 | 0.13 | 0.40 |
| | 75th | 0.47 | 1.54 | 2.26 | 0.21 | 0.63 |
| | 95th | 0.80 | 2.59 | 3.70 | 0.35 | 1.04 |
| Heavy exercise (30 attendees) | 5th | 0.15 | 2.20 | 2.73 | 0.37 | 3.59 |
| | 25th | 0.66 | 4.95 | 6.30 | 0.80 | 7.72 |
| | median | 2.59 | 8.52 | 10.99 | 1.37 | 12.89 |
| | 75th | 4.27 | 14.01 | 18.33 | 2.21 | 19.99 |
| | 95th | 7.62 | 24.44 | 31.85 | 3.82 | 32.29 |

5.3.1.2 Risk at weddings with one contagious individual

We estimated one case arising (90% CI: 0.2-1.6) in the wedding scenario with 200 attendees. Infection cases increase to 2 (90% CI: 0.5-5.2) when the attendees are not wearing masks, and three (90% CI: 0.8-7.4) when the room is also poorly ventilated. If a similar event would happen three times in the same location, the number of cases increases to 8 (90% CI: 2.4 – 21.3), e.g., if the contagious person worked at the wedding venue over a weekend. We qualify these seemingly low estimates with the fact that 1) we assumed there was only one contagious individual, 2) everyone kept ~2 m distance at all times, c) the room was large enough to dilute the virus to concentrations that have

low probability of delivering enough virus to cause several infections. The assumption that people would keep ~2 m of distance with each other at all times at a wedding is probably unrealistic. Our model would benefit from observational studies characterizing the behavior of attendees at weddings or similar social and professional gatherings to better calibrate our assumptions.

A previous study characterized two COVID-19 outbreaks at weddings that took place in Jordan and Uruguay. The wedding event in Jordan had 400-450 attendees and dozens of infected attendees were detected soon after. The wedding event in Uruguay had 500 attendees and 44 (8.8%) infection cases were traced back to the wedding, with the index case having attended the wedding hours after arriving from Spain (Saidan et al., 2020). In our modelled scenario, one contagious individual could infect 0.5-1.5% of 200 people through long range aerosol transmission. Comparing our model to the outbreak in Uruguay, it is likely that short range aerosol transmission played a role in the outbreak. This hypothesis is supported by the finding that fomite transmission plays a minor role for COVID-19 (Mondelli et al., 2021; Pitol and Julian, 2021).

5.3.1.3 Risk at heavy exercise sessions with one contagious instructor

The heavy exercise scenario presented the highest risk compared to the wedding and classroom scenarios. We estimated 1 case arising (90% CI: 0.0 – 2.2) even when the 30 attendees were wearing masks and the room had ventilation rates of 1-3 ACH. Cases increased to 2 (90% CI: 0.6 – 7.1) when the attendees were not wearing masks and 3 (90% CI: 0.8 – 9.2) when the room was also poorly ventilated. We estimated that 20 (90% CI: 7.0 – 28.4) cases would arise if the index case was a contagious instructor teaching this exercise class ten times in a poorly ventilated room and without masks. Seven cases would arise (90%CI: 0.4 – 15.9) if everyone wears a mask in these ten

exercise classes and the room has ventilation rates of 1-3 ACH. In this scenario, the number of cases after ten fitness classes (with everyone wearing a mask) can be reduced to 4 (90% CI: 1.0 – 9.4) by increasing the room's ventilation rates to 6-8 ACH.

Previous investigations of COVID-19 transmission in heavy exercise classes in South Korea found an overall attack rate of 26.3% (Jang et al., 2020). These outbreaks were associated with a 4 hour fitness workshop held in February 2020 that identified 8 out of 27 (29.6%) infected attendees (Jang et al., 2020). Using our heavy exercise model with a similar event duration, and assuming a ventilation rate of 4-6 ACH (equivalent to 2-4-fold the minimum ventilation rate for aerobics rooms (ANSI/ASHRAE, 2019)), we estimated 7 (90 CI: 2.2 – 13.92) cases arising out of the 27 attendees. Our estimated attack rate of 25% is close to the overall attack rate in the South Korean outbreaks, illustrating the practical use of our web application to retrospectively evaluate events where long-range aerosol transmission of COVID-19 is suspected. Unfortunately, the report by Jang et al. did not have details on the geometries or ventilation rates of the indoor spaces where the fitness class took place. If the ventilation rates were higher in reality, it is likely that short-range droplet and aerosol transmission played a bigger role in the South Korean outbreak, as long-range aerosol transmission would have been reduced.

5.3.2 The effect of ventilation in risk mitigation

Figure 5 demonstrates the effect of ventilation in long-range aerosol transmission risk reduction for different exposure times in a heavy exercise scenario with one contagious individual and attendees not wearing masks. Here we show that ventilation has a relatively low effect on risk reduction when exposure is limited to 30 minutes, with median risks of infection <5% regardless of the ventilation rate. The effect of ventilation

is more noticeable as time of exposure increases. Risk of transmission can be reduced by ~40-60% with ventilation rates of 5 ACH for 1-4 h exposure events, and ~70% with ventilation rates of 10 ACH for 4 h exposure events. However, the marginal benefits in risk reduction are smaller as ventilation rates increase beyond 5 ACH.

The previous examples and our findings suggest that heavy exercise activities would require high ventilation rates, and/or masks with higher filtration efficiencies to reduce the risk of long range aerosol transmission. The use of high filtration efficiency masks could be uncomfortable when doing heavy exercise, prompting people to avoid them or misuse them. Therefore, heavy exercise activities should probably not be performed in indoor settings during the COVID-19 pandemic.

