

Enteric pathogens in outdoor urban aerosols: knowns and unknowns

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Key input from

Olivia Ginn, Notre Dame

Lucas Rocha-Melogno, Duke

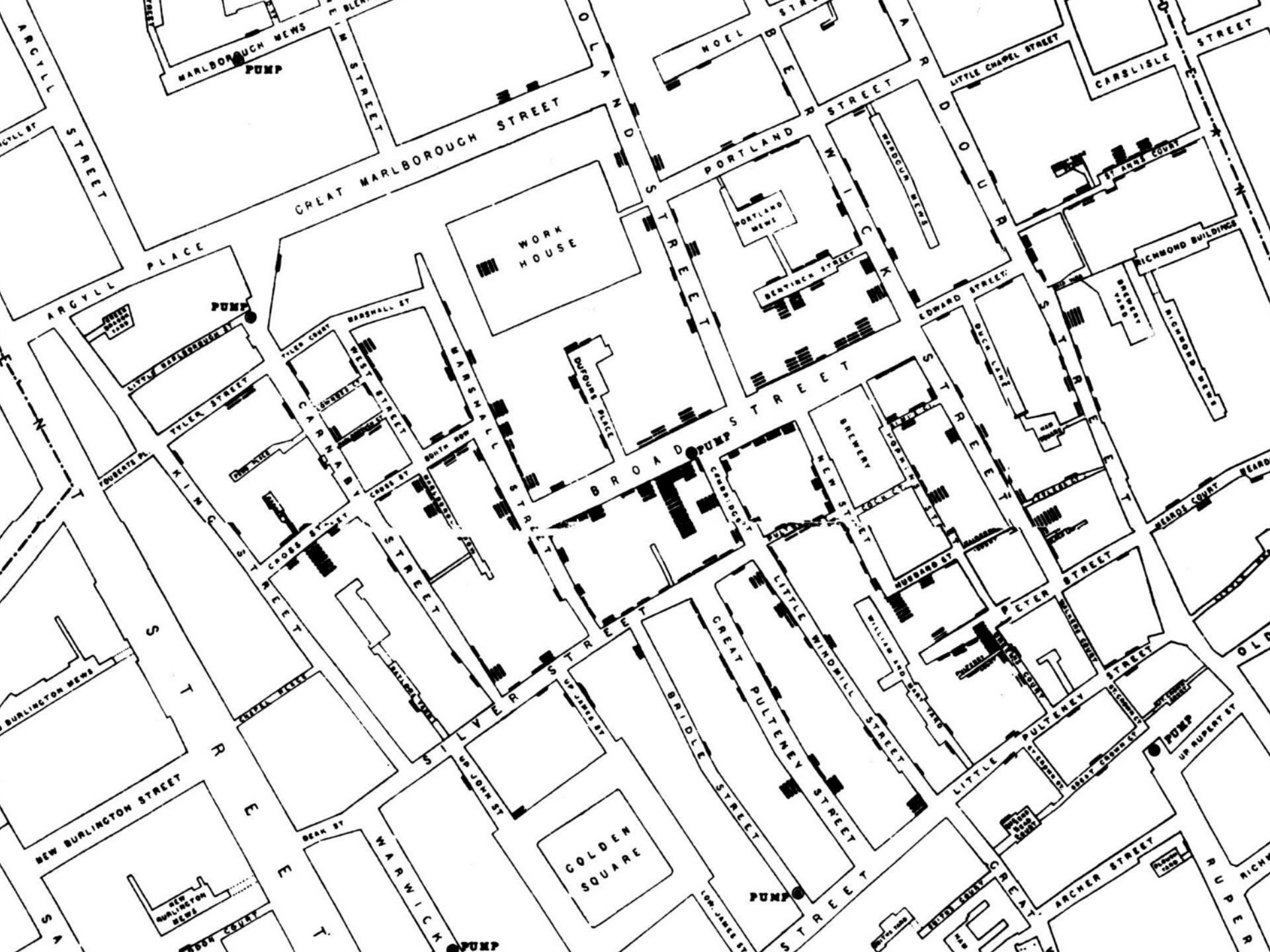
Freddy Soria, Universidad Católica Boliviana

Marc Deshusses, Duke

Sachi Tripathi, IIT-Kanpur



GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH



**CHOLERA MAP
OF THE METROPOLIS.**

1849.

EXHIBITED IN THE REGISTRATION DISTRICTS.

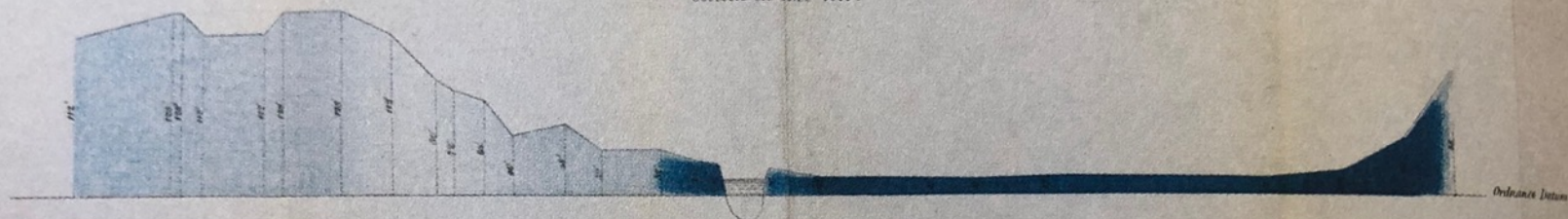


SECTIONS

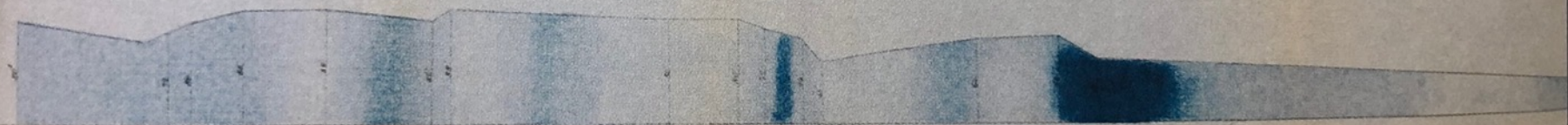
*Shewing the relative intensity of the attack of Cholera at
the various levels along the lines marked on*

THE CHOLERA MAP.

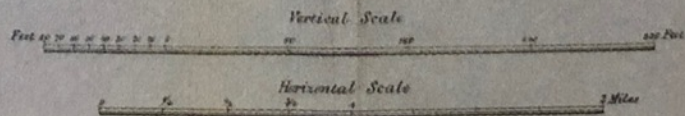
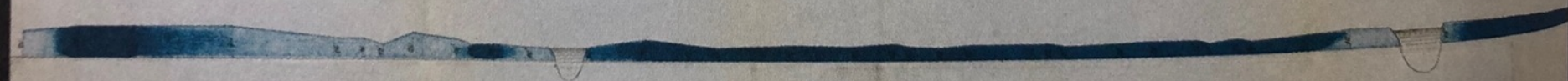
Section on Line A A.



Section on Line B B.



Section on Line C C.



Number of Districts.	Elevation in Feet above Trinity High-water mark.	OBSERVED AVERAGE.						Poor Rate in the £ of House- rent, 1842-43.
		Annual Mortality to 10,000 Persons Living.	Number of Persons to		Average Annual Value of			
		Cholera (1849).	All Causes (1838-44).	An Acre.	A House.	Houses.	House and Shoproom to each Person.	
						£	£	£
16	Under 20 ft.	102	251	74	6·8	31	4·645	·072
7	20 — 40 „	65	237	105	7·6	56	7·358	·071
8	40 — 60 „	34	235	184	8·5	64	7·342	·056
3	60 — 80 „	27	236	152	8·8	52	6·374	·049
2	80 — 100 „	22	211	44	7·7	38	5·183	·036
1	100 „	17	227	102	9·8	71	7·586	·043
1	350 „	8	202	5	7·2	40	5·804
All London		62	252	29	7	40	5·419	·063



EXPERIMENTS MADE TO DETERMINE THE CONDITIONS UNDER WHICH "SPECIFIC" BACTERIA DERIVED FROM SEWAGE MAY BE PRESENT IN THE AIR OF VENTILATING PIPES, DRAINS, INSPECTION CHAMBERS, AND SEWERS.*

BY MAJOR W. H. HORROCKS, R.A.M.C.

MOST sanitarians at the present time believe that when sewage is in a putrefactive condition and gas bubbles rising through it are bursting at the surface, bacteria may be carried into the air of

BACTERIA OF SEWER AIR.

Dr. F. W. Andrewes presents a third report on the bacteria of sewer air, with special reference to the conveyance of sewage bacteria by air currents. A departmental committee of the Local Government Board has been considering the question of intercepting traps in house drainage, and experiments were undertaken to gain information on the subject. In five experiments the sewer of a thoroughfare was found to contain enormous numbers of *Bacillus prodigiosus*. The intercepting traps were rendered useless by removing the cap of the raking arm. The air inlets and ventilating shafts of the sewer were stopped up. Everything, in fact, was done to secure a flow of sewer air through the house drain and up the ventilating shaft of the soil pipes. Agar culture plates were suspended in the inspection chambers of the house drains and in some cases in the ventilating shafts of the soil pipes. Three out of the five experiments were negative. Considering that the infection of the sewer was nothing less than colossal, Dr. Andrewes's conclusion that organisms conveyed from a sewer are so small in number that they cannot be prejudicial to health is amply justified. Experiments on contamination of drains, as opposed to sewers, showed that sewage organisms may be carried by the convection currents for a long distance—in one case to the top of a soil pipe 73 ft. above the point of effective splashing.

Airborne Enteric Bacteria and Viruses from Spray Irrigation with Wastewater

B. TELTSCH AND E. KATZENELSON*

Environmental Health Laboratory, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received for publication 19 August 1977

The relationship between bacterial concentrations in wastewater used for spray irrigation and in the air was examined. Aerosolized coliforms were detected when their concentration was 10^3 /ml or more in the wastewater. Relative humidity and solar irradiation appeared to affect viable bacteria in the air; a positive correlation was found between relative humidity and the number of aerosolized bacteria. The correlation between solar irradiation and bacterial level, on the other hand, was negative. During night irrigation, up to 10 times more aerosolized bacteria were detected than with day irrigation. Wind velocity did not play an important role in the survival of aerosolized bacteria. Echovirus 7 was isolated in 4 out of 12 air samples collected 40 m downwind from the sprinkler.

Agricultural spray irrigation with sewage effluents, as a way to increase the water potential and as an important alternative to advanced wastewater treatment, is widely practiced throughout the world.

One of the disadvantages of spray irrigation is the aerosolization of pathogens that may be present in domestic sewage (2, 11) even after secondary treatment and chlorination (12). Essentially, very few quantitative data are available to evaluate the possible public health risks

terial aerosol concentrations occurred under conditions of relative atmospheric stability and darkness. About 50% of the particles that bore viable bacteria were of human respirable size (range 1.0 to 5.0 μ m); chlorination reduced bacterial aerosol levels by close to three orders of magnitude.

In the present study, controlled experiments utilizing marker bacteria were carried out to evaluate the quantitative relationship between enteric bacteria in the effluent used for irrigation

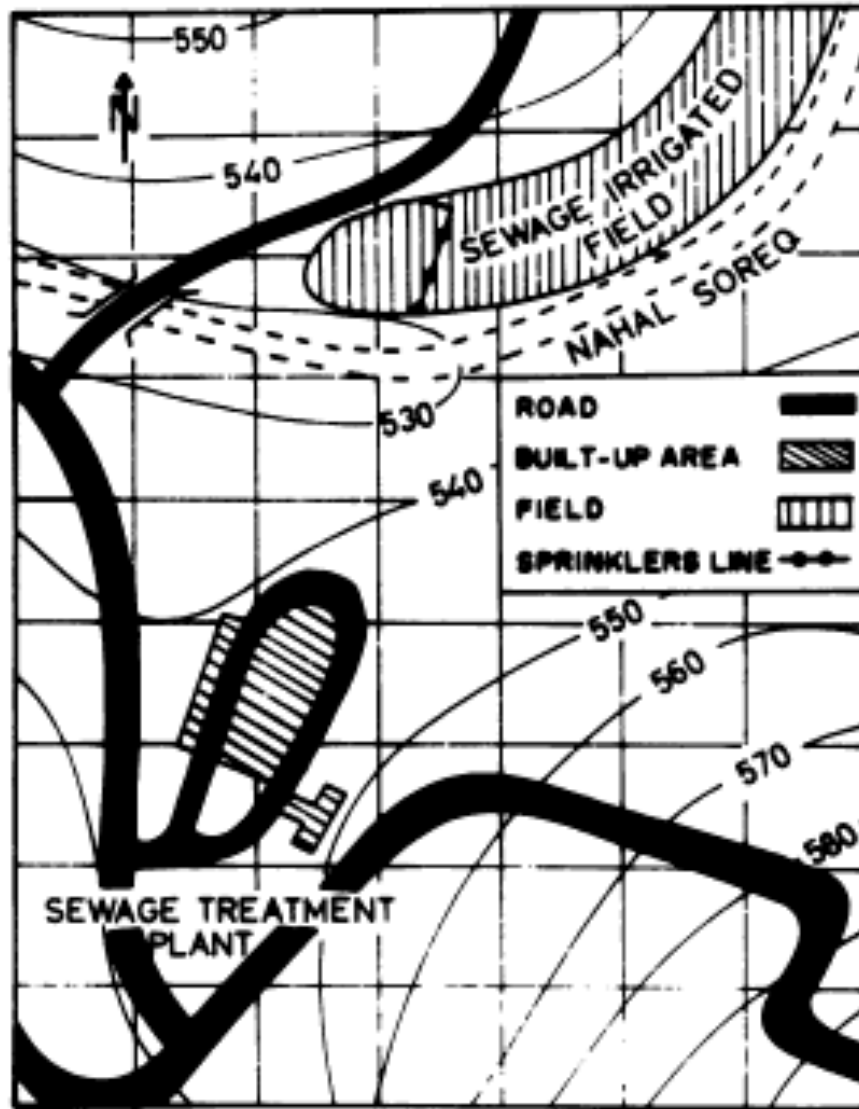


FIG. 1. *Experimental field at Ein Kerem.*

“This study does not prove that enteric bacterial and viral diseases are transmitted through the air as a result of spray irrigation with sewage. It strongly indicates, however, that such a possibility exists”

Microbiological Hazards of Household Toilets: Droplet Production and the Fate of Residual Organisms

CHARLES P. GERBA, CRAIG WALLIS, AND JOSEPH L. MELNICK*

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

Received for publication 12 March 1975

Large numbers of bacteria and viruses when seeded into household toilets were shown to remain in the bowl after flushing, and even continual flushing could not remove a persistent fraction. This was found to be due to the adsorption of the organisms to the porcelain surfaces of the bowl, with gradual elution occurring after each flush. Droplets produced by flushing toilets were found to harbor both bacteria and viruses which had been seeded. The detection of bacteria and viruses falling out onto surfaces in bathrooms after flushing indicated that they remain airborne long enough to settle on surfaces throughout the bathroom. Thus, there is a possibility that a person may acquire an infection from an aerosol produced by a toilet.

The transmission of disease by aerosols from toilets has received only limited study. It has been suggested that, aside from coughing and sneezing, this must be the most common process involved in the generation of infectious aerosols (6). Darlow and Bale (6) demonstrated the production of bacterial aerosols, with the aid of both a liquid impinger and a slit sampler, from flushed bowls containing *Serratia marcescens*. These aerosols were found to persist for at least 12 min after the flush. The generation of aerosols by toilets seeded with coliform bacteria has been demonstrated by Bound and Atkinson (3) and more recently by Newsom (14). The size of particles produced by the flushing toilet was found to be in the range that was capable of reaching the lower respiratory tract (6). In addition, pathogenic fecal contaminants, such as *Escherichia* and *Salmonella*, have been isolated from the respiratory tract of infected humans (6).

