

Wastewater Surveillance to Inform Hyperlocal Trends in Antimicrobial Resistance (AMR) and Community Level Antimicrobial Stewardship (AMS) in Urban and Peri-Urban Settings: A Pilot Study from Bengaluru, India

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Abstract

We sought to explore the potential of wastewater surveillance to detect hyperlocal AMR patterns of clinically relevant Gram-negative bacteria against key antimicrobial classes and examine implications for community-level antimicrobial stewardship (AMS).

Methods

Intracity open drains: Between March and April 2022, samples from 66 open drain sites (containing untreated wastewater and rainwater) across the eight administrative zones of Bengaluru city in southern India were tested for antimicrobial sensitivity profiles against key antibiotic classes using standard microbiological methods. **Intercity treated-wastewater (TWW) channels:** TWW from Bengaluru is pumped to peri-urban areas to rejuvenate dried lakes and increase groundwater levels. We tested the lake waters and surrounding open wells for AMR, antibiotic levels and heavy metals in June and September 2023.

Results

Intracity drains: About 28% of *Enterobacteriaceae* isolates (82% of total isolates) were susceptible to all antibiotic classes while 12% were resistant to all. Carbapenem resistant microorganisms were found in several sites. Testing cost was ~INR 5,000 (USD 53)/sample for consumables. **Intercity channels:** Certain antibiotics (amoxicillin, clavulanic acid, isoniazid, fluconazole and linezolid) were detected. However, no heavy metals were present. No isolates resistant to all antibiotic classes tested were found. Additional costs for antibiotics and heavy metal testing were ~INR 20,000 (USD 206)/sample.

Discussion

Our results indicate: a) drug resistant bacteria are found in significant numbers in intracity open drains b) Significant geographical variation in AMR patterns, underscoring the need for hyperlocal AMR intelligence and community-relevant stewardship approaches.

Conclusion

Wastewater surveillance is a feasible and cost-effective method for generating hyperlocal community-level AMR intelligence in urban and peri-urban settings.

Keywords: Antimicrobial Resistance (AMR), Wastewater Surveillance, Antibiotics, Hyperlocal Signals, Community level Antimicrobial Stewardship (AMS), Hyperlocal Surveillance, Community Health, One Health, Environmental Surveillance

1. Introduction

Approximately 1.2 million people died in 2019 globally due to antimicrobial resistant infections making it one of the major public health issues of the 21st century [1]. Yet, antimicrobial resistance, contrary to popular belief, is not just a problem created in the rooms of doctors' clinics and hospitals. It is a "wicked" problem spanning human, animal and environmental realms, which epitomizes volatility, uncertainty, complexity and ambiguity and the need for nuanced understanding and interpretation- all the characteristics that make it incredibly challenging to solve through broad public health directives or vertical programs [2,3].

1.1. The Need for Community-Level Antimicrobial Resistance and the Potential of Wastewater Testing

Antimicrobial resistance (AMR) both contributes to and is heavily influenced by local factors at the community and the individual levels [4]. Antimicrobial stewardship (AMS), the process of fighting antibiotic resistance and preserving antibiotics, is, at its foundation, a learning guided behavioral change at the levels of individuals (micro), local organizations (meso), and overall systems (macro). Behavioral changes need the problem to be personalized, for individuals to believe in a sense of personal urgency without which it is impossible to keep efforts sustained for altering lifelong habits. However, currently, there is a perceptible translation gap between the data and directive policies of AMR and the day-to-day practice of antibiotic prescribing. Without a sense of personal agency or responsibility, antimicrobial stewardship on the ground remains theoretical. This could be due to the fact that much of the data on AMR is derived from clinical specimens (human or animal) received at large, tertiary care hospitals attached to clinical academic research centers, thus increasing the chances that it is seen as irrelevant at the ground level. Thus, while national databases and trend reports for antibiotic resistance have their place and uses, there are potential and significant harms, even if unintended, possible from using them as primary guidelines to inform local prescriptions at the community level for humans and animals.

1.2. Community Level AMR should be Viewed as Social-Ecological-Systems Problem using the One Health Approach

One important implication of hyperlocal AMR surveillance is that antimicrobial resistance needs to be understood not merely as a hospital-based microbiological phenomenon, but as a dynamic ecological process shaped by local prescribing cultures, healthcare fragmentation, sanitation systems, environmental circulation, and community antibiotic exposure. In this framing, wastewater becomes a population-level reflection of how antibiotics, pathogens, healthcare systems, and urban infrastructures interact over time. Such an ecological understanding may help bridge the persistent translation gap between national AMR policies and the everyday realities of antimicrobial use in community settings.