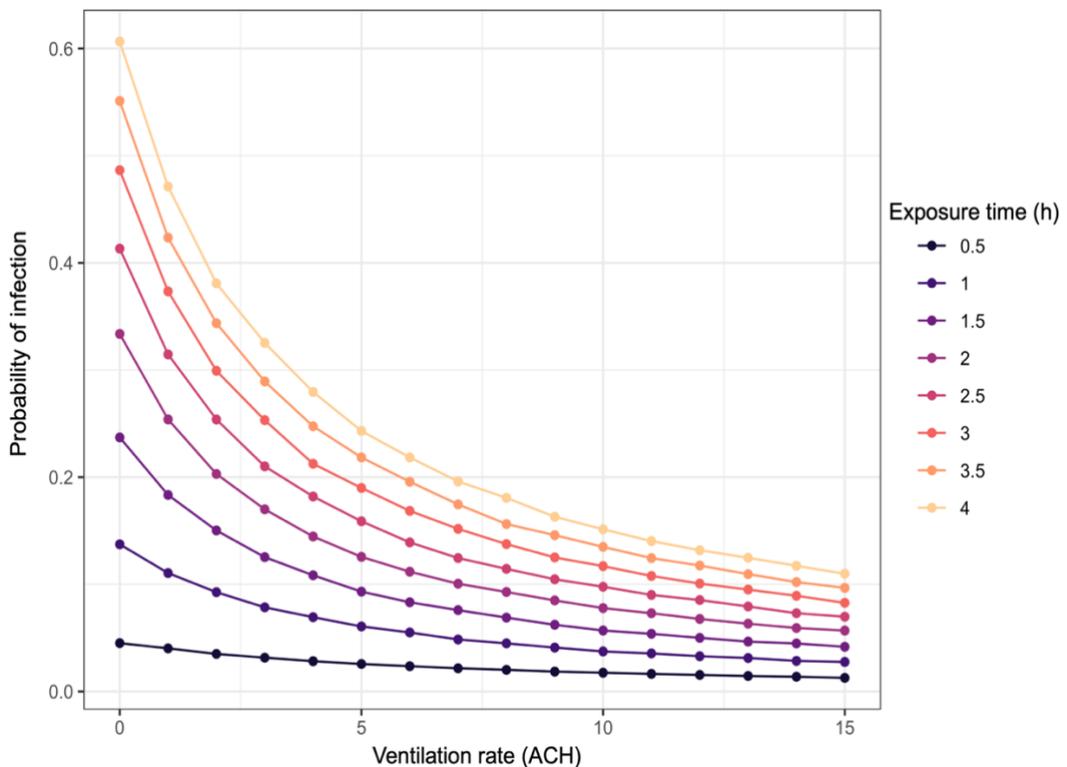


Figure 5: Probability of infection as a function of time and ventilation rates in an indoor heavy exercise scenario with one contagious instructor.

5.3.3 Study limitations and recommendations

Our stochastic risk assessment model has the following limitations that require further investigation. First, the model was based on quanta, a theoretical amount of infectious virus calculated from previous outbreaks (Buonanno et al., 2020b; Miller et al., 2020), instead of measurements of viral concentrations in emissions or aerosol samples e.g., plaque forming units or TCID50 assays. Buonanno et al. attempted to integrate the use of quanta with viral RNA copies to illustrate their relationship in risk assessments and highlight the importance of ventilation (Buonanno et al., 2020b). Their limitation, however, was the need to assume a 0.01-0.1 ratio of infectious virions to RNA copies (Buonanno et al., 2020b; Watanabe et al., 2010). Future studies would ideally measure the amount of infectious virus found in different aerosol sizes released during different activities, at the source and in the indoor environment. These measurements would reduce the uncertainty in the amount of infectious virus present in aerosols.

Second, our model did not include aerosol dynamics in the assessed scenarios. Future studies would benefit from including computational fluid dynamics (CFD) modelling to consider airflow and aerosol dispersion to find locations inside a room where air may stagnate and present higher risk of exposure to respiratory viruses. Private companies already use such models to develop personalized ventilation systems to improve comfort indoors, or clean rooms in healthcare settings. Building design and construction after the COVID-19 pandemic are likely to emphasize good ventilation and air filtration. The main challenge will be the system's optimization to provide clean air, with the lowest use of energy, maintenance, and construction cost.

Third, our model did not include short-range aerosol transmission modelling. Chen et al. showed that short-range aerosol transmission dominates exposure to

respiratory pathogens at close-contact (Chen et al., 2020). Typical indoor ventilation systems cannot prevent short-range aerosol transmission because their flow velocity (~0.2 m/s) cannot significantly modify the expired airflow trajectory with higher velocities (Chen et al., 2020; Liu et al., 2017) e.g., 3.9 m/s when speaking, 11.7 m/s when coughing (Chao et al., 2009). Masks interrupt this airflow trajectory allowing the human's body heat plume to carry the leaked aerosols upwards and diluting them in the room's air (Chen et al., 2020). Personalized ventilation systems may be an alternative tool to reduce short-range aerosol transmission, especially where people remain stationary for extended periods of time (Melikov, 2004). Innovative personalized ventilation systems are likely to receive increased research and development in the next decade.

Fourth, the decay constant for infectious SARS-CoV-2 was obtained from a regression model based on laboratory experiments that tested the virus against UV radiation, temperature and relative humidity changes (Dabisch et al., 2021; Schuit et al., 2020). A recent pre-print discussing the mechanistic theory behind viral inactivation was published online in December 2020 (Morris et al., 2020). The current science of viral decay suggests that enveloped viruses (including SARS-CoV-2) are more susceptible at a relative humidity (RH) of 40-65%, but more stable at <40% RH and >85% RH, regardless of the temperature conditions (Morris et al., 2020). Future risk assessment models would benefit from using this mechanistic theory to estimate the decay constant and improve its accuracy.