The fallout of droplets containing pathogens on bathroom surfaces is also of concern, since hand contact with contaminated surfaces can

MATERIALS AND METHODS

Viruses and virus assays. *E. coli* bacteriophage MS-2 and a plaque-purified line of type 1 poliovirus (strain LSc) were used in this study. MS-2, like poliovirus, is a small (25-nm diameter) icosahedral ribonucleic acid virus. All bacteriophage assays were done by a modification of the agar overlay method as described by Adams (1). Overlay agar and broth used for bacteriophage samples were prepared according to Davis and Sinsheimer (8). Stock poliovirus was grown in baboon kidney cells, concentrated 10-fold, and partially purified by membrane chromatography (22). Poliovirus samples were diluted in tris(hydroxymethyl)aminomethane-buffered saline containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Poliovirus assays were made with BSC-1 cells by the plaque-forming unit method as used in this laboratory (20).

Bacteria and bacterial assays. A strain of *E. coli* isolated from domestic sewage was used (identification based on ImVic test). All coliform assays were performed on Levine eosin methylene blue (EMB) agar. Total aerobic bacterial counts were performed on Standard Methods agar (BBL, Cockeysville, Md.). Cultures of *E. coli* used in seeding experiments were grown overnight in Trypticase soy broth (TSB) (BBL,

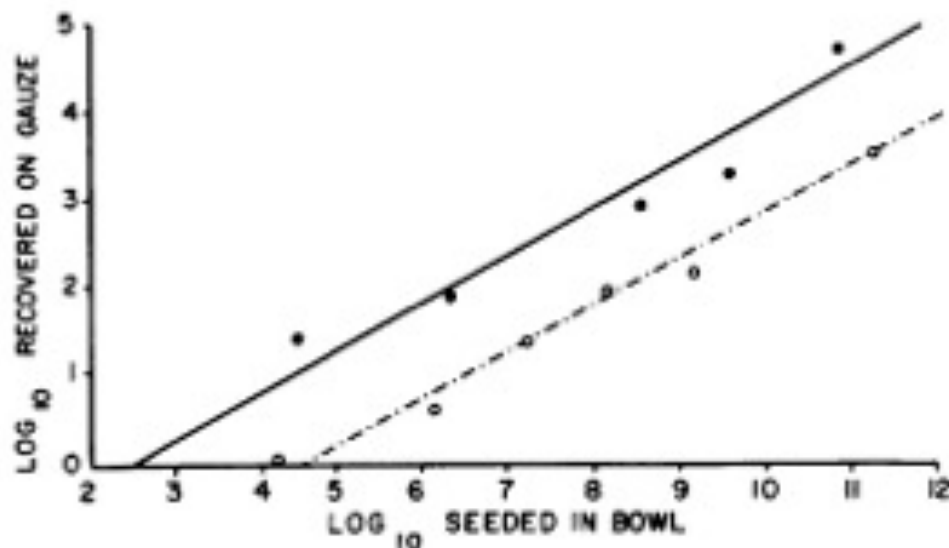


FIG. 4. Concentration of bacteria and virus in toilet bowl and numbers ejected during flushing. The \log_{10} number of *E. coli* or MS-2 phage indicated above was placed in the bowl water, a gauze wetted with TSB was placed over the bowl, and the toilet was flushed. The \log_{10} of the number of organisms recovered from the gauze is indicated in the ordinate of the above figure. Symbols: ●, MS-2 phage; ○, *E. coli*.

Bioaerosols & enteric microbes: modern era

Wherever you have concentrated waste & mechanisms for aerosolization!

Wastewater treatment and land application of biosolids
(Cronholm 1980; Fannin et al. 1985; Gangamma et al. 2011;

Close to urban surface waters (Dueker et al. 2012;);
concentrated animal feedlot operations (Ko et al. 2008); toilet
flushing (Barker and Jones 2005); water features (de Man et
al. 2014); pavement cleaning using non-potable water (Seidl et
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Indoor exposures in high-risk environments, such as in hospitals
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Stability of airborne microbes in the Louvre Museum over time

Abstract The microbial content of air has as yet been little described, despite its public health implications, and there remains a lack of environmental microbial data on airborne microflora in enclosed spaces. In this context, the aim of this study was to characterize the diversity and dynamics of airborne microorganisms in the Louvre Museum using high-throughput molecular tools and to underline the microbial signature of indoor air in this human-occupied environment. This microbial community was monitored for 6 month during occupied time. The quantitative results revealed variations in the concentrations of less than one logarithm, with average values of 10^3 and 10^4 *Escherichia coli*/*Aspergillus fumigatus* genome equivalent per m^3 for bacteria and fungi, respectively. Our observations highlight the stability of the indoor airborne bacterial diversity over time, while the corresponding eukaryote community was less stable. Bacterial diversity characterized by pyrosequencing 454 showed high diversity dominated by the *Proteobacteria* which represented 51.1%, 46.9%, and 38.4% of sequences, for each of the three air samples sequenced. A common bacterial diversity was underlined, corresponding to 58.4% of the sequences. The core species were belonging mostly to the *Proteobacteria* and *Actinobacteria*, and to the genus *Paracoccus* spp., *Acinetobacter* sp., *Pseudomonas* sp., *Enhydrobacter* sp., *Sphingomonas* sp., *Staphylococcus* sp., and *Streptococcus* sp.

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H. Blanquart², S. Ferreira²,
S. Moularat¹, J.-J. Godon³,
E. Robine¹**

¹Université Paris-Est, Centre Scientifique et Technique du Bâtiment (CSTB), Laboratoire de Recherche et d'Innovation pour l'Hygiène des Bâtiments, Mame-la-Vallée Cedex 2, France, ²Genoscreen, Genomic Platform and R&D, Lille, France, ³Laboratoire de Biotechnologie de l'Environnement (LBE), INRA, UR50, Narbonne, France

Key words: Bioaerosols; indoor air quality; Pyrosequencing; qPCR; Capillary electrophoresis single strand conformation polymorphism; Louvre museum.

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... and another data point

“dog feces are likely the dominant source of aerosolized bacteria in the winter months in Cleveland, Detroit, and, to a lesser extent, Chicago.”
(Bowers et al. 2011)



Summary: trends over time

Miasma – “bad air” – causes many diseases

Miasma causes no diseases: germs do

Airborne transport of enteric bacteria documented

Low relative concentrations

Airborne transmission of some diseases happens

Measles, TB, influenza, covid-19

Enteric microbes in aerosols documented routinely

Risk from modern wastewater infrastructure is low

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Most evidence for airborne transport of enteric microbes has been:

Culture of bacteria that do not persist in aerosols or molecular detection of fecal indicators that are everywhere we look

Near modern wastewater / waste sites in rich countries

In low-burden settings where pathogens are rare

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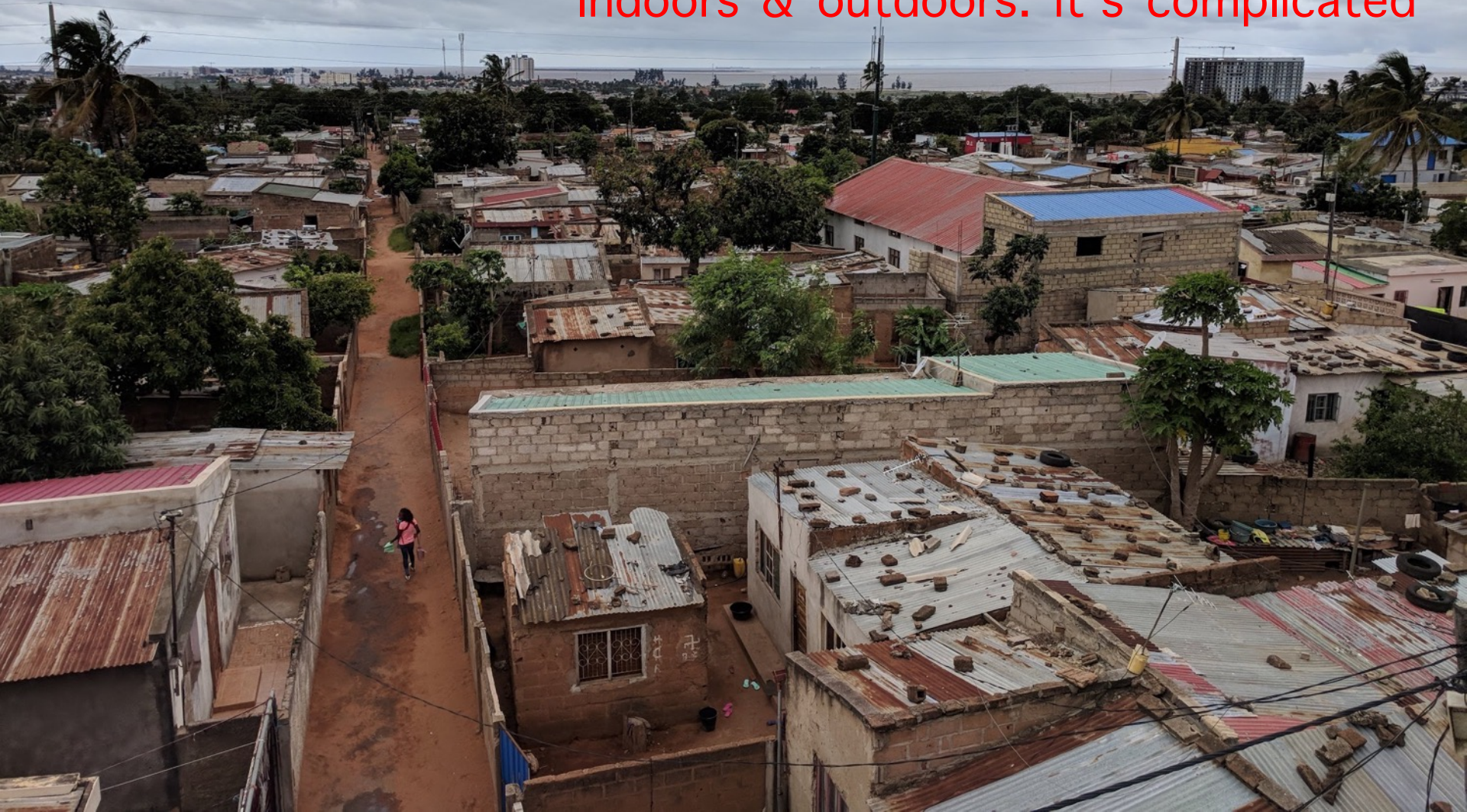
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Indoors & outdoors: it's complicated





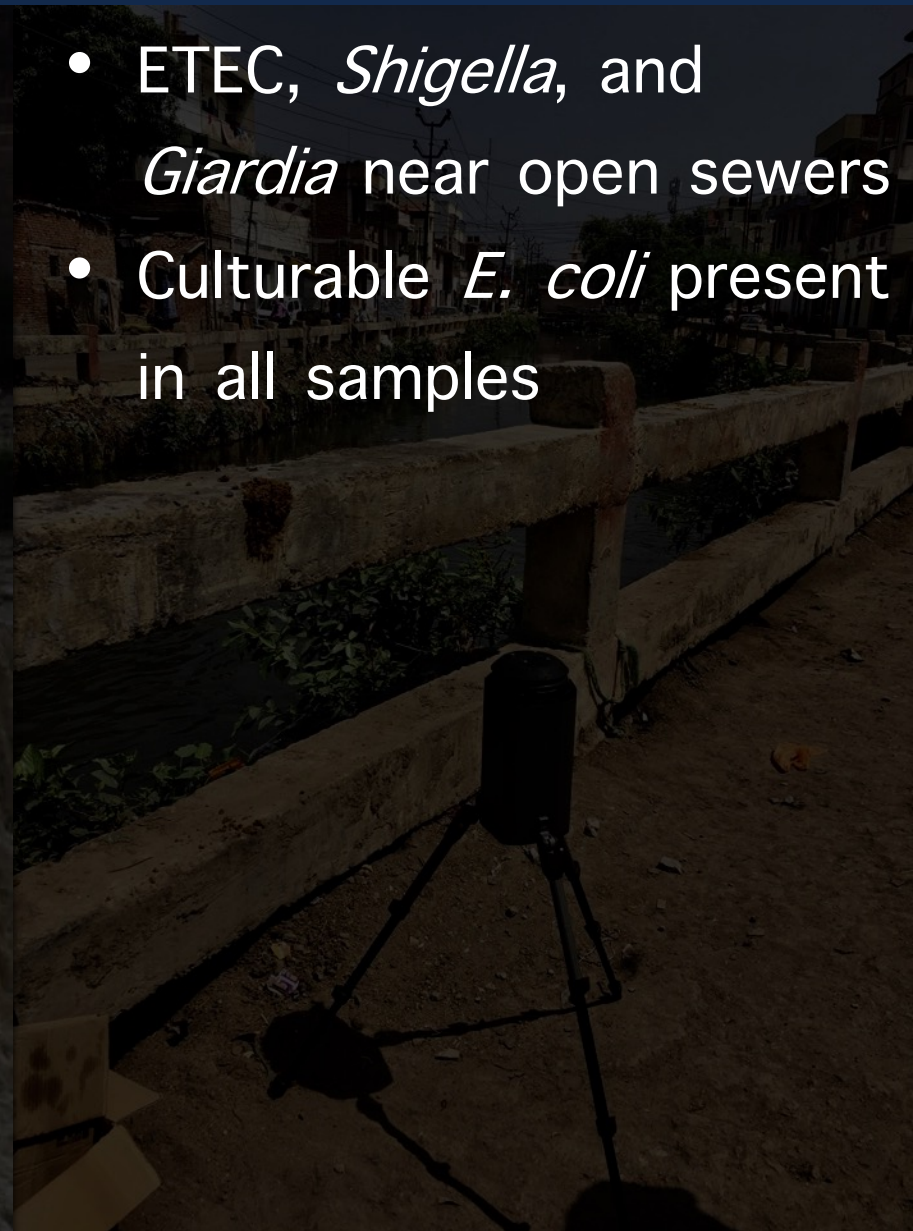
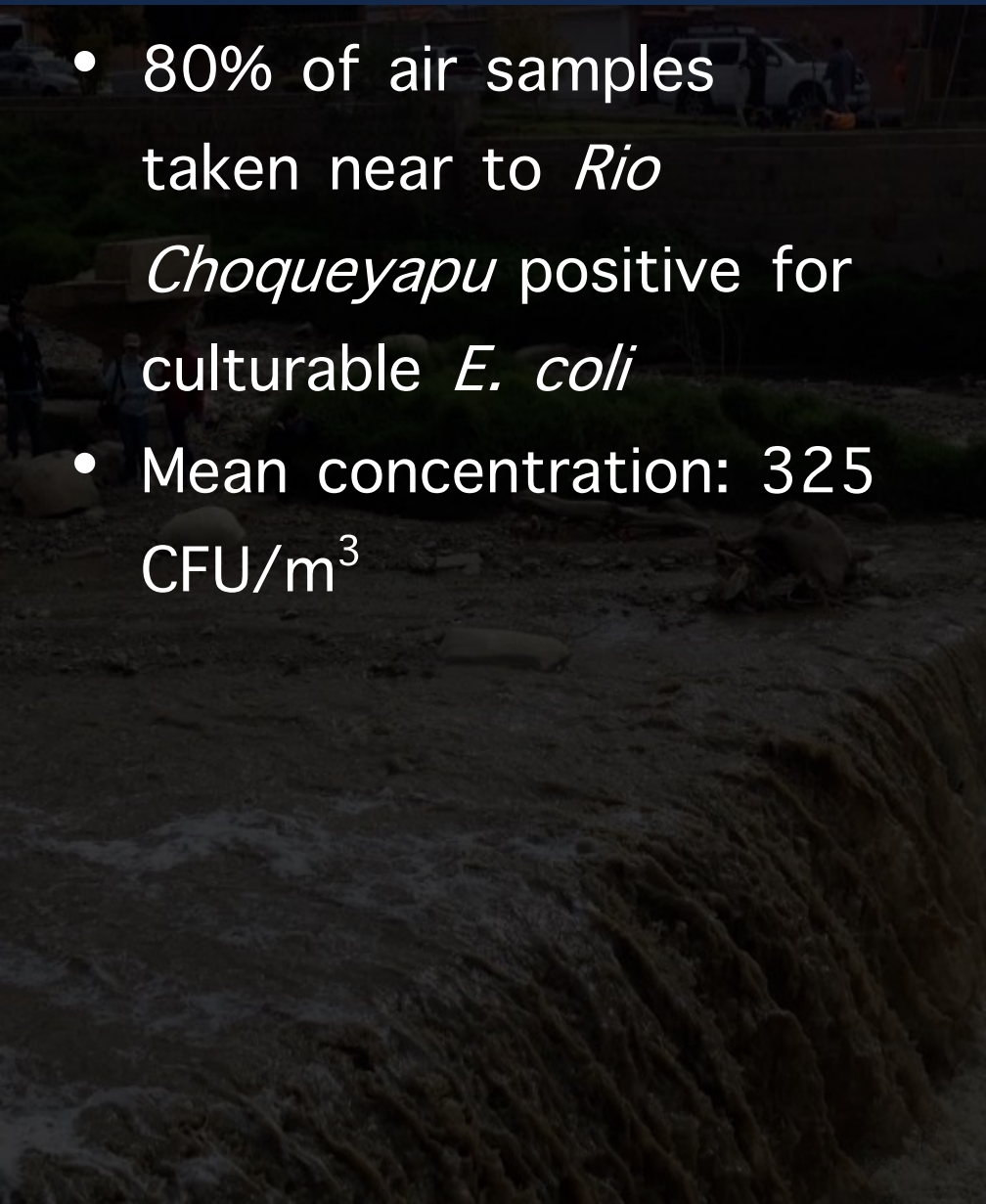
Piloting: March & June 2016, La Paz & Kanpur