1.3. Wastewater as a Surrogate for Measuring Community Burden of AMR

Community level antibiotic resistance data is notoriously difficult to obtain. Testing individual clinical samples from communities for the purpose of surveillance is labor-intensive and expensive, not to mention mired in ethical dilemmas about privacy and availability/ accessibility of subsequent treatment. While each hospital is expected to generate its own antibiograms (which track antibiotic usage and antimicrobial resistance against the antibiotics), which may or may not be regularly updated, this data is usually not shared publicly or entered into a central database for policy analysis and surveillance [5]. How then to generate local community-relevant, yet anonymized, data that could enable bottom-up, local-context based initiatives which are driven by motivations that arise not just from the cognitive domain but also in the affective domain? Wastewater could be a potential solution. Wastewater surveillance has been used as far back as 1900 to track *Mycobacterium tuberculosis* from sanatoriums, in the 1930s to detect typhoid outbreaks in villages, is now most well-known for usage as a monitoring method for polio virus and in the recent past has seen a revival as an early warning system for Covid-19 [6-10]. Wastewater is a significant environmental reservoir of AMR. It also contains high levels of antibiotics from various sources (Antibiotic manufacturers, human, veterinary, surface runoff from animal husbandry industries etc.) and thus represents an ideal environment for various populations of susceptible and resistant bacteria (ARB) to co-exist, exchange antimicrobial resistant genes (ARGs) and amplify and spread AMR [11]. Many studies in the past decade have shown the presence of ARGs against beta lactams, fluoroquinolones, cephalosporins, colistin, carbapenems and more, using methods ranging from Kirby Bauer method, PCR and metagenomic approaches [11-13]. AMR reservoirs in the environment could be a driver of persistence of AMR, even in countries with strong antimicrobial stewardship. Microbes can acquire antibiotic resistant genes from each other between human, animal and environmental domains. Crucially, wastewater-based AMR data isn't restricted to only human or animal-derived information but is a composite of both.

1.4. The added Complication of Lake Rejuvenation in Peri-Urban Areas using Urban Treated Wastewater

In recent years, with increasing global warming and unregulated urban growth, lakes around cities have been drained and groundwater levels steadily decreasing. In several cities across the world, including Bengaluru, the site of this study, efforts to rejuvenate dried lakes and replenish the shallow aquifers and groundwater levels have been initiated using treated wastewater (TWW) from the city. In the context of this study, we studied an ecosystem where treated wastewater (TWW) from the outlet of a sewage treatment plant is pumped to two lakes and the water from these lakes enters neighboring open wells and other lakes through underground gravitational flow. These waters were tested for the presence of AMR pathogens, antibiotics and heavy metals, to determine if the rejuvenation efforts were

inadvertently contributing to transmission of AMR and other risks from an urban to a peri-urban site. In this paper, our objectives were to explore the potential of wastewater to detect local AMR patterns by characterizing the phenotypic patterns of specific Gram-Negative Bacteria (GNB) against 4 key antimicrobial classes and 5 commonly used exemplar antibiotics across Bengaluru city. We have also included the costs incurred for the same to enable other groups, including government agencies, to conduct similar studies in their geographical contexts.

2. Methods

2.1. Geographical Context

Bengaluru city is India's third most populous city, with a total population of about 13.1 million individuals, spread over 2000 square kilometers [14]. Bengaluru, like most urban cities, has a high antibiotic usage pattern through the large numbers of tertiary and quaternary care hospitals within its boundaries, as well as animal husbandry units nearby. Intracity wastewater network relevant to this study: About 60-70% of its wastewater is underground and part of a formal sewage network, while 30% is disposed through open storm water drains, typically passing uncovered and exposed through lower socioeconomic areas of the city, occasionally accessible to cattle or pigs. These drains open into intracity lakes without treatment and if easily accessible, are then used for local agriculture and animal husbandry. Thus, these

drains contain a mix of untreated sewage (mostly domestic, although small industries could also be illegally emptying their waste into these channels) and rain water. Thus, they are both a source of water and disease for animals and humans alike. Intercity wastewater network relevant to this study: In parallel, treated wastewater (TWW) from Bengaluru, since 2018, has been pumped to lakes around the city in order to rejuvenate them and increase the groundwater levels. Thus, open wells and other shallow aquifers, in several areas around Bengaluru have also, through the process of gravitational flow of water, been revived. The lake waters are used for livestock (especially washing/bathing of cattle) and the water from open wells, for agricultural purposes. While studies on the impact of TWW on agricultural production and animal husbandry exist, no studies have looked at the potential for inadvertent transmission of AMR pathogens, antibiotics or heavy metals through this process.