Fifth, our model does not consider aerosol resuspension from surfaces. Previous studies suggested that SARS-CoV-2 could resuspend from surfaces such as floors and protective equipment when people move (Liu et al., 2020; Tang et al., 2020). This resuspension mechanism has been observed for Influenza A virus before (Asadi et al.,

2020). These findings suggest the potential transmission of respiratory pathogens through aerosolized fomites (Asadi et al., 2020; Liu et al., 2020; Prussin and Marr, 2015). Our model currently assumes a permanent deposition of infectious virions, being removed from the air. Future investigations characterizing the impact of aerosol resuspension and its impact on airborne transmission of diseases are needed.

In conclusion, this study presented a practical stochastic model and user-friendly application to understand the risk of long-range aerosol transmission of COVID-19 in indoor settings. We compared previous outbreaks characterized in the literature to three modelled scenarios using our web application, demonstrating its usability in retrospective and prospective assessments. Overall, we found that the risk of infection through aerosols indoors increased 309-332% when people were not wearing masks, and 424-488% when the room had poor ventilation in addition to no masks being worn across the scenarios. We also showed the impact of exposure time, mask use and ventilation in reducing the risk of long-range aerosol transmission of COVID-19. Our findings highlight the importance of a layered risk reduction strategy to control respiratory disease transmission indoors. Our web application remains free to use as an educational tool, helping engineers, public health professionals, and space planners conduct screening risk assessments efficiently.

6. Conclusions

This thesis focused on the development of practical methodologies to facilitate the monitoring of urban drinking water quality, bioaerosols in cities with poor sanitation, and risk assessment frameworks to translate these monitoring data into potential public health outcomes. The work presented in Chapters 2, 3 and 4 had the primary objective of providing useful tools in low-resource settings. While new water or bioaerosol monitoring technologies keep emerging in high-income countries, little attention had been placed on the optimization of available monitoring methods to help urban populations without safely managed drinking water or sanitation services. This thesis tries to address this gap, with Chapters 2 and 3 already published in peer-reviewed scientific journals. Chapter 5 was later added to address the COVID-19 pandemic, providing a practical tool for engineers, public health professionals, and space planners to conduct screening risk assessments of long-range aerosol transmission of COVID-19. The web application developed in this final chapter was among the first available worldwide, based on a collaborative effort between engineers, microbiologists, and infectious disease researchers.

For the research reported in Chapter 2, I co-led a team of 20 undergraduate students to characterize drinking water samples at the point-of-consumption in Cochabamba, Bolivia. We took drinking water samples from 80 households at the point of consumption i.e., water people would use for personal consumption. We tested the samples for turbidity, conductivity, total dissolved solids, free and total chlorine, and conducted basic microbial testing in a local laboratory. Chemical water quality analysis of the samples was done in the United States. We also conducted a household survey to characterize the service level of drinking water, sanitation and hygiene the citizens had

according to the SDG 6 terminology. Consumables had a cost of US \$51 per household, and we estimated an average time requirement of 6.4 person-hours per sample site (n=80). A 200-household survey would have a consumable cost of US \$25 per sample site, and a time requirement of 3 weeks with a team of 6 researchers. Our cross-sectional study also found that 71% of the water samples from Cochabamba met the WHO microbial safety criteria, 96% met the WHO chemical safety criteria, and 100% met the WHO aesthetic quality criteria. That said, our self-reported survey only categorized 18% of the households having safely managed drinking water services, 22% did not have basic hygiene facilities, and none had safely managed sanitation services. These results suggested that testing water quality at the point of consumption allows a more accurate characterization of drinking water safety. The methodology presented in Chapter 2 can generate basic water quality data using widely available technologies, allowing inter-city analyses to track their progress towards SDG 6.

In Chapters 3 and 4, my objective was to optimize a strategy to monitor bioaerosols near open wastewater canals in La Paz, Bolivia, and understand their potential impact on public health. Chapter 3 focused on field sampling requirements, challenges, and limitations during two sampling campaigns in the rainy and dry seasons. For this work, we conducted passive and active bioaerosol sampling, while monitoring on-site environmental conditions including wind speed, particulate matter (PM_{2.5}), temperature and relative humidity. We also obtained UVB irradiance data for the city based on the hypothesis that different environmental conditions could affect the fate and transport of bioaerosols. Median bacterial coliform fluxes were 71 CFU m⁻²h⁻¹ and 64 CFU m⁻²h⁻¹ during the rainy and dry season, respectively, with 38% of the samples taken in the dry season testing positive for *E. coli*. Regression analyses indicated that wind

speed, relative humidity and UVB irradiance were significant covariates affecting bioaerosol fate and transport. Active bioaerosol sampling indicated the presence of human adenovirus (HadV) in one sample at the site with highest bacterial flux, and influenza A in a separate sample in the rainy season. Only Influenza A was detected in 4 samples in the dry season (winter in La Paz). Our findings suggested that the citizens of La Paz could be at risk of exposure to human pathogens through bioaerosols near open wastewater canals. The methodology presented in Chapter 3 could be used in low-resource settings to monitor bioaerosols in places relevant to human exposure, despite the limitations of passive sampling.