Piloting: March & June 2016, La Paz & Kanpur

- 80% of air samples taken near to *Rio Choqueyapu* positive for culturable *E. coli*
- Mean concentration: 325 CFU/m³

- ETEC, *Shigella*, and *Giardia* near open sewers
- Culturable *E. coli* present in all samples



Malawi pit emptying: December 2016

HPC → +3 orders
of magnitude
during emptying

E. coli and total
coliforms 350 /
790 CFU per m³

Detection of ETEC
by PCR



Farling, S., Rogers, T., Knee, J., Tilley, E., Brown, J., and Deshusses, M. 2018. Bioaerosol emissions associated with pit latrine emptying operations. *Science of the Total Environment* <https://doi.org/10.1016/j.scitotenv.2018.08.147>.

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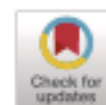
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Which pathogens are there, are they infectious, how did they get there, and what are the risks (if any) of exposure?



Review article

A systematic review of enteric pathogens and antibiotic resistance genes in outdoor urban aerosols

Olivia Ginn^{a, **}, Sarah Lowry^b, Joe Brown^{c, *}^a Department of Civil & Environmental Engineering & Earth Science, University of Notre Dame, Notre Dame, IN, 46556, USA^b Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, United States^c Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, 27599, United States

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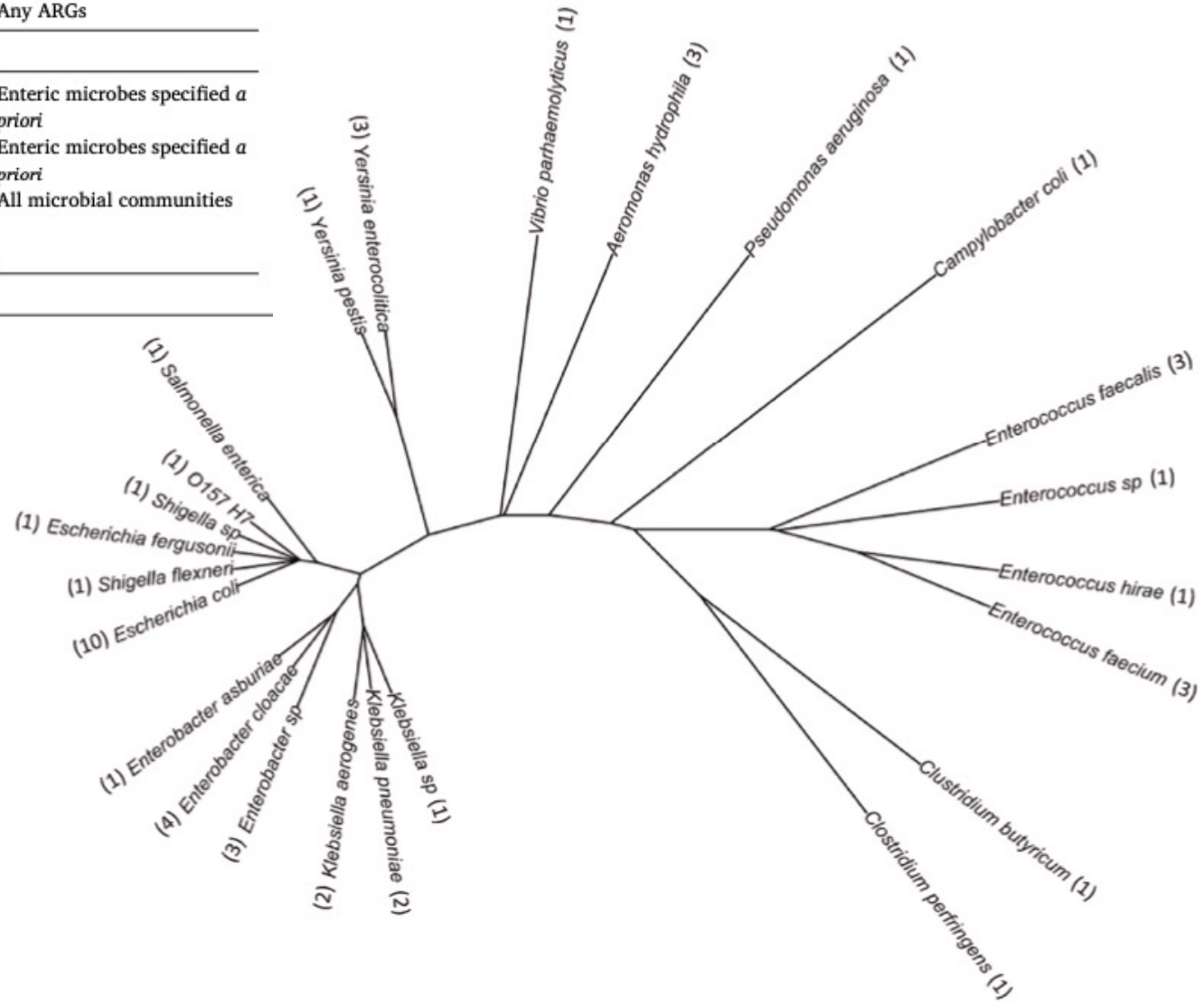
Enteric pathogens
Antibiotic resistance
Bioaerosols
Urban air
Public health
Sanitation

ABSTRACT

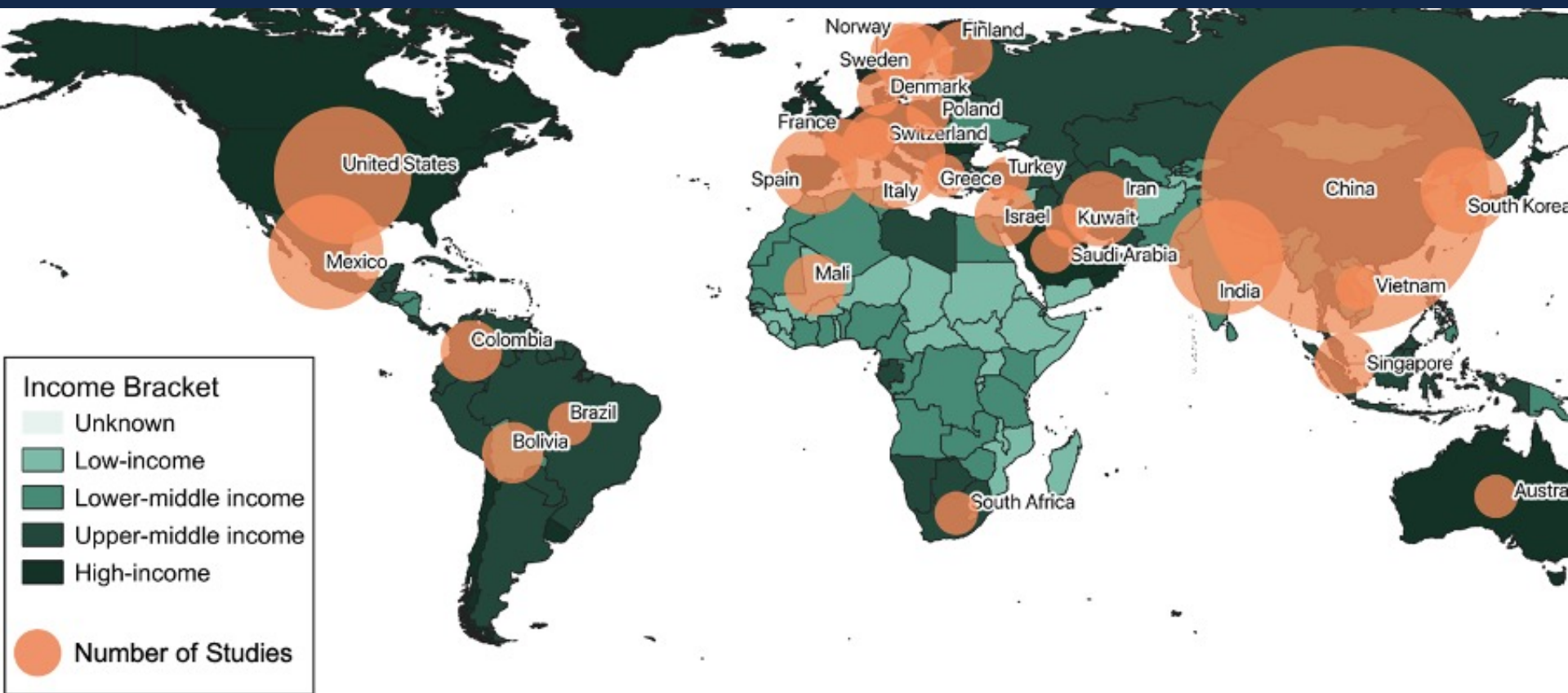
Aerosol transport of enteric microbiota including fecal pathogens and antimicrobial resistance genes (ARGs) has been documented in a range of settings but remains poorly understood outside indoor environments. We conducted a systematic review of the peer-reviewed literature to summarize evidence on specific enteric microbiota including enteric pathogens and ARGs that have been measured in aerosol samples in urban settings where the risks of outdoor exposure and antibiotic resistance (AR) spread may be highest. Following PRISMA guidelines, we conducted a key word search for articles published within the years 1990–2020 using relevant data sources. Two authors independently conducted the keyword searches of databases and conducted primary and secondary screenings before merging results. To be included, studies contained extractable data on enteric microbes and AR in outdoor aerosols regardless of source confirmation and reported on qualitative, quantitative, or viability data on enteric microbes or AR. Qualitative analyses and metric summaries revealed that enteric microbes and AR have been consistently reported in outdoor aerosols, generally via relative abundance measures, though gaps remain preventing full understanding of the role of the aeromicrobiological pathway in the fate and transport of enteric associated outdoor aerosols. We identified remaining gaps in the evidence base including a need for broad characterization of enteric pathogens in bioaerosols beyond bacterial genera, a need for greater sampling in locations of high enteric disease risk, and a need for quantitative estimation of microbial and nucleic acid densities that may be applied to fate and transport models and in quantitative microbial risk assessment.

Results

	Data Type	Method	Count	Intended Targets
AR	Qualitative	PCR	1	Antibiotic Resistance Genes (ARGs) specified <i>a priori</i>
	Quantitative	Culture	6	AR associated microbes specified <i>a priori</i>
		PCR	13	ARGs specified <i>a priori</i>
	Semi-quantitative	PCR	1	
		Sequencing	7	Any ARGs
	Total AR		28	
Enteric	Qualitative	Culture	2	Enteric microbes specified <i>a priori</i>
	Quantitative	Culture	10	
		PCR	2	Enteric microbes specified <i>a priori</i>
	Semi-quantitative	PCR	1	
		Sequencing	68	All microbial communities
	Total Enteric		83	
	Repeats		10	
	TOTAL		101	



Results



Summary: systematic review

Limited range of enteric microbes, primarily indicator bacteria
via culture: *Enterococcus*, *Enterobacter*, *Escherichia*

Some pathogenic genera reported: *Yersinia*, *Salmonella*

qPCR detects of same plus human adenovirus, enteroviruses,
rotavirus (one study)

Our study in Malawi detected ETEC by PCR

16S rRNA analysis most common: 37 to genus, 13 to species

Many detects via 16S: *Clostridium*, *Vibrio*, *Campylobacter*,
Shigella, *Aeromonas*, *Klebsiella*, *E. coli* O157:H7

Relative abundance measures only (fecal typically <1% total)

28 AR studies, wide range reported, but 80% in HICs

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What's missing?

Quantitative estimation of a wide range of pathogens where risks are greatest

Bacteria, virus, protozoa

Viability estimation alongside molecular detection

New technologies!

Highly sensitive, quantitative molecular detection

Portable, high-volume samplers with good & known recovery of DNA/RNA



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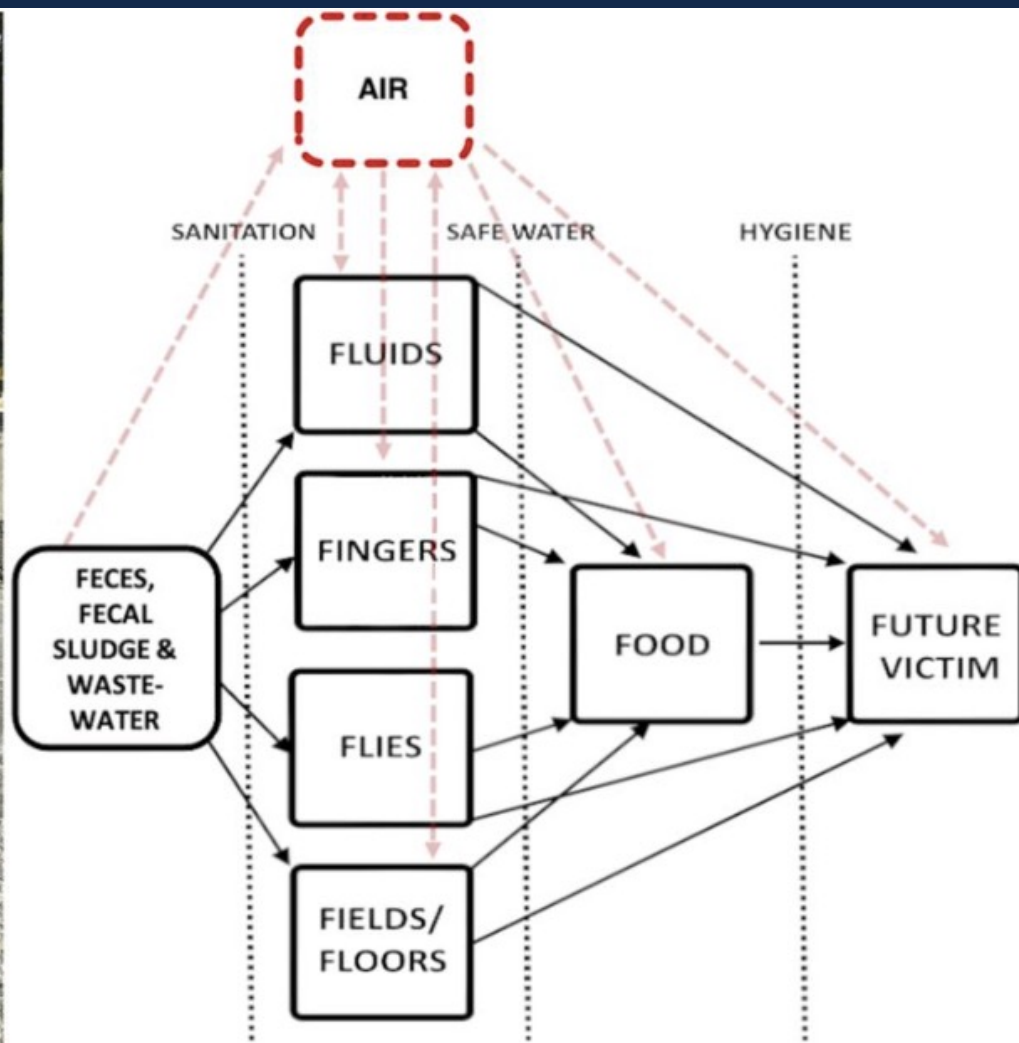
New technologies!