2.2. Sampling

Intracity sites: 66 open drain sites belonging to 8 municipal administrative zones, as designated by the Municipal Corporation of Bengaluru city (Figure 1A), were sampled over a period of 2 months from March to April 2022. The average temperature in Bengaluru during this time of the year is between 23- 26°C. Of the 66 sites sampled, 14 were sampled in Mar 22 and the remaining in April 22.

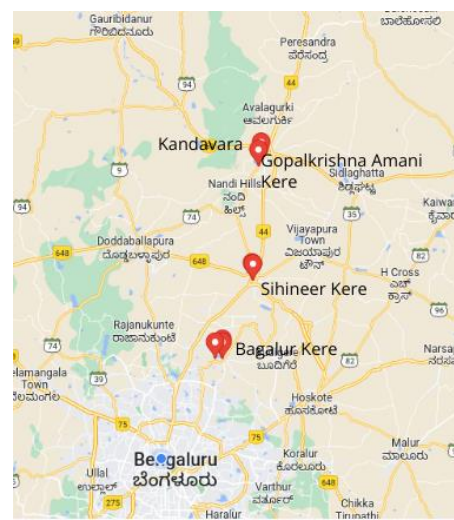
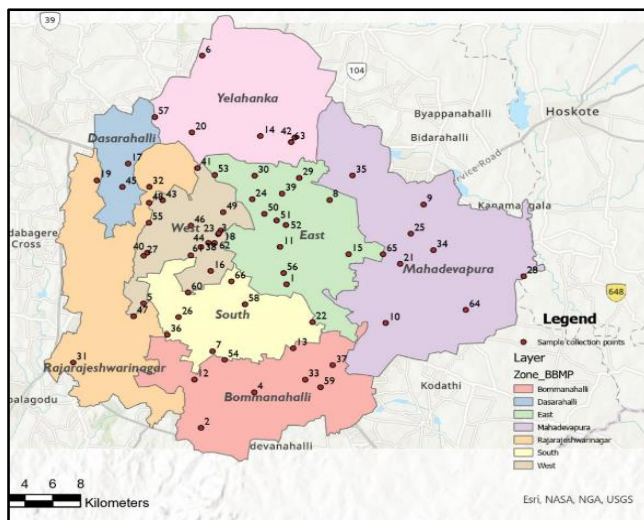


Figure 1A (left): Map of Bangalore, Showing the Administrative Zones and the Collection Sites
Figure 1B (right): Location of the 4 Selected Lakes North of Bengaluru

2.3. Intercity Sites

Between June and September 2023, water from 4 lakes receiving TWW north of Bengaluru, and the open wells near them were collected (Fig 1B), based on the TWW pumping strategy given in Fig 1C. As the water from the middle of the lake was not accessible for collection, we collected lake water samples from two opposite banks, ensuring that the depth was at least 2 feet and that no sediment from the bed entered the collection bottle.

2.4. Water Collection

About 500mL of water samples from open drains, lakes, and open wells were collected in clean low density polyethylene bottles using the grab and collect method. pH was measured at the time of collection and, if found to be between 7 and 7.2, the samples were transported to the lab on ice packs, within one to two hours of collection.

2.5. Intracity Samples Microbiological Culture and Sensitivity Assays

Serially diluted samples were plated onto Mac Conkey and Salmonella-Shigella (SS) agar plates separately. Colonies were counted and randomly picked for further subculturing and identification using Himedia Biochemical strip test kits [HiAssorted™ Biochemical Test Kit and HiIMViC™ Biochemical Test Kit]. Colonies were classified in two different ways: A) Clinically Relevant or Environmental (based on published literature on where it is commonly isolated from) and b) Enterobacteriaceae or Non - Enterobacteriaceae.

For antimicrobial resistance, Kirby Bauer disc diffusion method was followed. Five antibiotics discs at standard concentrations- Meropenem (MER) 10mcg (SD727), Ertapenem (ERT) 10mcg (SD280), Ciprofloxacin (CIP) 5mcg (SD060), Cefixime (CF) 5mcg (SD266) and Piperacillin/Tazobactam (TZP) 100/10mcg (SD210) (Himedia Labs) were placed on the MHA plates equally spaced and the plates were incubated overnight to calculate the zone of inhibition was calculated. Clinical and Laboratory Standards Institute (CLSI) standards were used to determine sensitivity based on the zone of inhibition.