In Chapter 4, my work focused on translating the data we collected for Chapter 3 into its potential health outcome, using Quantitative Microbial Risk Assessment (QMRA). For this work, I selected three reference pathogens to build stochastic models of transmission: enterotoxigenic *Escherichia coli* (ETEC), *Shigella flexneri*, and *Campylobacter jejuni*. Three scenarios were modelled using bacterial fluxes and genome copies of human pathogens: food contamination, fomite transmission in playgrounds, and inhalation followed by ingestion. Inhalation followed by ingestion had the highest median infection risk per event e.g., 3×10^{-5} *Campylobacter jejuni* infections per exposure compared to contaminated food with *Campylobacter jejuni* e.g., 5×10^{-6} and fomite transmission e.g., 2×10^{-7} . Our sensitivity analysis using Spearman rank correlations showed that bacterial fluxes from the air were the most influential factor on risk estimation regardless of the transmission route. Median annual infection risks of *Campylobacter jejuni* infections were 18 (food), and 100 (inhalation) times greater than the EPA's standard for drinking water (1×10^{-4}). This screening risk assessment indicated that fecal bacterial aerosols from open wastewater canals present non-negligible risks of

infection in La Paz. We included the food contamination and fomite transmission models in the web application we developed for aerosol-mediated infectious disease risk assessments.

The fifth chapter of this dissertation focused on developing a practical web application to estimate the risk of long-range aerosol transmission of COVID-19 in indoor settings. For this work a stochastic model using the Wells-Riley model of respiratory disease transmission was developed. This approach was selected because of the absence of dose-response data for SARS-CoV-2. Previous studies had estimated quanta emission rates, which allowed us to make assumptions about their possible distributions given a certain type of activity e.g., emissions when resting, speaking, or doing exercise. Three settings were modelled using the web application: a classroom with a contagious student, a wedding with a contagious attendee, and a heavy exercise session with a contagious instructor. The highest median risk of infection was 2.6×10^{-2} infections/personal exposure events in the heavy exercise scenario. The wedding and classroom scenarios presented 1-2 orders of magnitude lower risk of infection compared to a heavy exercise session, respectively. Risk of infection increased 3 times when the susceptible individuals weren't wearing masks, and 4-5 times when the room also had poor ventilation, regardless of the type of activity. Ventilation rates of 6-8 ACH reduced the risk of infection by ~50% when people were wearing masks. We compared our model to previously characterized outbreaks, showing that long-range aerosol transmission may have played a role in them given the observed attack rates. Our web-based risk assessment tool can be used for retrospective and prospective analyses to understand and mitigate the risk of COVID-19 aerosol transmission.

In conclusion, this thesis presented practical drinking water, bioaerosols' monitoring, and risk assessment strategies to address global health challenges including access to safe drinking water, sanitation services, and indoor spaces. This work highlighted that: 1) water monitoring at the point of consumption can provide accurate information regarding access to safe drinking water, 2) standardized monitoring methodologies allow performance comparisons, leading to better contextual responses, 3) bioaerosols may play a role in enteric pathogen disease transmission in cities with poor sanitation, and 4) microbial risk assessment builds a communication channel between scientists and public health professionals, translating monitoring data into potential public health outcomes. Future work would ideally focus on the integration of environmental monitoring, epidemiology and QMRA to guide public health policies to more effectively decrease the risk of enteric and respiratory disease epidemics.

Appendix A: Supplementary material for Chapter 2 – surveying instructions

The respondent should be a member of the household from which the water sample is being taken. It is okay if more than 1 individual of the household is present for the survey. The detailed steps of surveying are as follows:

1. Before beginning the questionnaire, it is important that our local partner from UCB connects with the respondent. This is vital, even under the pressure of timing, in order to collect complete and accurate data from the responses. Additionally, throughout the process of surveying and sampling, the surveyor must never complain, show anger, or argue.

2. The surveyor will give an introduction to the survey. A model of this introduction can be found at the beginning of the questionnaires, but it should not be read directly to the respondent. This introduction should include what the respondent will gain from participating in the survey (such as communicating to authorities about the status of their infrastructure). It should also flow naturally into the asking of the survey questions.

3. The surveyor will go through each of the questions of the survey. He or she should not provide the answer options that are available on the response forms so that the responses received are unprompted. In the circumstance that the person interviewed cannot think of a response, the interviewer should first reword the question then give them the sample options. In this scenario, it should be noted in the comments section which questions were prompted.

4. If an answer given by a respondent is unclear, then the surveyor should probe for more information or ask to see what the respondent is talking about. Throughout this

process, be sure to stay engaged with the client, relaying all information out loud so that the respondent stays interested in the survey.

5. If an answer given is not included in the options of the response form provided, then the surveyor should indicate that in the comments section. Any abnormal occurrences that come up throughout the survey should also be noted in the comments section.

6. The surveyor will thank the respondent and ask if they have any questions. A phone number will be given to the household that will connect them with a UCB affiliate if the household wants the results of the water quality testing.

Appendix B: Supplementary material for Chapter 2 – survey

The following questions will be addressed to every household within a particular locale participating in the water and sanitation quality survey.

The questions are separated into 3 categories:

- ID questions (ID1 - ID7) that identify the City, Locale number, Pre-selected House Coordinates, Street name, Cross Street 1 and Cross Street 2 and Selected House Coordinates.
 - Information for ID1 - ID6 will be already prepared and pre-indicated for each locale.
 - If the pre-selected House will be different than the Selected House, the enumerators will provide the coordinates of the selected house.
- Water quality questions (W1 -W6) pertaining to source, location of the source, storage, treatment and frequency.

- Sanitation questions (S1 - S5) pertaining to sanitation type, location, whether facilities are shared and by how many households and whether there are hand-washing options available.

The enumerators were provided with:

- A 1-page questionnaire per household
- A 1-page map of the urban extent of the city indicating all the numbered locales.
- A 1-page sheet with pre-filled information for City, Locale Number, Pre-selected House Coordinates, Street Name, Cross Street 1 and Cross Street 2.