Highly sensitive, quantitative molecular detection

Portable, high-volume samplers with good & known recovery of DNA/RNA



Aerosol transport of enteric pathogens in cities with poor sanitation (2017 – present)



Detection and Quantification of Enteric Pathogens in Aerosols Near Open Wastewater Canals in Cities with Poor Sanitation

Olivia Ginn, Lucas Rocha-Melogno, Aaron Bivins, Sarah Lowry, Maria Cardelino, Dennis Nichols, Sachchida Nand Tripathi, Freddy Soria, Marcos Andrade, Mike Bergin, Marc A. Deshusses, and Joe Brown*

Cite This: *Environ. Sci. Technol.* 2021, 55, 14758–14771

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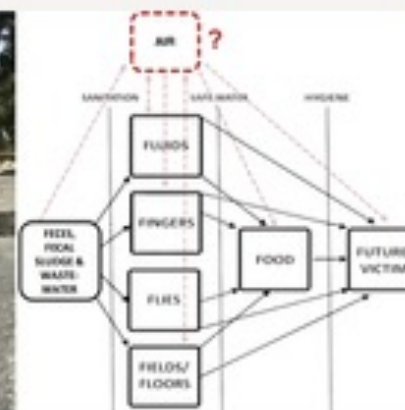
ACCESS |

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Urban sanitation infrastructure is inadequate in many low-income countries, leading to the presence of highly concentrated, uncontained fecal waste streams in densely populated areas. Combined with mechanisms of aerosolization, airborne transport of enteric microbes and their genetic material is possible in such settings but remains poorly characterized. We detected and quantified enteric pathogen-associated gene targets in aerosol samples near open wastewater canals (OWCs) or impacted (receiving sewage or wastewater) surface waters and control sites in La Paz, Bolivia; Kanpur, India; and Atlanta, USA, via multiplex reverse-transcription qPCR (37 targets) and ddPCR (13 targets). We detected a wide range of enteric targets, some not previously reported in extramural urban aerosols, with more frequent detections of all enteric targets at higher densities in La Paz and Kanpur near OWCs. We report density estimates ranging up to 4.7×10^3 gc per m^3_{air} across all targets including heat-stable enterotoxigenic *Escherichia coli*, *Campylobacter jejuni*, enteroinvasive *E. coli*/*Shigella* spp., *Salmonella* spp., norovirus, and *Cryptosporidium* spp. Estimated 25, 76, and 0% of samples containing positive pathogen detects were accompanied by culturable *E. coli* in La Paz, Kanpur, and Atlanta, respectively, suggesting potential for viability of enteric microbes at the point of sampling. Airborne transmission of enteric pathogens merits further investigation in cities with poor sanitation.



Methods

Non-random sampling, reference sites close to & far from sources

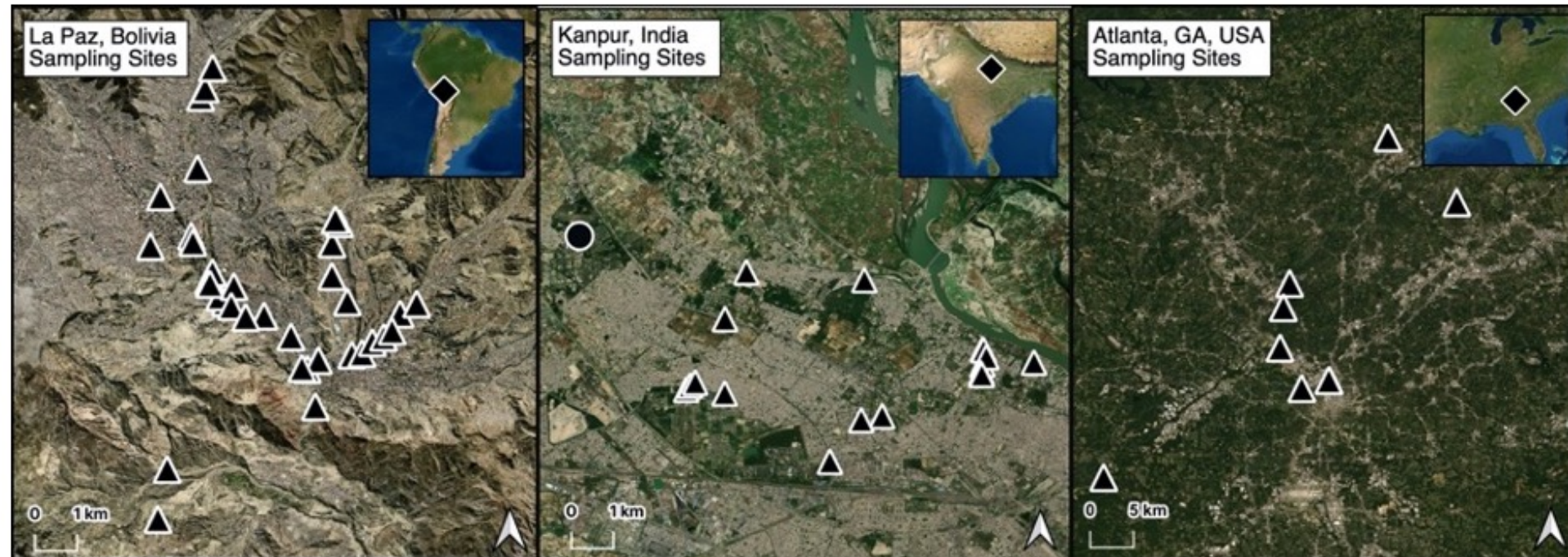
150-200 lpm sampling over ~4 hours with Bobcat (up to 48m³)

impingement (12.5 lpm) & ACI (28 lpm) for culture

qPCR & ddPCR, ~50 targets (indicators, pathogens, AMR)

RH, temperature, wind, UV, other data

2 seasons



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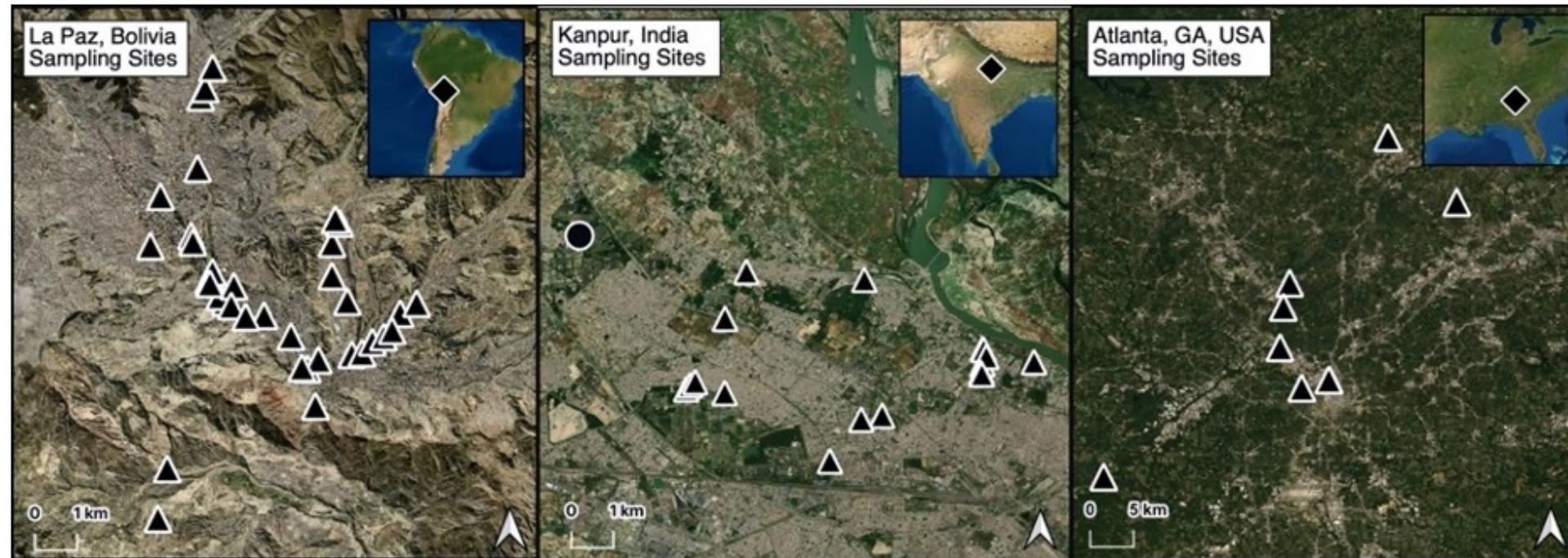
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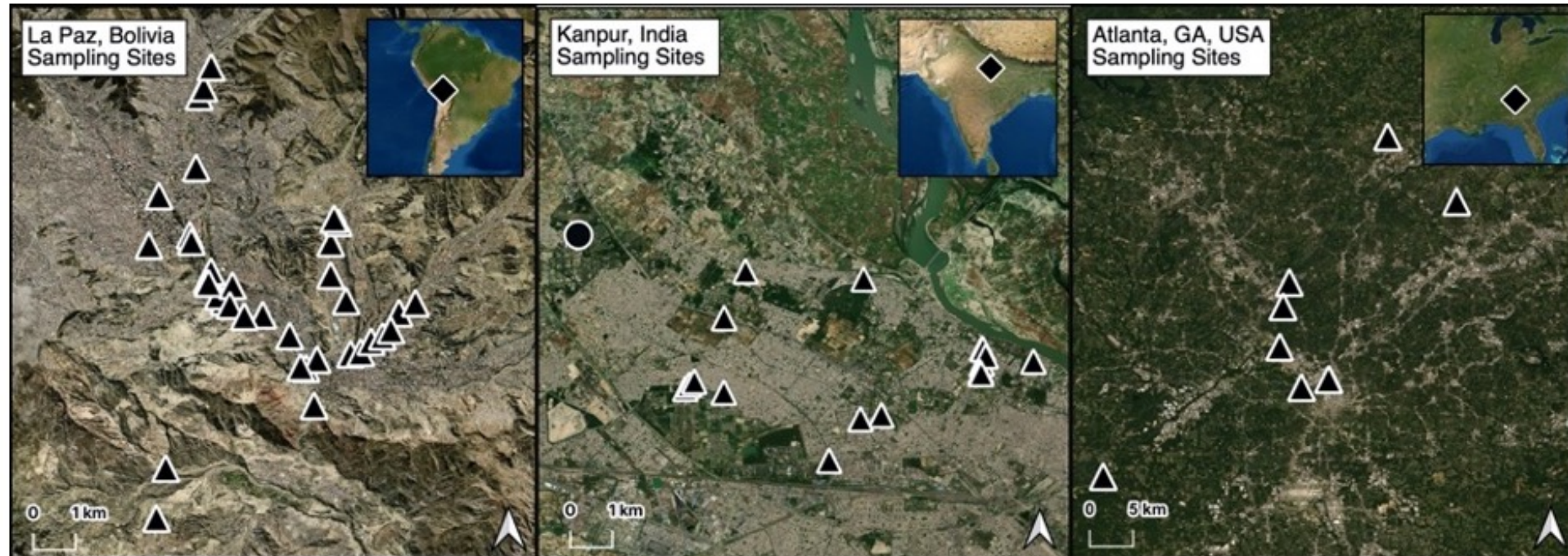
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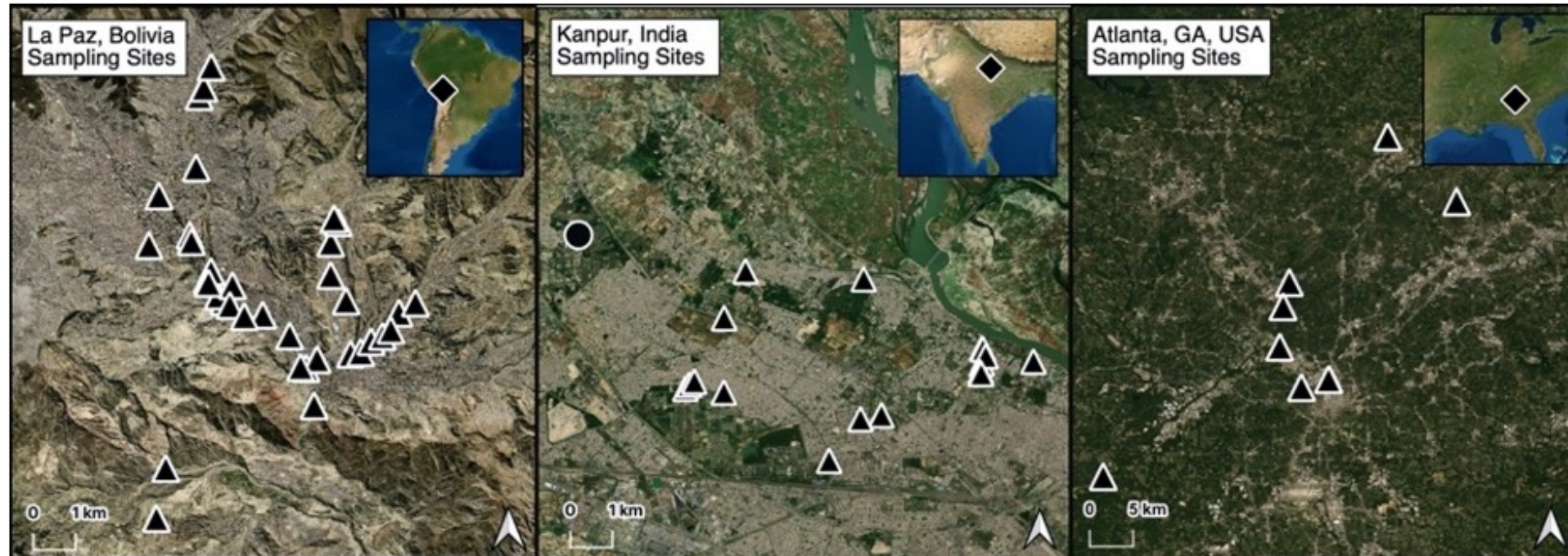
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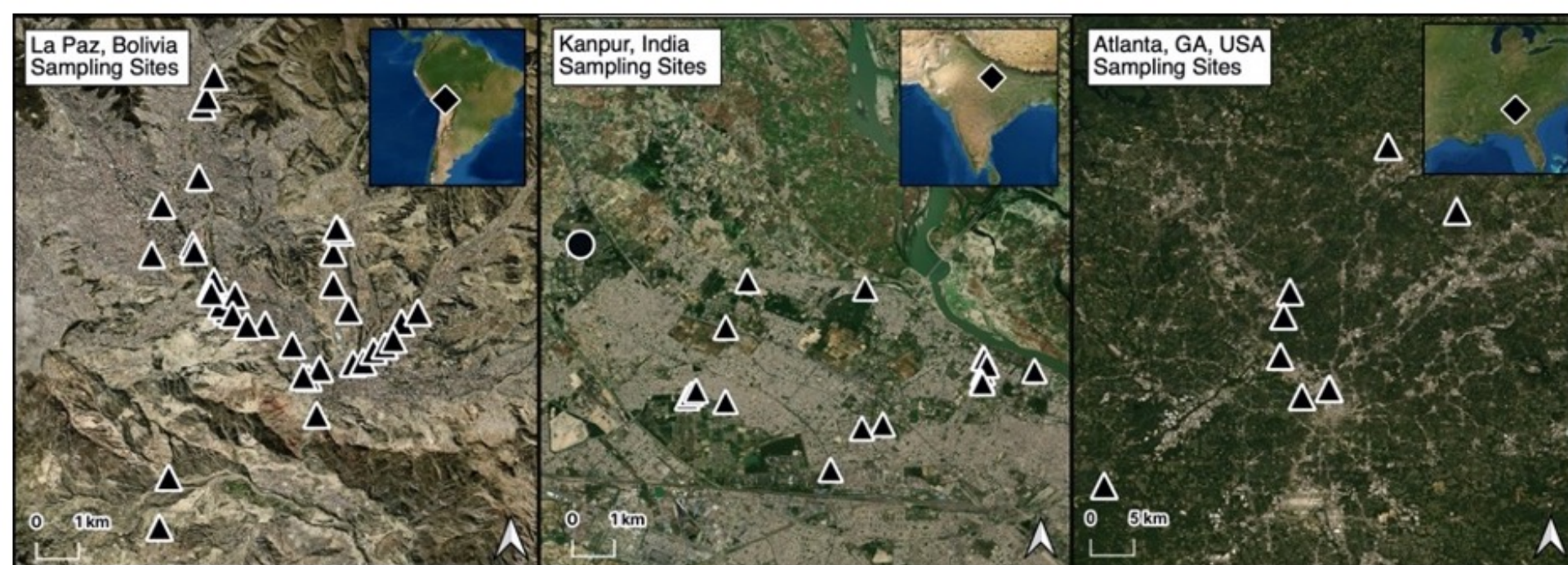
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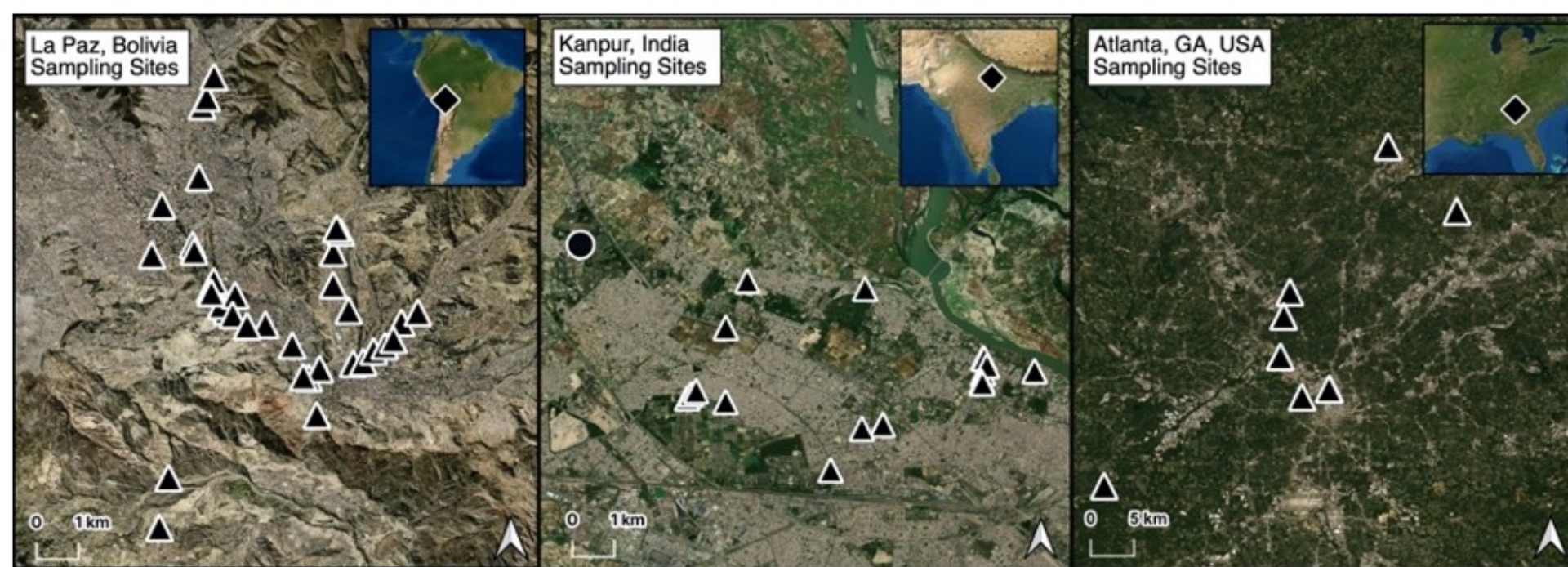
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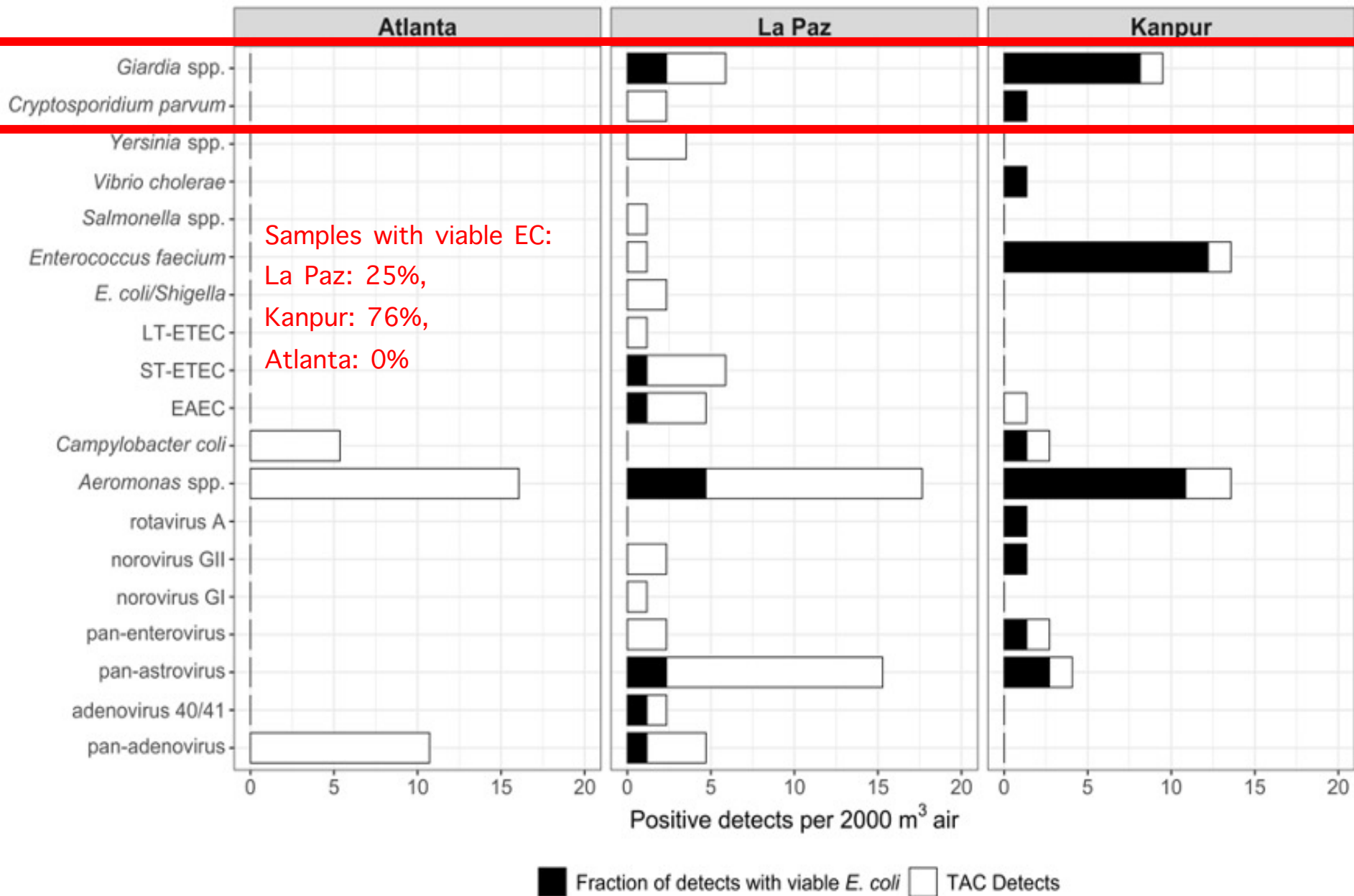