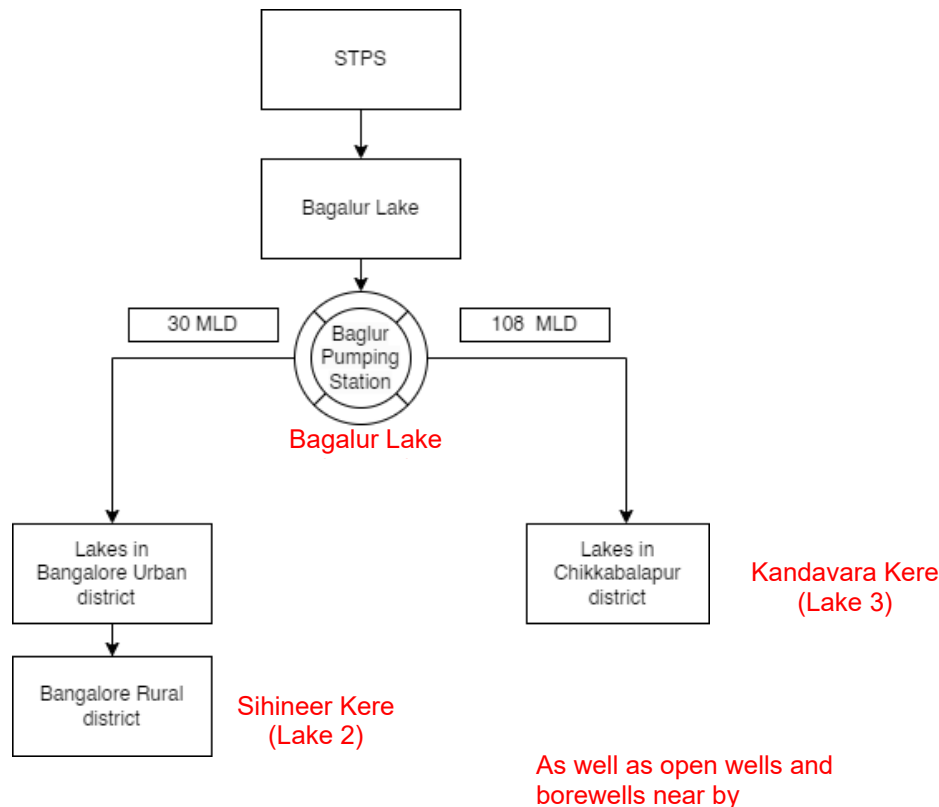


Figure 1C: Direction and Quantity of Flow of Treated Wastewater into the Lakes. STP: Sewage Treatment Plant MLD: Million Liters Daily. In Red: The Lakes and Surrounding Sites Sampled

The isolates were mapped into their respective municipal zones. Clinically relevant Enterobacteriaceae family isolates were summarized into those that are resistant against only ciprofloxacin, cefixime, TZP or both carbapenems, then a combination of antibiotic pairs, and antibiotic triads (Table 1). For the purposes of this summary, intermediate resistance was considered to be susceptible as the antibiotic in question still acts against the pathogen, only at higher concentrations.

2.6. Data Visualization

The data obtained in this study has been mapped and summarized here:

<https://storymaps.arcgis.com/stories/011a7411f6ed44c-5840971cda1b6f0b9> for easy browsing.

2.7. Intercity Samples Pharmaceutical Compound Testing

For antibiotic testing, 15mL of water was additionally collected in 15mL falcon tubes, labeled and transported to the antibiotic testing facility on ice within 4h of collection. The following compounds were tested: Amoxicillin, Azithromycin, Clarithromycin, Clavulanic acid, Clofazimine, Erythromycin, Ethambutol, Isoniazid, Favipiravir, Fluconazole, Ketoconazole, Linezolid, Moxifloxacin, Rifampicin, Roxithromycin, Remdesivir and Oseltamavir by LC/MS-MS.

2.8. Intercity Samples Metal Testing

1L of water was collected from each site in low density polyethylene bottles, kept on ice and transported to the IISc Center for Earth Sciences for mass spectrometry for a set of the following metals: Aluminum, Boron, Barium, Calcium,

Cadmium, Cobalt, Chromium, Copper, Iron, Gallium, Indium, Potassium, Lithium, Magnesium, Manganese, Sodium, Nickel, Lead, Samarium, Strontium, and Zinc.

2.9. Intercity Samples Culture and Sensitivity

Samples were plated immediately upon receipt on McConkey plates (MCC) at increasing serial dilutions. Randomly selected colonies from various dilutions were picked for identification and sensitivity testing using Vitek (BioMérieux) in a standard NABL-accredited diagnostic laboratory.

3. Results

3.1. Intracity Samples

Data Description

A total of 160 randomly picked colonies were isolated during the study period. These 160 isolates fell into 29 species. 150 out of the 160 were considered clinically relevant, with 132 belonging to the Enterobacteriaceae family (Supplementary Table 1). The most common species found were *Klebsiella pneumoniae* (18 *Klebsiella pneumoniae* subsp. *pneumoniae* and 3 *Klebsiella pneumoniae* subsp. *Ozaenae* isolates) and *Escherichia coli* (15 isolates).