Suggested brief introduction to the household about the survey

“We would like to do a simple test of your drinking water and would like to ask a few questions about the drinking water and sanitation facilities you are using. The purpose is to inform a global study at the city level. Results will be reported at the city level and you will not be identified as a household. Your participation in this study is voluntary.”

Table 7: Survey used for the rapid assessment of urban water quality in Cochabamba

| | | |
|-------------|---------------------------------------|---|
| ID1. | <i>City:</i> | City name |
| ID2. | <i>Locale No</i> | The locale number from which the sample was taken from. |
| ID3. | <i>Pre-selected House Coordinates</i> | Geographic coordinates of a central dwelling unit within the specific locale. |

| | | |
|-------------|--|--|
| ID4. | <i>Street</i> | Street where the central dwelling unit within the specific locale is found. |
| ID5. | <i>Cross Street 1</i> | Cross street 1 where the central dwelling unit within the specific locale is found. |
| ID6. | <i>Cross Street 2</i> | Cross street 2 where the central dwelling unit within the specific locale is found. |
| ID7. | <i>Selected House Coordinates</i> | Geographic coordinates of the dwelling from which the sample was collected. |
| W1. | SOURCE: What is the <u>main</u> source of drinking water used by members of your household? (Could you provide me with a glass of water as you would provide a thirsty child?) | Piped water tap Tube Well / Borehole Protected well Unprotected well Protected spring Unprotected spring Rainwater Tanker-truck Cart with small tank Surface water Bottled water Sachet water Other (specify in comment section) |
| W2. | WATER AVAILABILITY: Within the past week, was drinking water from your (piped) water source always available? | Water service every day At least 1 day without water service Don't know |
| W3. | PIPED WATER FREQUENCY: On average, how often is drinking water from your water source available? | Continuous 24 hours/day service Average 12 - 24 hours/day service Average 6 -12 hours/day service Average less than 6 hours/day service Don't know |

| | | |
|-------------------|---|---|
| <p>W4.</p> | <p>STORAGE: How do you store your drinking water?</p> | <p>No storage Covered container Uncovered container Other (specify in comment section)</p> |
| <p>W5.</p> | <p>TREATMENT: Have you or members of your household treated this water to make it safer to drink? If yes, how have you treated the water?</p> | <p>No treatment Boiled it Added bleach/chlorine Strained it through a cloth Used a water filter (ceramic, Sand, composite, etc.) Solar disinfection Left to stand and settle Other (specify in comment section)</p> |
| <p>W6.</p> | <p>LOCATION: Where is your <u>main</u> water source located?</p> <p>If unclear, probe to identify the place from which members of this household most often collect drinking water (collection point).</p> | <p>Inside dwelling In compound / yard / plot At neighbor's dwelling Elsewhere (specify in comment section)</p> |

| | | |
|-------------------|---|--|
| <p>S1.</p> | <p>TYPE: What kind of toilet facility do members of your household usually use?</p> | <p>Flush to piped sewer system Flush to septic tank Flush to pit latrine Flush to somewhere else Flush to DK where Ventilated Improved Pit latrine Pit latrine with slab Pit latrine without slab / Open pit Composting toilet Bucket Hanging toilet / Hanging latrine No facility / Bush / Field Other (specify in comment section)</p> |
| <p>S2.</p> | <p>HANDWASHING: Is there a hand-washing facility in proximity to your sanitation facility?</p> | <p>No handwashing facility Handwashing facility with water Handwashing facility with water & soap</p> |
| <p>S3.</p> | <p>LOCATION: Where is the toilet facility located?</p> | <p>In own dwelling In own yard / plot Elsewhere (specify in comment section)</p> |
| <p>S4.</p> | <p>SHARING: Do you share your toilet facility with other households?</p> | <p>Not sharing with other households Sharing with 1 - 5 households Sharing with 5 - 10 households Sharing with more than 10 households</p> |
| <p>S5.</p> | <p>DISPOSAL (if no sewer): a. Is your sanitation facility ever emptied? b. If it was emptied, it was emptied by whom?</p> | <p>Yes No Don't know Service provider Household Don't know</p> |

| | | |
|--|---|--|
| | c. If it was emptied, where was the waste disposed? | Away from neighborhood In covered pit on or near plot In uncovered pit on or near plot |
|--|---|--|

Appendix C: Supplementary material for Chapter 2 – Lab testing procedure

AquaTest Medium provided by Oasis plc:

<https://www.oasiswatertest.com/>

1. Label the 10 mL test tube and the 100 mL Whirl-Pak bag.
2. Add 0.11 g of AquaTest medium to test tube using small pre-sized measuring spoon.
3. Add 1.1 g of AquaTest Medium to Whirl-Pak bag using large pre-sized measuring spoon.
4. Pour 10 mL (or to line indicated on Whirl-Pak bag) of sample into the test tube with the medium already present.
5. Incubate for 24 hours at 36-37°C.
6. Shake to mix and aerate the sample.
7. See/check if test tube is orange or pink. Pink indicates a positive result (presence), while orange indicates a negative result (absence).
8. Record results.

Colilert Test:

<https://www.idexx.com/en/water/water-products-services/colilert/>

1. Label 10 mL and 100 mL Colilert test tubes.
2. Add 10 mL of sample to the 10 mL Colilert tube; add medium.

3. Add 100 mL of sample to the 100 mL Colilert tube; add medium.
4. Incubate for 24 hours at $35 \pm .5$ °C.
5. Use UV light to see if it's fluorescent to determine if positive for *E. coli*.
6. Record results.

Compact Dry Test:

https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CompactDryEC.html

1. Label Compact Dry Dish.
2. Use 1 mL syringe to collect 1 mL of sample.
3. Evenly spread the sample on the Compact Dry Dish.
4. Incubate for 24 hours at 35-37 °C.
5. Count number of red and blue colonies.
 - a. Red colonies indicate total coliforms
 - b. Blue colonies indicate *E. coli*
6. Record results.