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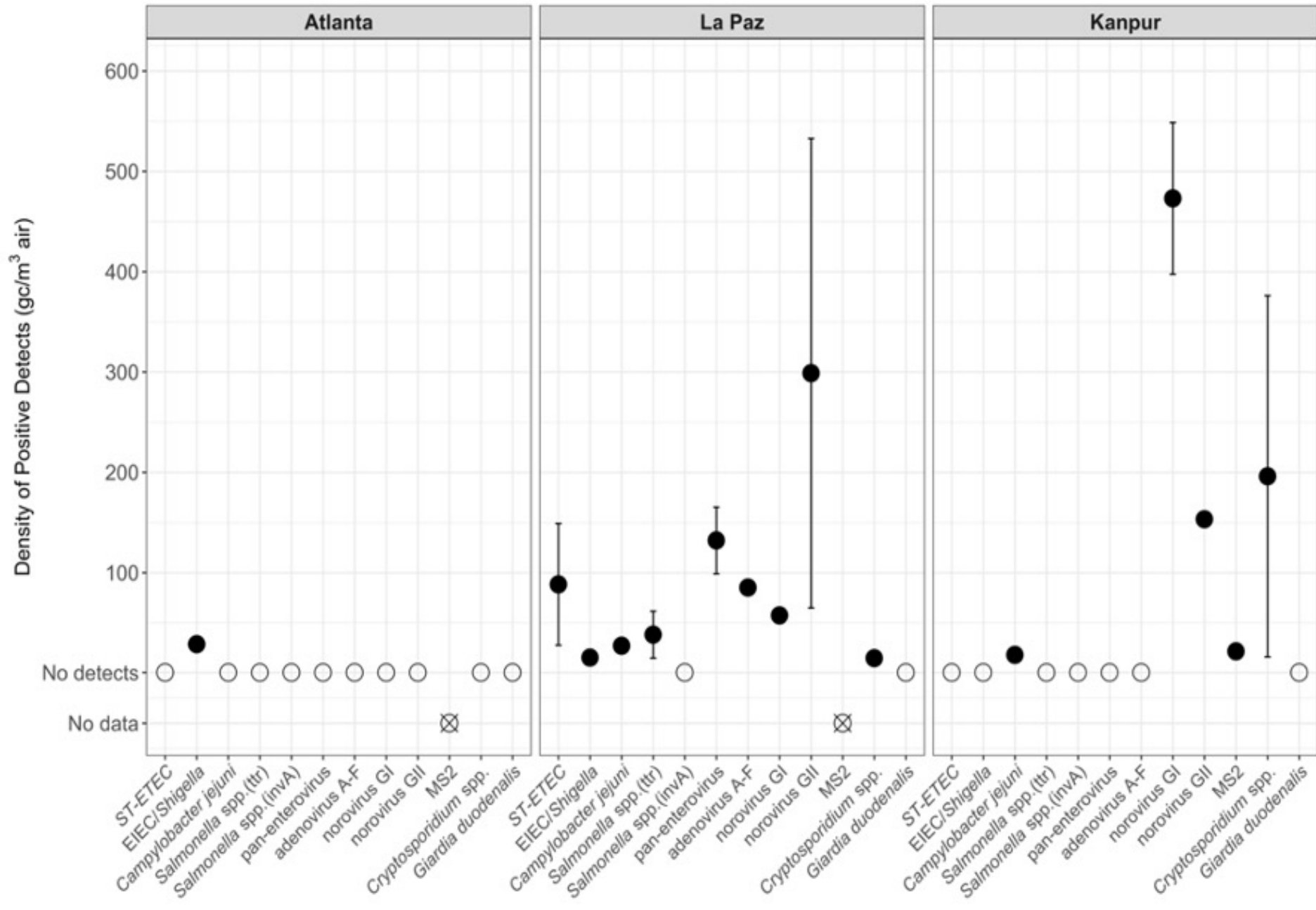
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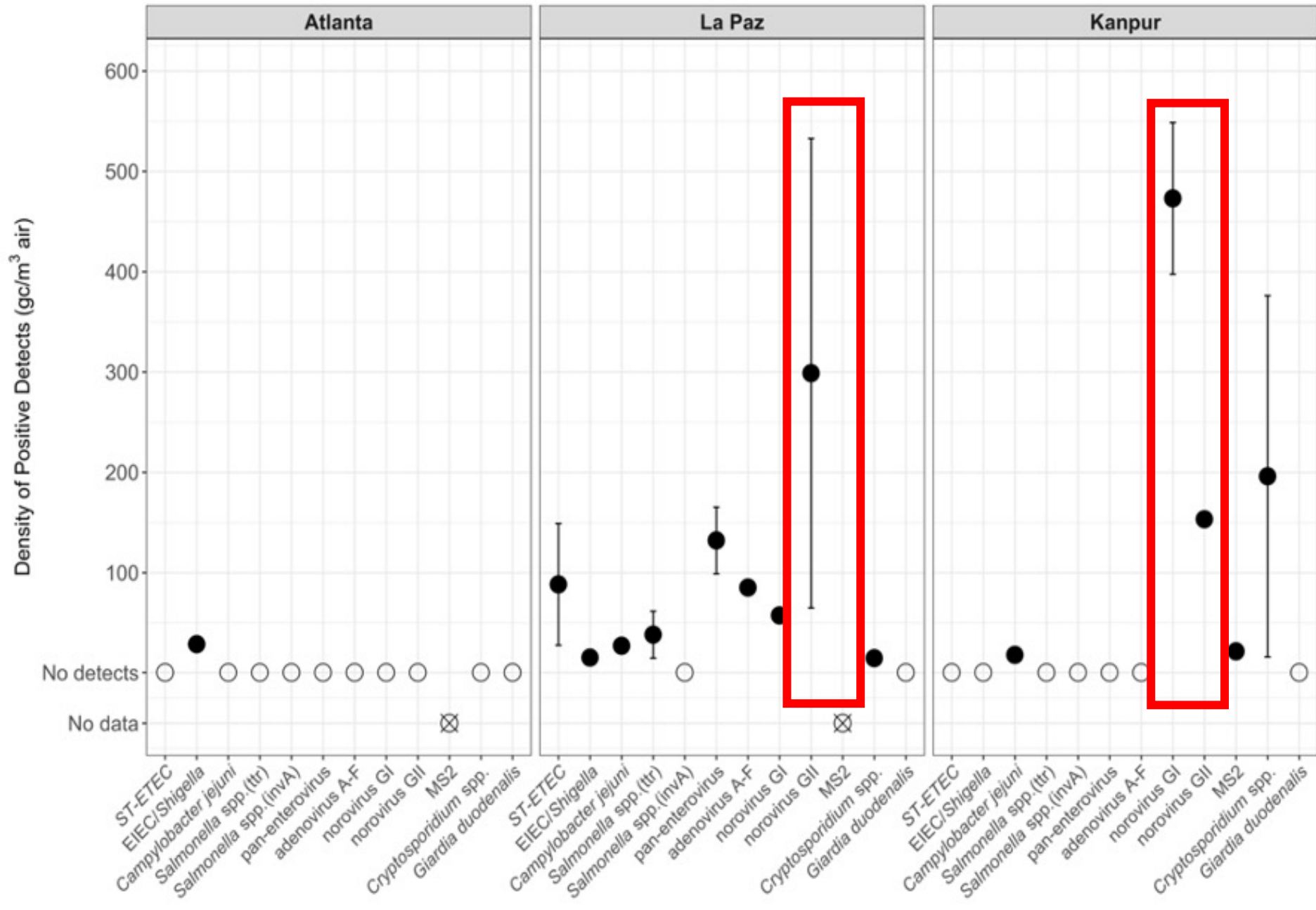
Multiplex pathogen screening via TAC