Sr no	Zone name	Ward name	Ward Number	Colony forming unit (CFU/ml) in millions		Species identified
				MacConkey	SS	
1	Bommanahalli	AREKERE	193	1.5	0.62	<i>E.coli</i> , <i>P.fluorescens</i> , <i>S.enterica</i>
2	Bommanahalli	BOMMANAHALLI	175	1.2	1.02	<i>E.hermannii</i> , <i>V.fluvialis</i> , <i>B.agrestis</i>
3	Bommanahalli	HONGASANDRA	189	1.38	0.6	<i>K.pneumoniae</i> , <i>C.freundii</i>
4	Bommanahalli	SINGASANDRA	191	1.24	0.98	<i>E.coli</i> , <i>S.enteritidis</i>
5	Bommanahalli	HSR LAYOUT	174	1.36	0.72	<i>Y.pseudotuberculosis</i> , <i>C.freundii</i>
6	Bommanahalli	ANJANAPURA	196	1.6	1.04	<i>L.amnigena</i> , <i>Shigella spp</i>
7	Dasarahalli	CHOKKASANDRA	39	0.9	1.2	<i>K.pneumoniae</i> , <i>V.furnissis</i> , <i>Serratia spp</i>
8	Dasarahalli	PEENYA	41	0.12	0.72	<i>K.pneumoniae</i> , <i>G.hollisae</i> , <i>P.fluorescens</i>
9	Dasarahalli	SHETTYHALLI	12	1.44	1.02	<i>E.coli</i> , <i>V.funissis</i> , <i>S.plymuthica</i>
10	East	HBR LAYOUT	24	1.18	0.8	<i>E.coli</i> , <i>A.craviae</i> , <i>P.vulgaris</i>
11	East	SHANTHALA NAGAR	111	0.82	0.82	<i>K.pneumoniae</i> , <i>G.hollisae</i> , <i>S.flexneri</i>
12	East	BANASWADI	27	0.92	1	<i>E.hermannii</i> , <i>G.hollisae</i> , <i>S.flexneri</i>
13	East	CV RAMAN NAGAR	57	0.72	0.2	<i>K.pneumoniae</i> , <i>V.funissis</i> , <i>K.ascorbate</i>
14	East	NAGAVARA	23	1.8	0.58	<i>P.dispera</i> , <i>P.pseudoalcaligenes</i> , <i>Shigella spp</i>
15	East	BHARATHINAGAR	91	0.82	1.38	<i>E.hermannii</i> , <i>K.ascorbate</i>
16	East	AGARAM	114	1.54	1.54	<i>K.pneumoniae</i> , <i>S.enteritidis</i>
17	East	SK GARDEN	61	0.98	1.78	<i>E.coli</i> , <i>S.plymuthica</i>
18	East	RAMASWAMPALYA	62	1.3	1.2	<i>E.aerogenes</i> , <i>S.enteritidis</i>