Petrifilm Test:

https://www.3m.com/3M/en_US/company-us/all-3m-products/~/ECOLICT-3M-Petrifilm-E-coli-Coliform-Count-Plates/?N=5002385+3293785155&rt=rud

1. Label Petrifilm
2. Add 1 mL of sample to Petrifilm with 1 mL syringe.
3. Incubate for 24 hours at 35-37°C.
4. Count number of colonies gas bubbles.
 - a. Red colonies indicate total coliforms
 - b. Blue colonies indicate *E. coli*
5. Record results.

Appendix D: Supplementary material for Chapter 3 – equations, sampling site map and data summary

Stokes Law equation with slip correction coefficient for theoretical deposition velocities (Seinfeld and Pandis, 2016):

$$Eq. A1: V_d = \left[\frac{D_p^2 \times \text{density} \times \text{gravity} \times \text{SCC}}{18 \times \text{viscosity}} \right]$$

Where V_d = deposition velocity; D_p = particle diameter; SCC = Slip Correction Coefficient

Table 8: Deposition velocities for particles with diameters 0.5-10 μm (Adapted from Seinfeld and Pandis, 2016).

| Particle diameter (D_p) [μm] | Particle diameter (D_p) [m] | Slip correction coefficient (SCC) [unitless] | Deposition velocity (V_d) [m/s] |
|--|------------------------------------|---|--|
| 0.5 | 5.00E-07 | 1.326 | 1.0E-05 |
| 1 | 1.00E-06 | 1.164 | 3.5E-05 |
| 1.5 | 1.50E-06 | 1.123 | 7.6E-05 |
| 2 | 2.00E-06 | 1.082 | 1.3E-04 |
| 2.5 | 2.50E-06 | 1.06325 | 2.0E-04 |
| 3 | 3.00E-06 | 1.057 | 2.9E-04 |
| 4 | 4.00E-06 | 1.0445 | 5.0E-04 |
| 5 | 5.00E-06 | 1.032 | 7.8E-04 |
| 6 | 6.00E-06 | 1.028 | 1.1E-03 |
| 7 | 7.00E-06 | 1.024 | 1.5E-03 |
| 8 | 8.00E-06 | 1.024 | 2.0E-03 |
| 9 | 9.00E-06 | 1.02 | 2.5E-03 |
| 10 | 1.00E-05 | 1.016 | 3.1E-03 |

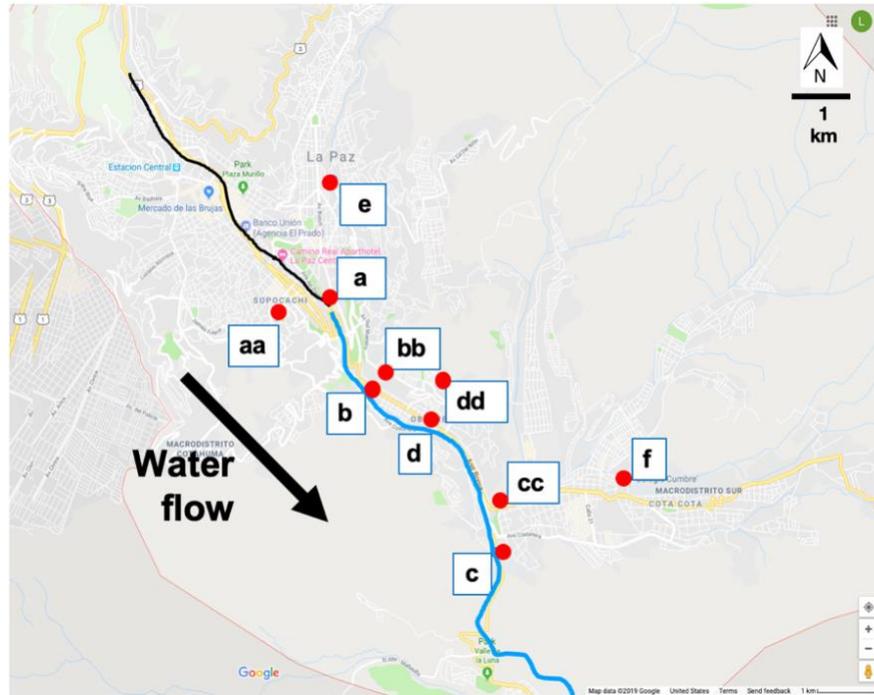


Figure 6: Map of the sampling locations, adapted from Google Maps.

Table 9: Data summary

| Season | Flux [CFU/(m ² *h)] | Wind Speed [m/s] | Daily UVB [kJ/m ²] | Temperature [°C] | RH [%] | PM _{2.5} [µg/m ³] |
|--------|-----------------------------------|---------------------|-----------------------------------|---------------------|---------------|---|
| Rainy | 71* (0-5411) | 0.8 (0.3-1.1) | 54 (33-76) | 20 (16-27) | 46 (34-54) | 5.6 (0.3-19.5) |
| Dry | 64* (0-3374) | 0.6 (0.1-1.0) | 51 (45-55) | 18 (14-28) | 30 (6-47) | 11.3 (0.5-26.8) |

Note: *median value (min-max), otherwise: average (min-max)

Appendix E: Supplementary material for Chapter 3 –

Passive sampling observations

MI agar and Compact Dry plates were tested in the field as alternatives to AT medium for bioaerosol passive sampling (n=60, in duplicates). However, they were outperformed by AT medium in determining CFU fluxes (Figure 7). While Compact Dry was deemed better in identifying *E. coli* qualitatively in the laboratory, its medium was damaged after 2 h of exposure to field conditions while sampling, regardless of the time in the day (Figure 7, image I), preventing the identification and quantification of *E. coli*. In addition, its dry surface may not allow accurate flux determination as particles may be more likely to bounce back into the air (Pepper and Dowd, 2009). MI agar *E. coli*-staining chromogen was damaged by extended sunlight exposure while sampling, as *E. coli* colonies did not turn blue (Figure 7, image II and III) consistent with the manufacturer's instructions to keep plates in the dark (BD Diagnostics, 2009). MI agar was also affected by the lower relative humidity in the Dry Season, losing surface area coverage (Figure 7, image III). Relative humidity (RH) during the rainy season campaign had a mean with standard deviation of $46\pm 6\%$ while the dry season campaign had a mean RH of $30\pm 12\%$.