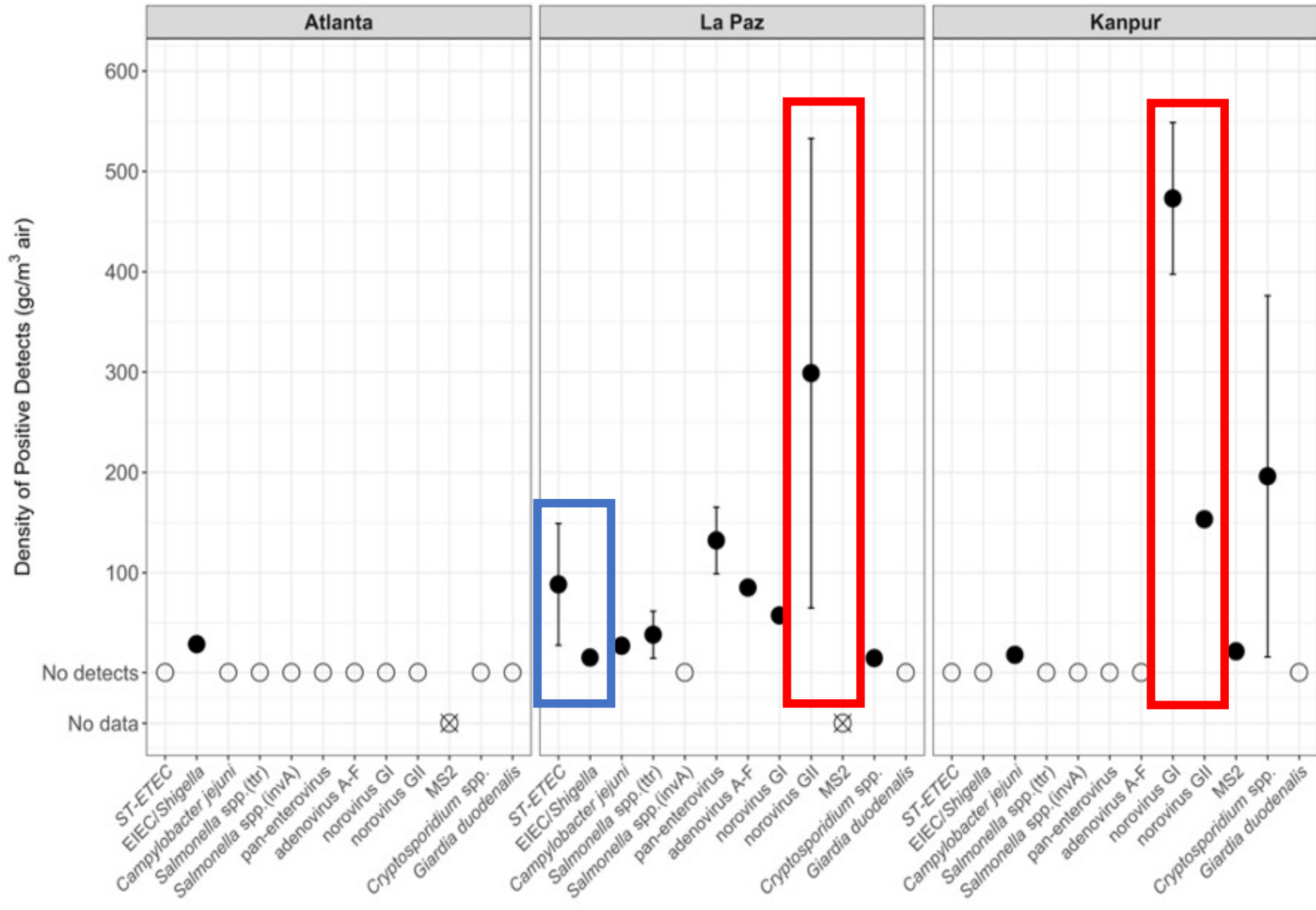
Density estimation via ddPCR



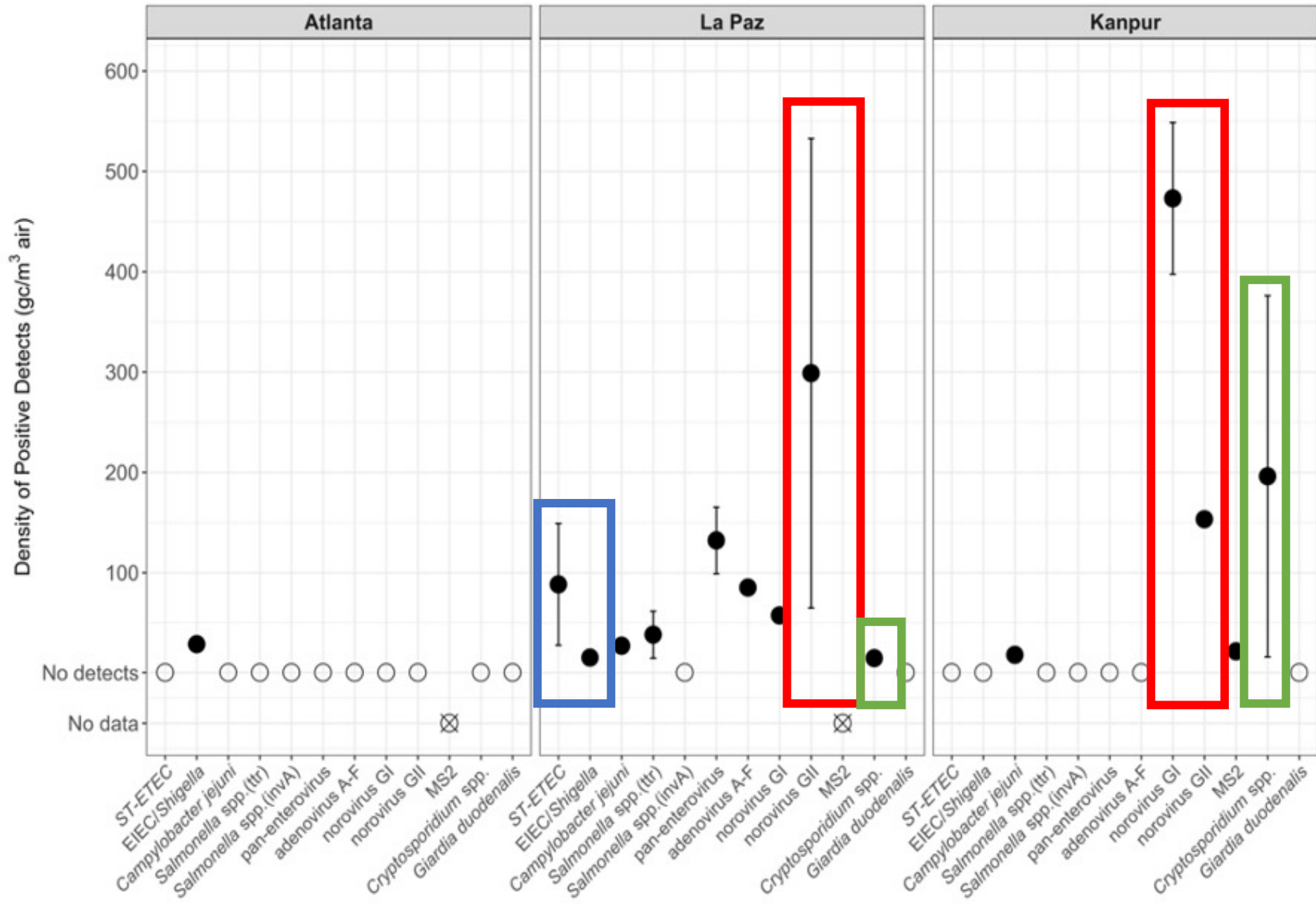
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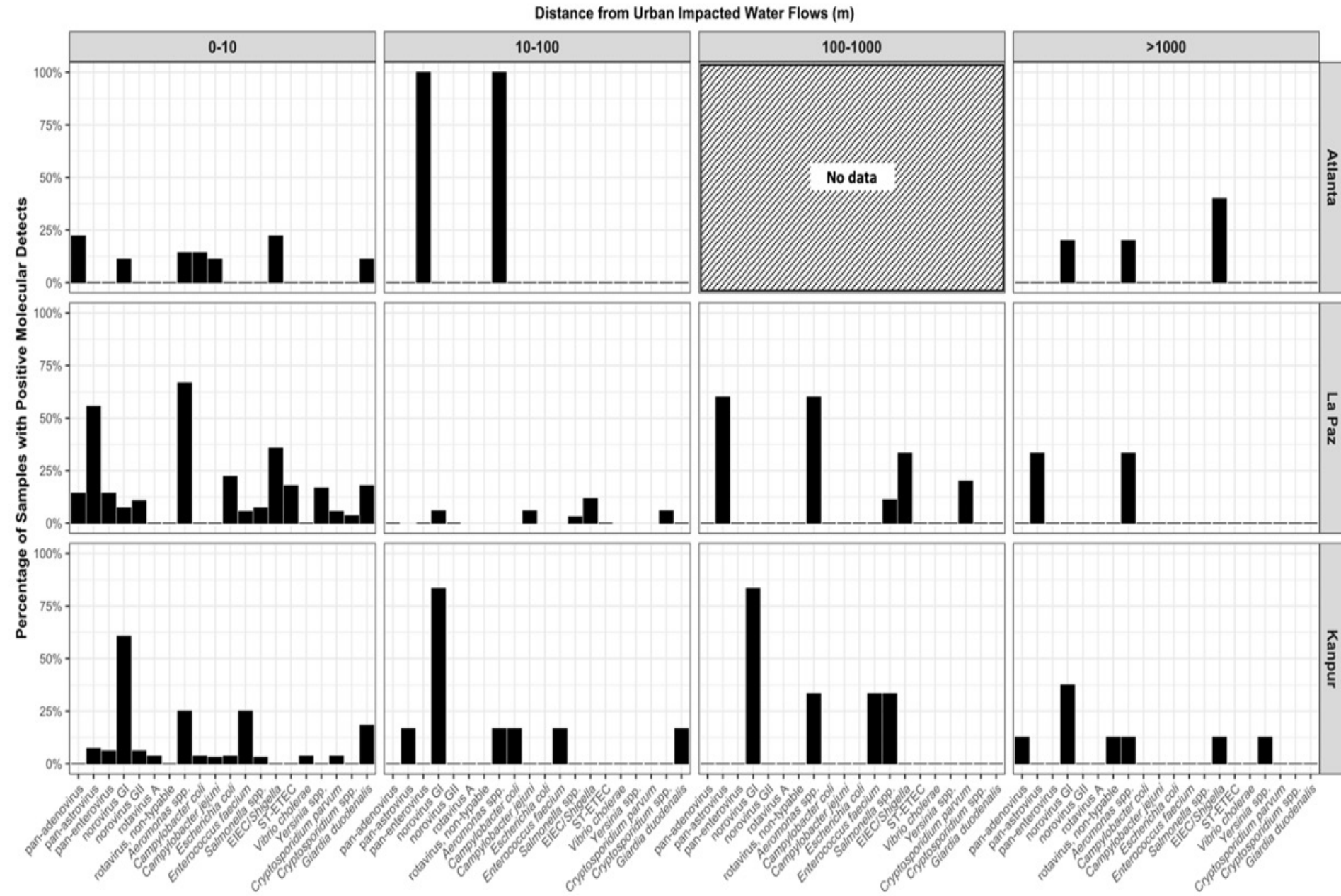
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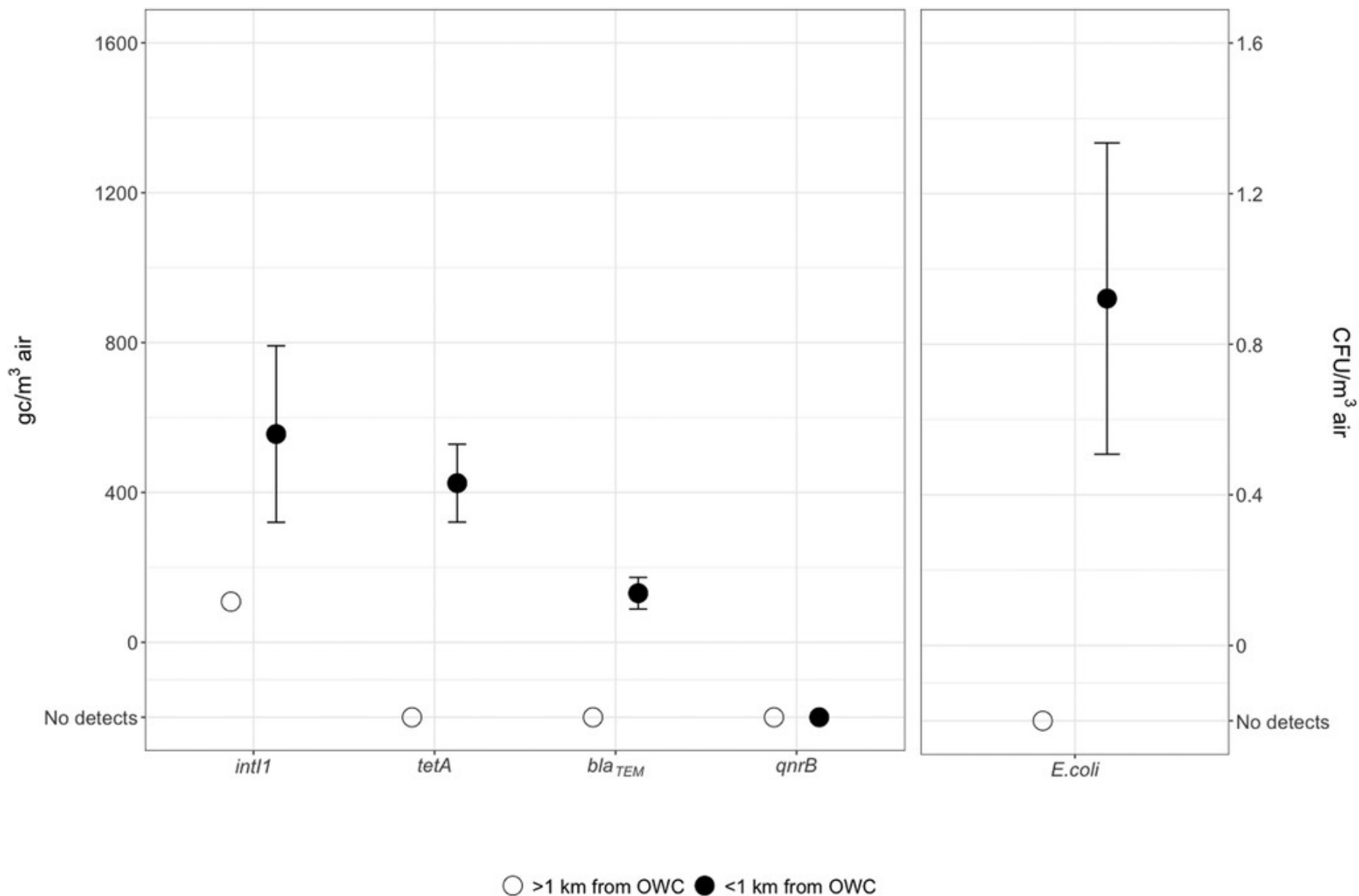
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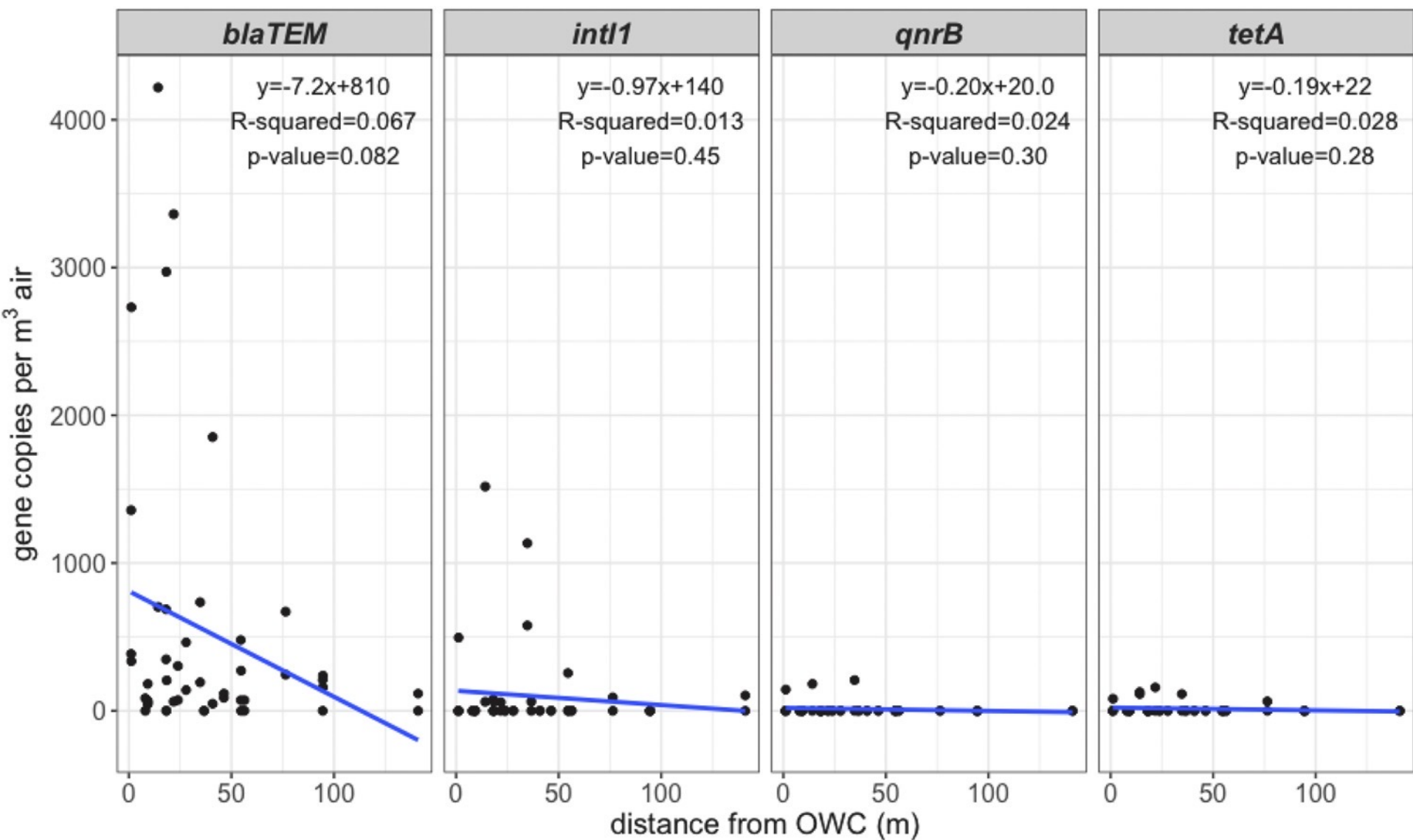
% samples with positive molecular detection