19	East	KAUSHALANAGAR	31	1.42	0.82	<i>K.pneumoniae, S.enterica</i>
20	East	GANGEHALLI	34	1.32	0.94	<i>Ypseudotuberculosis, C.freundii</i>
21	East	SAGAYAPURAM	60	0.48	1.32	<i>K.pneumoniae, Shigella spp</i>
22	Mahadevapura	VARTHUR	149	1.38	1.36	<i>E.amnigenus, Vfurnissis, S.plymuthica</i>
23	Mahadevapura	HOODI	54	0.9	0.2	<i>K.pneumoniae, Vfurnissis, P.mirabilis</i>
24	Mahadevapura	VIGNANANAGAR	81	0.92	0.48	<i>Ypseudotuberculosis, P.fluorescens, P.dispera</i>
25	Mahadevapura	DODDANEKUNDI	85	1.1	0.58	<i>K.pneumoniae,, Vfurnissis,S.enterica</i>
26	Mahadevapura	BASAVANAPURA	53	1.44	0.54	<i>E.hermanii, G.hollisae, S.entomophila</i>
27	Mahadevapura	BELLANDUR	150	0.94	0.46	<i>K.pneumoniae, A.craviae, K.ascorbate</i>
28	Mahadevapura	HAGADURU	84	1.4	0.62	<i>K.pneumoniae, G.hollisae, K.ascorbate</i>
29	Mahadevapura	GARUDACHARPALYA	82	1.6	1.6	<i>K.pneumoniae, S.entomophila</i>
30	Mahadevapura	HORAMAVU	25	1.74	1.76	<i>E.amnigenus, S.dysenteriae</i>
31	RRNagar	DOODABIDARAKALLU	40	1.2	0.82	<i>K.pneumoniae, Vfurnissis, P.mirabilis</i>
32	RRNagar	HEMMIGEPURA	198	0.84	0.84	<i>P.dispera, G.hollisae, P.mirabilis</i>
33	RRNagar	RAJARAJESHWARI NAGAR	160	0.92	1.28	<i>E.amnigenus, Vfurnissis, S.enteritidis</i>
34	RRNagar	HMT	38	1.46	1.1	<i>P.dispera, G.hollisae, P.mirabilis</i>
35	RRNagar	RAJGOPALNAGAR	70	1.66	1.66	<i>P.dispera, P.fluorescens</i>
36	South	EJIPURA	148	1.8	1.48	<i>K.pneumoniae,P. fluorescens, S.plymuthica</i>
37	South	BTM LAYOUT	176	1.76	1.8	<i>E.coli, C.freundii</i>
38	South	SARAKKI	178	1.78	1.48	<i>E.hermannii, S.enterica</i>
39	South	BANASHANKARI TEMPLE	180	1.92	1.98	<i>K.pneumoniae,Shigella spp</i>
40	South	VISVESVARAPURAM	143	1.18	1.86	<i>E.coli, S.enterica</i>
41	South	SIDDAPURA	144	0.62	1	<i>P.dispera, Shigella spp</i>
42	South	GIRINAGAR	162	1.48	0.94	<i>E.coli, S.entomophila</i>
43	South	SRINAGAR	156	1.8	1.46	<i>P.dispera, Serratia spp</i>
44	South	HOSKEREHALLI	161	1.56	1.68	<i>E.coli, S.flexneri</i>
45	West	ATTIGUPE	132	0.18	0.38	<i>E.hermanii, G.hollisae, K.ascorbate</i>
46	West	NANDINI LAYOUT	43	1.26	1.52	<i>K.pneumoniae,A. craviae, K.ascorbate</i>

47	West	ARAMANENAGAR	35	1.32	0.9	<i>E.coli, K.ascorbate</i>
48	West	SRI RAM MANDIR	108	1.34	1.62	<i>E.aerogenes, S.enterica</i>
49	West	RAJAJINAGAR	99	1.1	1.52	<i>K.pneumoniae, P.mirabilis</i>
50	West	OKALIPURAM	96	1.4	1.8	<i>E.hermannii, S.flexneri</i>
51	West	MATTIKERE	36	1.78	1.44	<i>E.coli, S.plymuthica</i>
52	West	KAMAKSHIPALYA	101	1.7	1.54	<i>Serratia spp., C.freundii</i>
53	West	DATTATREYA	77	1.1	1.8	<i>E.hermannii, K.ascorbate</i>
54	West	RAJMAHAL	64	0.9	1.02	<i>Shigella spp., K.ascorbate</i>
55	West	GANDHINAGAR	94	1.24	0.92	<i>E.amnigenus, P.fluorescens</i>
56	West	CHALAVADIPALYA	138	0.84	1.02	<i>E.amnigenus, K.ascorbate</i>
57	West	GOVINDRAJNAGAR	104	1.76	1.26	<i>E.aerogenes, S.flexneri</i>
58	West	KAVERIPURA	103	0.6	1.1	<i>K.pneumoniae, Serratia spp</i>
59	West	SHANKARMATHA	75	1.08	1.18	<i>E.coli, S.enterica</i>
60	West	SUBASHNAGAR	95	1.46	1.08	<i>K.pneumoniae, Serratia spp.</i>
61	Yelahanka	ATTUR	3	1.2	0.82	<i>E.coli, V.furnissis, S.enteritidis</i>
62	Yelahanka	THANISANDRA	6	0.78	1.6	<i>K.pneumoniae, Paeruginosa, S.enteritidis</i>
63	Yelahanka	BYATANARYANAPURA	7	1.18	1.66	<i>E.cloacae, P.fluorescens, S.enteritidis</i>
64	Yelahanka	HEBBAL	21	1.34	0.8	<i>E.amnigenus, V.furnissis, C.freundii</i>
65	Yelahanka	DODDABOMMASANDRA	10	1.26	1.22	<i>E.coli, P.mirabilis</i>
66	Yelahanka	SANJAYNAGAR	19	1.26	1.34	<i>E.aerogenes, P.mirabilis</i>

Table 1: Colony Counts

Significant Presence of Multi Drug Resistant Bacteria in the Wastewater System Across All Geographical Zones in the City

We found that 28% of all clinical Enterobacteriaceae isolates were susceptible to all the tested antibiotics, whereas 12% were resistant to all the tested antibiotics. About 4% of all isolates were resistant only to Ciprofloxacin and cefixime,

while 12% were resistant only to ciprofloxacin or cefixime or TZP; about 9% were resistant to the higher order antibiotics only (TZP and carbapenems). The remainder had a mixed combination of resistance profiles (Table 1). We then classified all clinically relevant bacteria, Enterobacteriaceae and non-Enterobacteriaceae, by zone and number of isolates resistant to increasing numbers of antibiotics.