Although *E. coli* colonies on AT medium were not as intense pink after sampling (Figure 7, image IV), the AT medium allowed CFU counting during both seasons and *E. coli* identification during the dry season. We observed that the average UVB daily dose (kJ/m^2) was similar during the rainy season sampling and the dry season sampling ($54\pm 14 \text{ kJ/m}^2$ vs. $51\pm 4 \text{ kJ/m}^2$) which could have affected AT's chromogen. Finally, we noticed a reduction in CFU in AT and MI positive controls when plated before and after

being taken to the field while sampling (Figure 7, images II and V), which was expected as solar inactivation of bacteria can happen (Paez-Rubio and Peccia, 2005). Our findings suggest that UVB irradiance plays an important role on AT's performance to identify and quantify *E. coli* accurately and that AT medium is more resilient to environmental conditions compared to Compact Dry plates and MI agar.

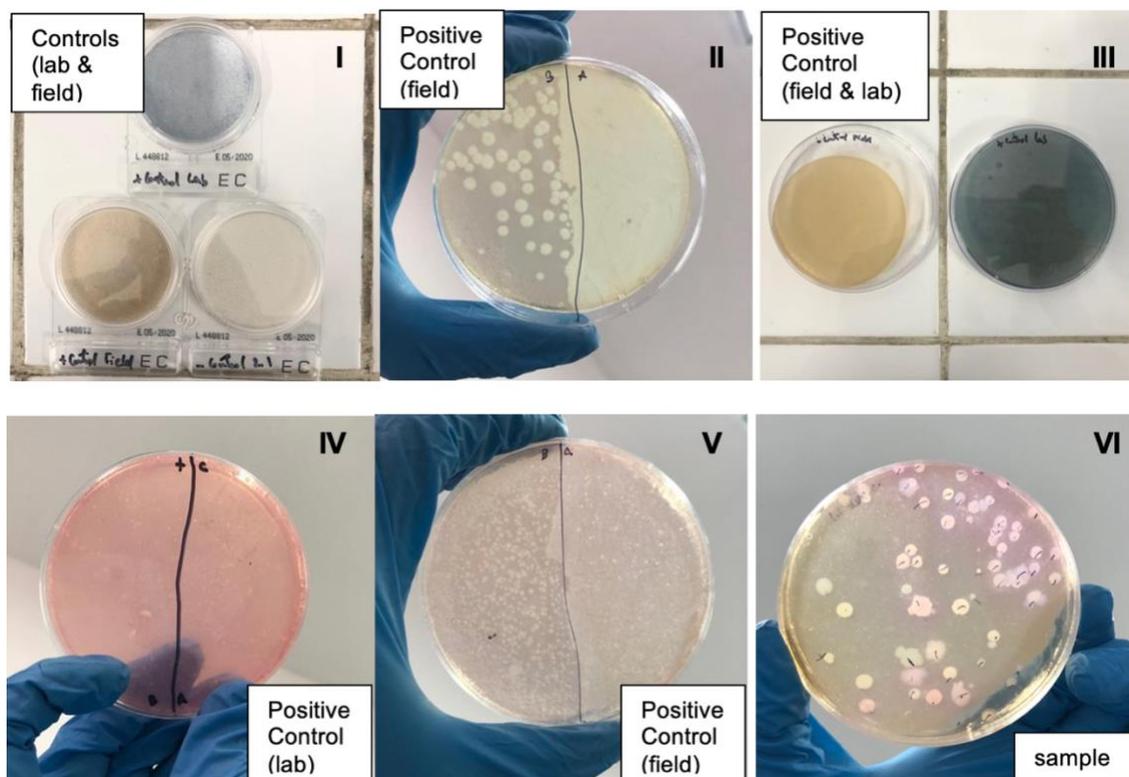


Figure 7: Passive sampling method comparison

I. Compact Dry plates positive controls next to negative control. II. MI medium positive control before and after sampling (notice how there are no blue colonies). III. MI medium positive controls taken to the field (left) and kept in the laboratory (right). IV. AT medium positive control kept in the laboratory. V. AT medium positive control inoculated before (B) and after sampling (A) VI. AT passive sample replicate at site with highest bacterial fluxes. Note 1: +c = positive control Note 2: Total coliforms form white colonies on MI agar and AT medium, and red ones on Compact Dry plates. *E.coli* forms blue colonies on MI agar and Compact dry plates, and pink colonies on Aquatest medium.