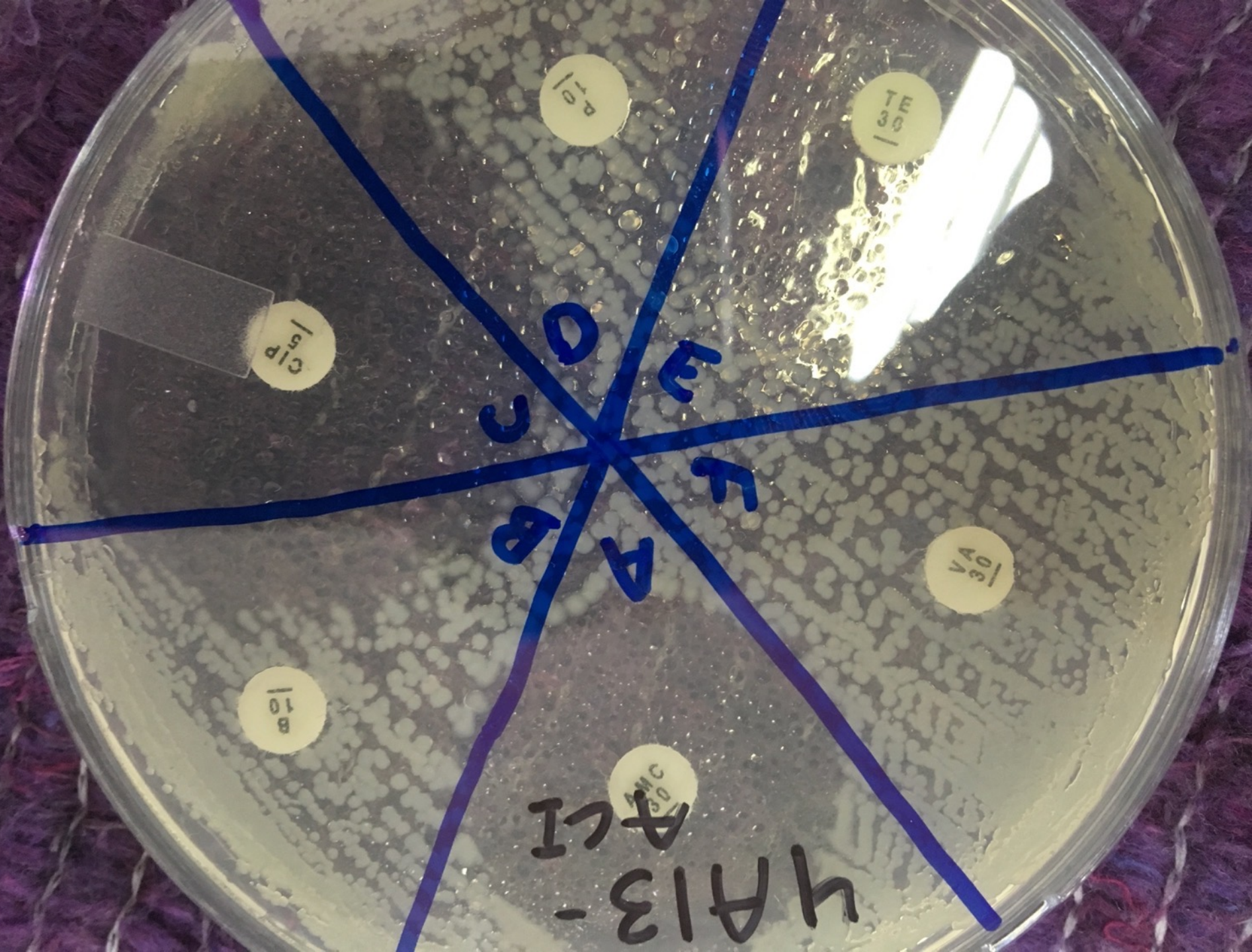


Kanpur



Mean antimicrobial resistance gene and mobile integron densities with mean standard error bars for the distribution in gene copies per cubic meter of air, where targets were detected at levels equal to or above the limits of detection (left). Estimated mean culturable *E. coli* as colony-forming unit per cubic meter of air with mean standard error bars for the distribution (right).





4A13-
ACI

AMC
30

B
10

V30

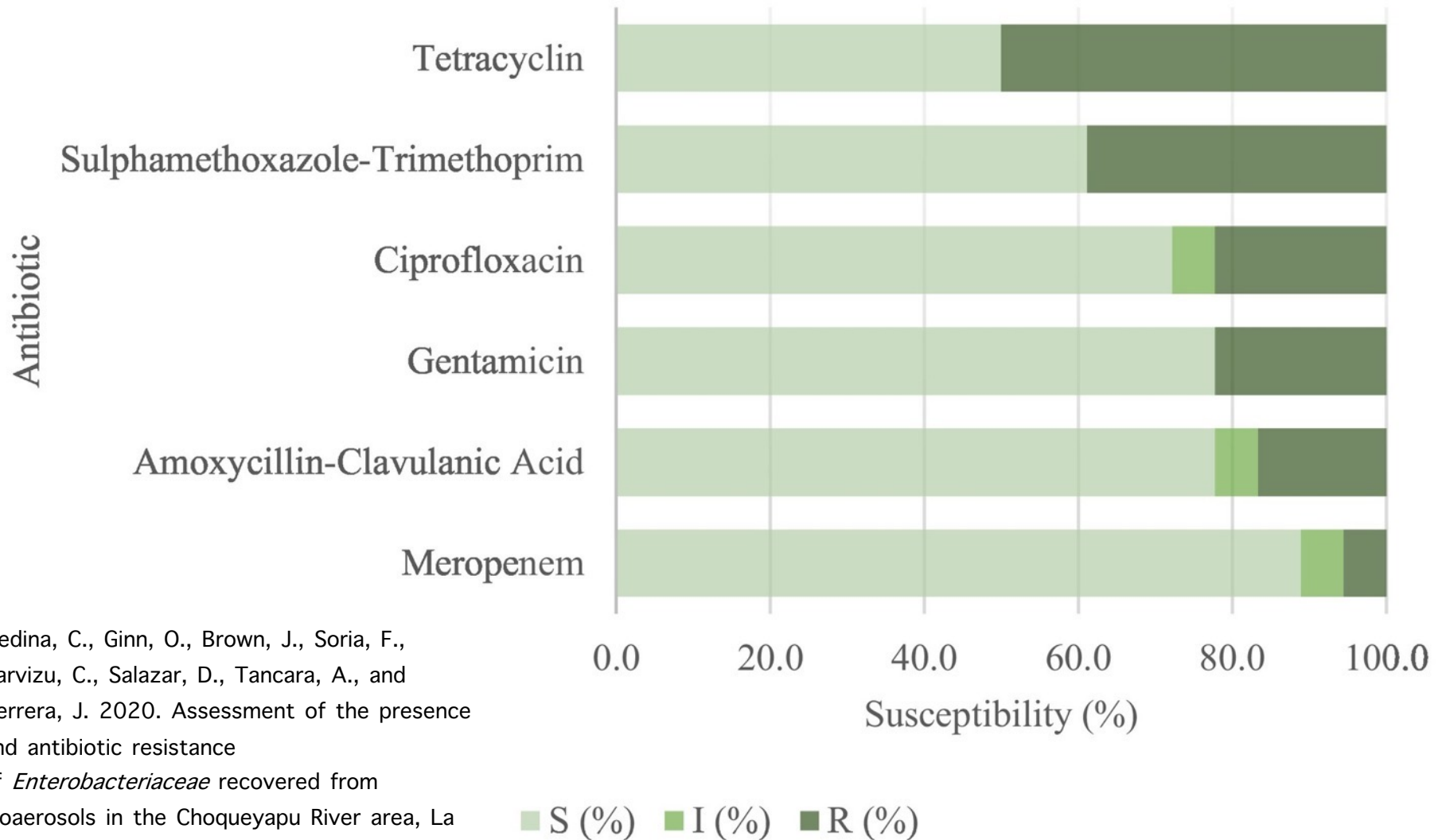
TE
30

10P

CIP
15P

A
B
C
D
E
F

La Paz



Medina, C., Ginn, O., Brown, J., Soria, F., Garvizu, C., Salazar, D., Tancara, A., and Herrera, J. 2020. Assessment of the presence and antibiotic resistance of *Enterobacteriaceae* recovered from bioaerosols in the Choqueyapu River area, La Paz, Bolivia. *Science of the Total Environment*



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



Lucas Rocha-Melogno^{a,b,c}, Katherine C. Crank^d, Olivia Ginn^e, Michael H. Bergin^a, Joe Brown^f, Gregory C. Gray^{b,g,h,i,j}, Kerry A. Hamilton^{k,l}, Kyle Bibby^d, Marc A. Deshusses^{a,b,*}

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^e School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332, United States

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^j Division of Infectious Diseases, University of Texas Medical Branch (UTMB), Galveston, TX 77555, United States

^k School of Sustainable Engineering and the Built Environment, Arizona State University, 770 S College Ave, Tempe, AZ 85281, United States

^l The Biodesign Institute Center for Environmental Health Engineering, Arizona State University, 1001 S McAllister Ave, Tempe, AZ 85281, United States

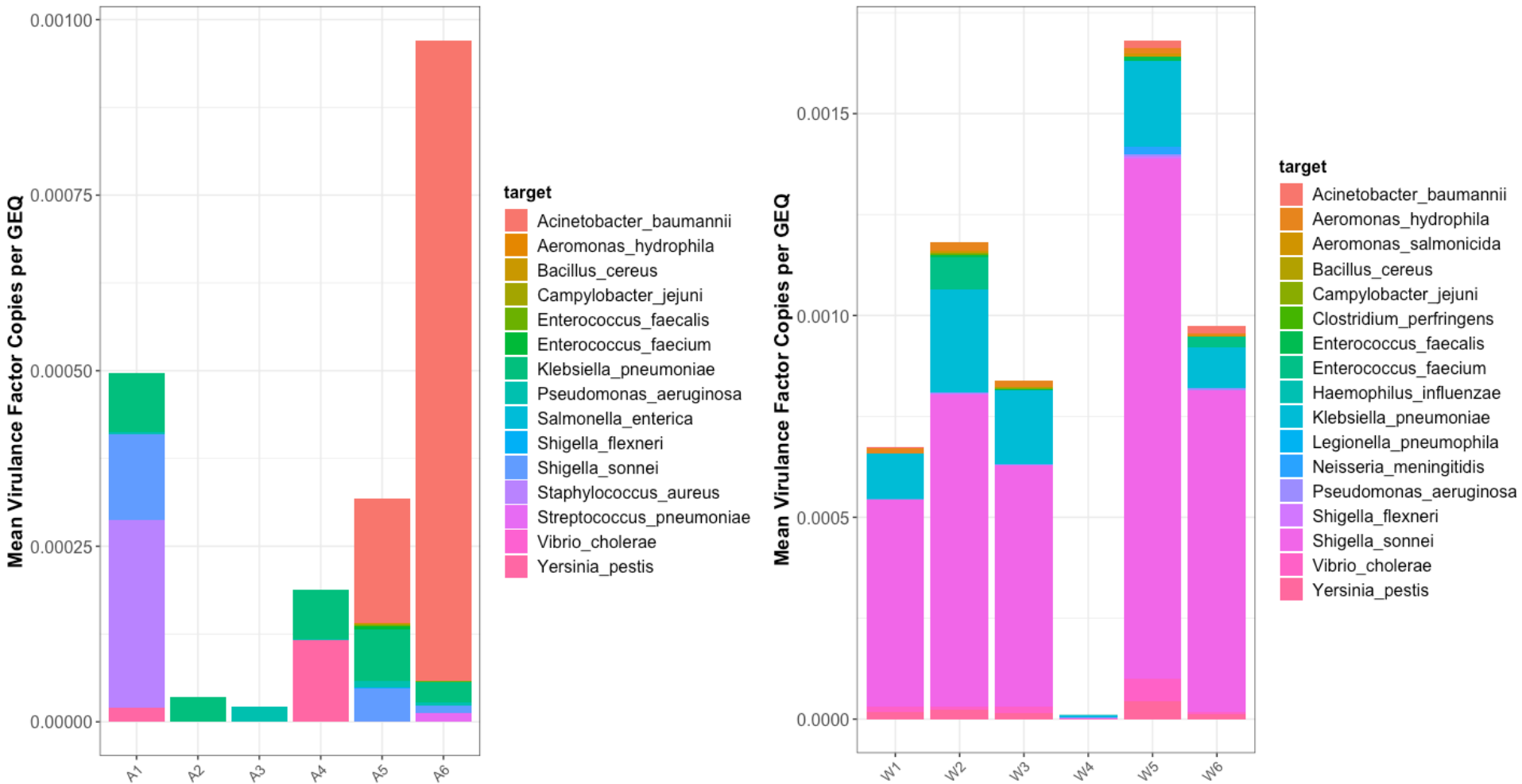
HIGHLIGHTS

- Infection risk of fecal bioaerosols near open waste canals (OWCs) was assessed.
- A Quantitative Microbial Risk Assessment (QMRA) model and a web app were developed.
- Fecal bacterial aerosols near OWCs pose non-negligible risks of infection.
- Bioaerosols near OWCs may not be a major cause of diarrheal disease in La Paz.
- The web application allows users to conduct QMRA in different contexts.