Zone	Number of isolates	Susceptible to all antibiotics n (%)	Resistant only to CIP n (%)	Resistant only to CF n (%)	Resistant only to TZP n (%)	Resistant to ERT OR MER only n (%)	Resistant to ERT AND MER only n (%)	Resistant to CIP and CF n (%)	Resistant to CIP, CF and TZP n (%)	Resistant to TZP, ERT and MER only n (%)	Resistance to CIP, CF, TZP, MER n (%)
Zone 1 (West Zone)	30	11 (37%)	1 (3.3%)	2 (6.7%)	1 (3.3%)	6 (20%)	0	2 (6.6%)	0	2 (6.7%)	1 (3.3%)
Zone 2 (South Zone)	20	4 (20%)	2 (10%)	0	0	2 (10%)	0	2 (10%)	0	3 (15%)	5 (25%)
Zone 3 (East Zone)	28	11 (39.3%)	3 (10.7%)	2 (7.1%)	0	6 (21%)	1 (3.5%)	0	1 (3.5%)	2 (7.1%)	0
Zone 4 (Yelahanka)	8	1 (12.5%)	1 (12.5%)	0	0	2 (25%)	1 (12.5%)	0	0	1 (12.5%)	0
Zone 5 (Mahadevpura)	18	5 (27.8%)	1 (5.6%)	2 (11.1%)	0	2 (11.1%)	1 (5.6%)	0	0	3 (16.7%)	3 (16.7%)
Zone 6 (Rajarajeshwari Nagar)	10	1 (10%)	0	0	0	5 (50%)	0	0	0	0	1 (10%)
Zone 7 (Bommanahalli)	12	2 (16.7%)	0	0	0	2 (16.7%)	0	1 (8.3%)	1 (8.3%)	0	3 (25%)
Zone 8 (Dasarahalli)	6	1 (16.7%)	0	0	0	2 (33.3%)	0	0	0	0	1 (16.7%)
Total	132	36 (27.2%)	8 (6.1%)	6 (4.5%)	1 (0.8%)	27 (20.4%)	3 (2.2%)	5 (3.8%)	2 (1.6%)	11 (8.3%)	16 (12.1%)

Table 1: Zone Wise Resistance Profiles of all Clinically Relevant Enterobacteriaceae Isolates CIP – Ciprofloxacin; CF – Cefixime; TZP – Piperacillin/Tazobactam MER- Meropenem; ERT – Ertapenem

Geographical Variation Within a City in the Patterns and Burden Of AMR

Multidrug resistance, as defined as resistance against 3 or more classes of antibiotics, was found in all eight zones, at varying levels (Figure 2) [15]. The proportion of resistant

organisms to each of the antibiotics tested varied between the 8 zones. The number of organisms with resistances to 2 or more antibiotics also showed significant spatial variation (Supplementary Table 2).

ZONE	SAMPLE SITE	BACTERIA IDENTIFIED	Zone of Inhibition									
			Meropenem		Ertapenem		Ciprofloxacin		Pipercillin/Tazobactam		Cefixime	
			Zone inhibition calculated (mm)	Inference	Zone inhibition calculated (mm)	Inference	Zone inhibition calculated (mm)	Inference	Zone inhibition calculated (mm)	Inference	Zone inhibition calculated (mm)	Inference
WEST	AARAMANENAGAR	<i>Escherichia coli</i>	1	R	1	R	1	R	0	R	0	R
		<i>Kluyvera ascorbate</i>	26	S	20	IR	30	S	26	S	10	R
	MATTIKERE	<i>Escherichia coli</i>	28	S	19	IR	1	R	20	IR	6	R
		<i>Serratia plymuthica</i>	24	S	25	S	20	R	20	IR	15	R
	NANDINI LAYOUT	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	20	IR	18	R	20	R	16	IR	15	R
		<i>Kluyvera ascorbate</i>	9	R	1	R	19	R	15	R	20	IR
		<i>Aeromonas craviae</i>										
	RAJMAHAL	<i>Shigella species</i>	50	S	50	S	26	S	30	S	35	S
		<i>Kluyvera ascorbate</i>	21	IR	28	S	32	S	22	S	18	IR
	SHANKARMATHA	<i>Escherichia coli</i>	26	S	22	S	24	S	20	IR	15	R
		<i>Salmonella choleraesuis subspecies choleraesuis</i>	21	S	19	IR	20	R	16	R	20	IR
	DATTATREYA	<i>Escherichia hermannii</i>	45	S	50	S	25	S	50	S	50	S
		<i>Kluyvera ascorbate</i>	20	IR	13	R	24	S	30	S	32	S
	GANDHINAGAR	<i>Enterobacter amnigenus</i>	39	S	38	S	30	S	40	S	37	S
		<i>Pseudomonas fluorescens</i>	19	IR	17	R	24	S	24	S	21	S
	SUBASHNAGAR	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	23	S	19	IR	23	S	20	IR	19	IR
		<i>Serratia sp</i>	15	R	8	R	35	S	17	R	26	S