Appendix F: Supplementary material for Chapter 3 – Bioaerosol sampling setup

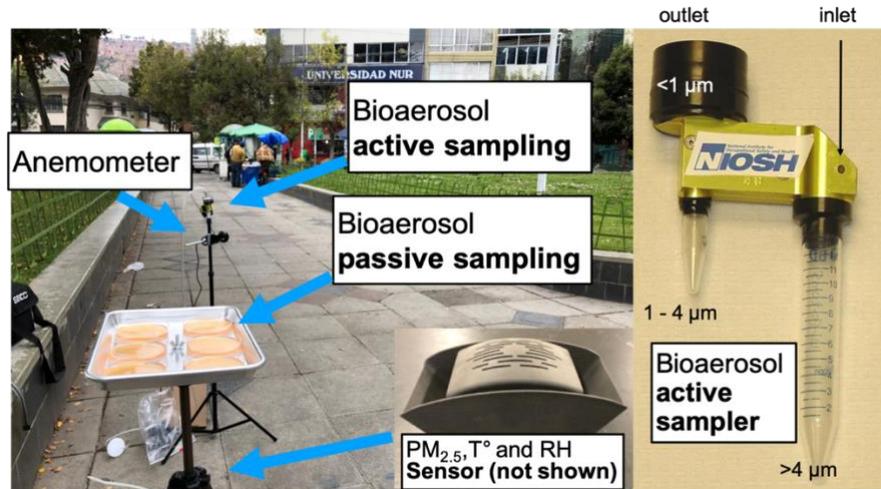


Figure 8: Bioaerosol sampling setup

Appendix G: Supplementary material for Chapter 4

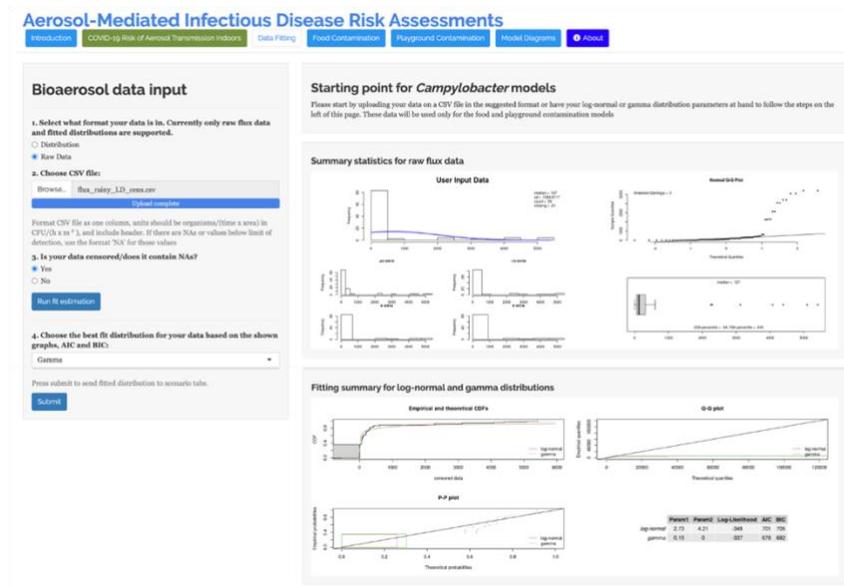


Figure 9: AMIDRA web application user interface: data fitting. Access link: tinyurl.com/aerosolrisk

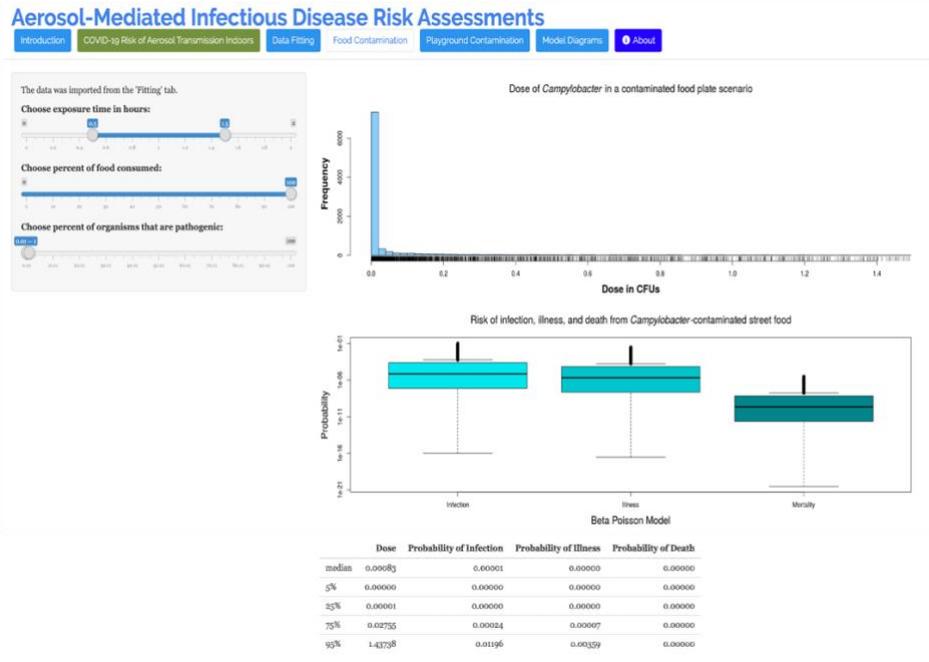


Figure 10: AMIDRA web application user interface: example of food contamination model

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Biography

Lucas Rocha Melogno earned a bachelor's degree (B.S.) in Development and Environmental Engineering from Zamorano University (Honduras) in 2015, and a master's degree (M.S.) in Environmental Engineering from Duke University in 2019. He holds 2 first-author publications at the time this thesis was written. He was awarded a graduate research assistantship to obtain his M.S. and Ph.D. degrees at Duke University. During his time at Duke, he received a D-SIGN grant to establish an interdisciplinary network of graduate students interested in water, sanitation, and hygiene. He was also a Duke Global Health Doctoral Scholar, receiving funding for tuition, stipend, and research expenses in Bolivia for 2 years.

Publications:

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