GRAPHICAL ABSTRACT



In progress: air & presumptive source metagenomes



Some observations

Enteric microbes, including pathogens & antimicrobial resistance, are present & enriched near open sewers

Some previously unreported in urban aerosols

Culturable indicators in many of the same samples

Could be many sources: ongoing work in source tracking

Probably lead to exposures in dense cities

Current & future transport and risk modeling

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UNC

GILLINGS SCHOOL OF
GLOBAL PUBLIC HEALTH

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Types of studies that will advance the science

Observational epi or risk assessment studies

Controlled laboratory experiments to examine the aerosolization and viability kinetics of fecal microbes

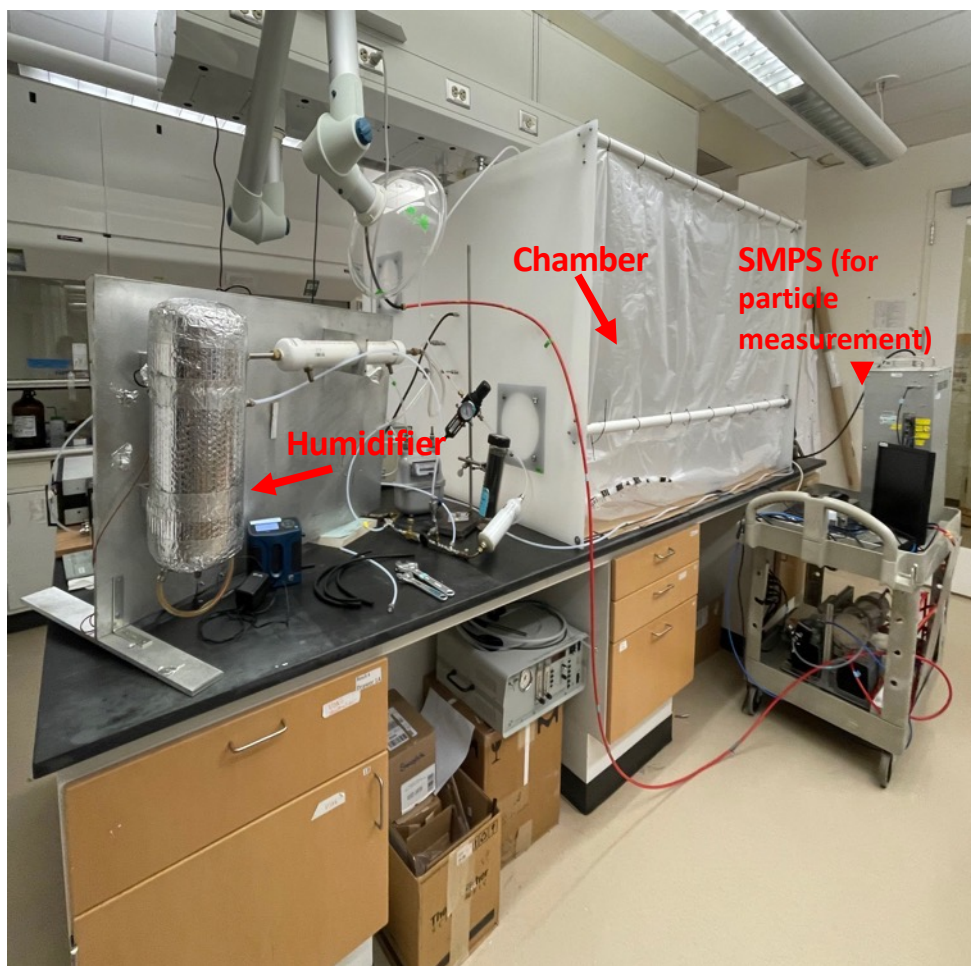
Controlled animal studies!

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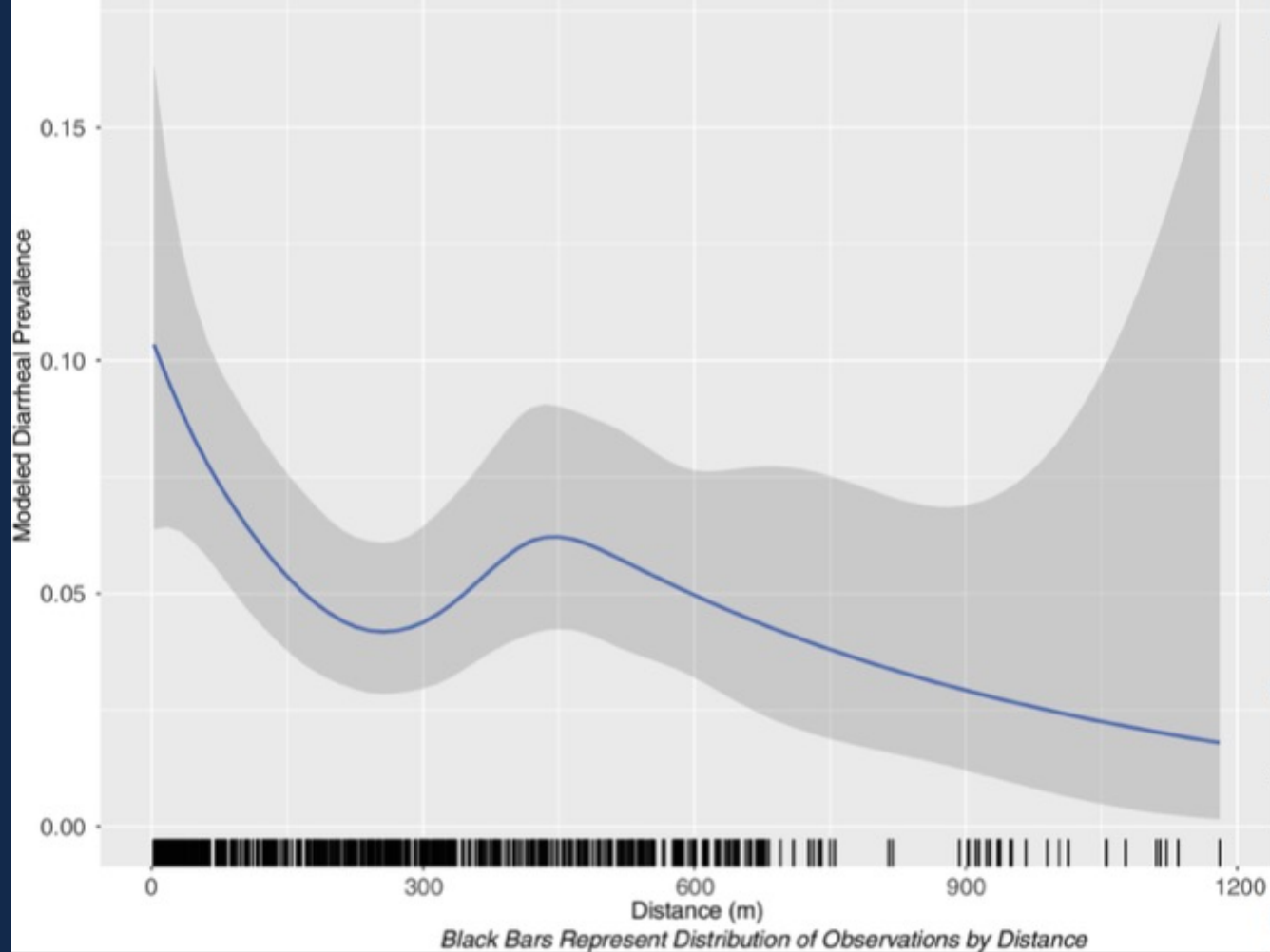
Controlled animal studies!

Contreras *et al.* 2020
Environmental Health Perspectives

Mezquital Valley in Mexico:

Compared to children under 5 living within 10 m from a canal, children living 100 m from a canal had 45% lower odds of diarrhea and children living 1000 m from a canal had 70% lower odds of diarrhea.

24% of all diarrheal cases in the study and 50% of all cases within 100 m from a canal were attributable to canal exposure

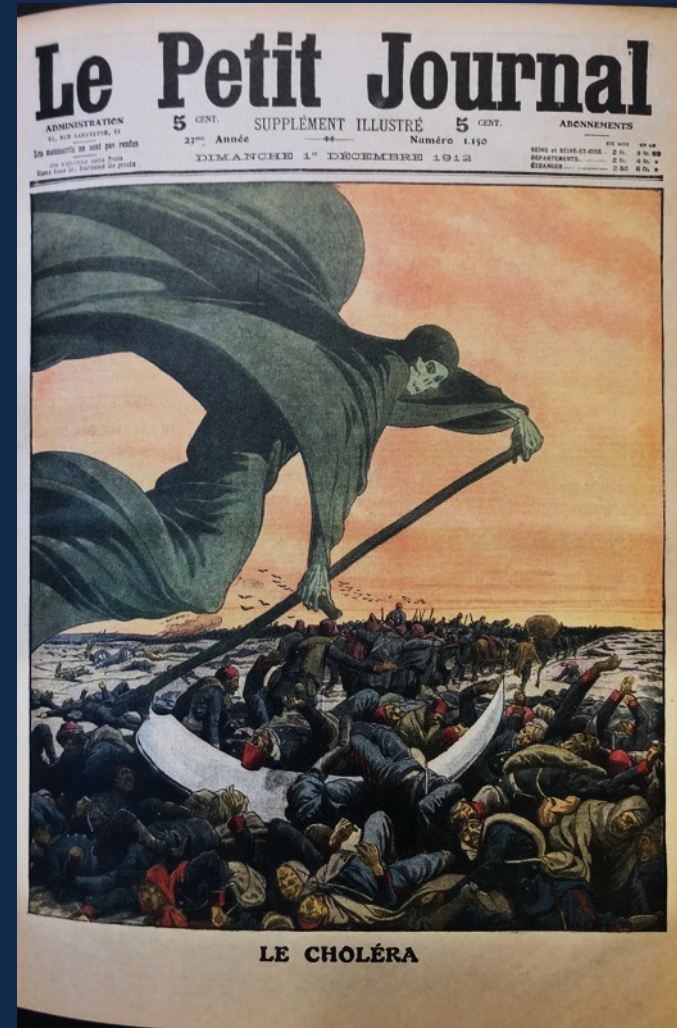


So what if it's raining (fecal) microbes in densely populated cities all over the world?

Aerosol transmission possible – *but is it probable?*

The source of the observed *microbial veneer*

Implications for all other environmental sampling

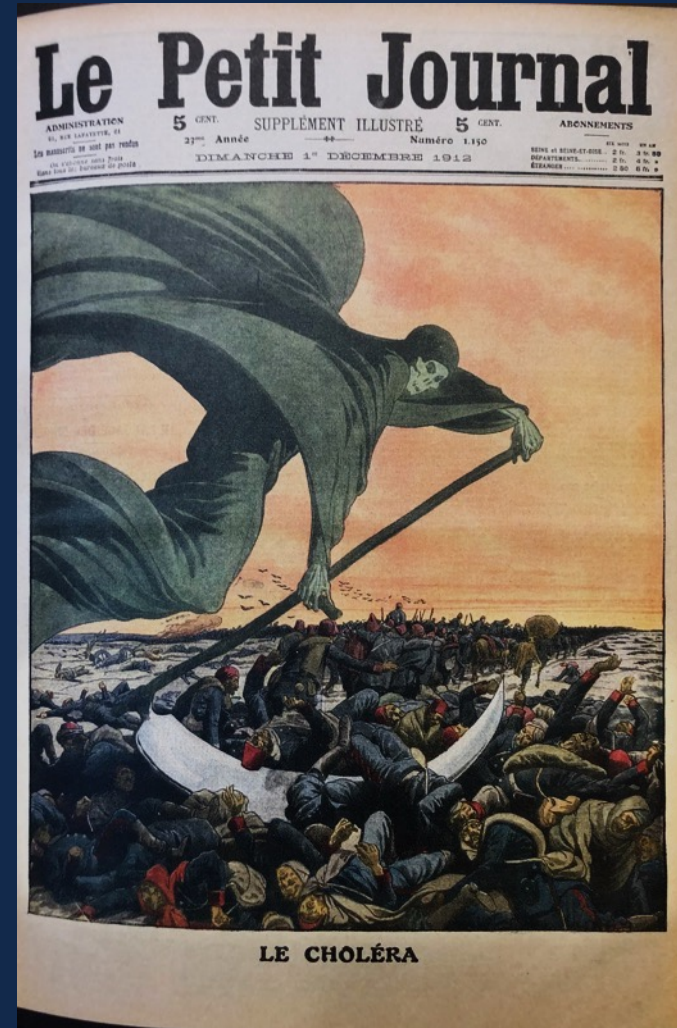


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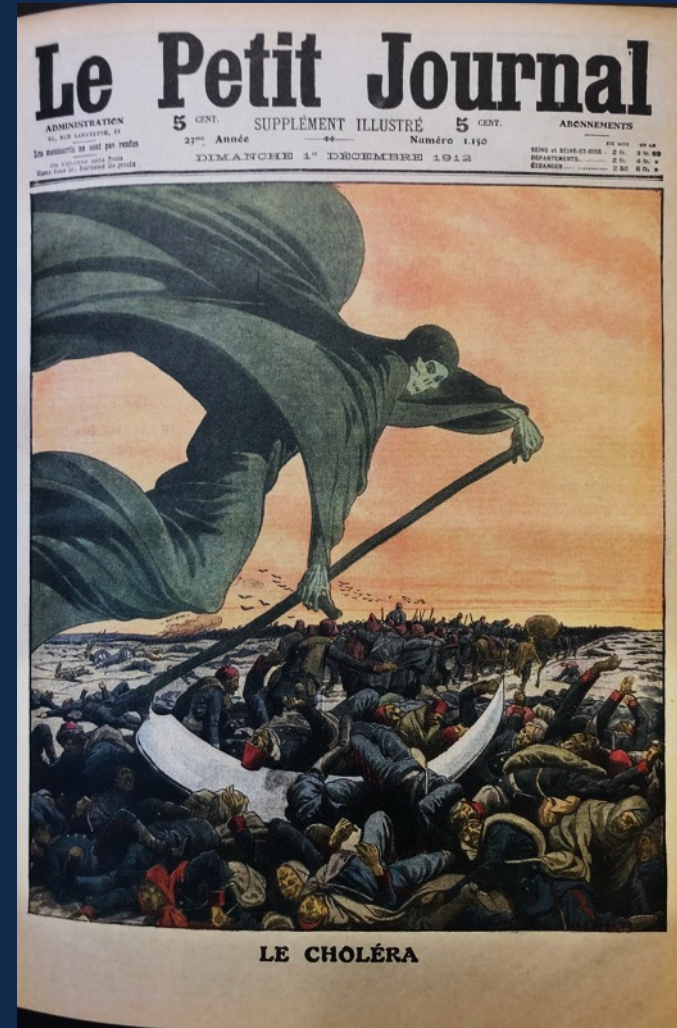


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David Holcomb PhD,
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Amanda Lai PhD PE,
post-doctoral fellow



Gouthami Rao, PhD
student



Erin Kowalsky, PhD
student



Yarrow Linden, PhD
student



Sarah Lebu, PhD student



Anjerul Islam, PhD
student



Troy Barker, MPH student



Abby Knoble, MPH
student



Jack Dalton, MPH student



Sierra Brantz, MPH
student



Toheedat Bakare,
undergraduate
researcher



Ryan Clark,
undergraduate
researcher



Abeoseh Fiemister,
undergraduate
researcher



Elizabeth Kim,
undergraduate



Alka Manoj,
undergraduate



Sam Pomper,
undergraduate



Victor Ilevbare,
undergraduate

Thanks!
More info:
tarheels.live/brown