	OOKALIPURAM	<i>Escherichia hermanii</i>	29	S	10	R	20	R	1	R	1	R
		<i>Shigella flexneri</i>	20	IR	21	S	20	R	21	S	8	R
	RAJAJINAGAR	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	40	S	35	S	40	S	32	S	35	S
	KAMAAKSHIPALYA	<i>Proteus mirabilis</i>	14	R	6	R	23	S	18	IR	9	R
		<i>Serratia sp</i>	40	S	31	S	39	S	25	S	34	S
		<i>Citrobacter freundii</i>	32	S	25	S	26	S	26	S	30	S
	KAVERIPURA	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	20	IR	16	R	21	R	22	S	5	R
		<i>Serratia sp</i>	25	S	18	R	8	R	15	R	1	R
	GOVINDRAJNAGAR	<i>Enterobacter aerogenes</i>	46	S	39	S	30	S	40	S	45	S
		<i>Shigella flexneri</i>	42	S	34	S	36	S	30	S	40	S
	SRI RAM MANDIR	<i>Enterobacter aerogenes</i>	26	S	23	S	13	R	19	IR	23	S
		<i>Salmonella choleraesuis subspecies choleraesuis</i>	26	S	16	R	25	S	25	S	15	R
	CHALAVADIPALYA	<i>Enterobacter amnigenus</i>	40	S	41	S	36	S	43	S	35	S
		<i>Kluyvera ascorbate</i>	20	IR	13	R	22	S	15	R	25	S
SOUTH	ATTIGUPE	<i>Escherichia hermanii</i>	20	IR	20	IR	1	R	0	R	15	R
		<i>Vibrio hollisae</i>	19	IR	12	R	1	R	9	R	1	R
		<i>Kluyvera ascorbate</i>										
	VISVESVARAPURAM	<i>Escherichia coli</i>	18	R	20	IR	18	R	15	R	14	R
		<i>Salmonella choleraesuis subspecies choleraesuis</i>	23	S	20	IR	20	R	18	IR	15	R
	SIDDAPURA	<i>Pantoea dispersa</i>	39	S	35	S	50	S	39	S	40	S
		<i>Shigella species</i>	20	IR	13	R	35	S	20	IR	24	S

	EJIPURA	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	15	R	17	R	11	R	0	R	10	R
		<i>Serratia plymuthica</i>	9	R	13	R	17	R	20	IR	30	S
		<i>Pseudomonas fluorescens</i>										
	SRINAGAR	<i>Pantoea dispersa</i>	17	R	10	R	27	S	15	R	31	S
		<i>Serratia sp</i>	20	IR	25	S	20	R	20	IR	21	S
	HOSKEREHALLI	<i>Escherichia coli</i>	18	R	6	R	19	R	14	R	6	R
		<i>Shigella flexneri</i>	39	S	40	S	1	R	35	S	45	S
	GIRINAGAR	<i>Escherichia coli</i>	35	S	25	S	25	S	26	S	35	S
		<i>Serratia entomophila</i>	22	S	16	R	16	R	26	S	26	S
	BTM LAYOUT	<i>Escherichia coli</i>	9	R	1	R	26	S	12	R	28	S
		<i>Citrobacter freundii</i>	26	S	26	S	27	S	26	S	30	S
	SARAKKI	<i>Escherichia hermanii</i>	1	R	1	R	1	R	1	R	1	R
		<i>Salmonella choleraesuis subspecies choleraesuis</i>	35	S	26	S	28	S	34	S	26	S
	BANASHANKARI TEMPLE	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	1	R	1	R	1	R	1	R	1	R
		<i>Shigella sp</i>	15	R	10	R	35	S	17	R	31	S
	EAST	SANJAYNAGAR	<i>Enterobacter aerogenes</i>	24	S	15	R	25	S	24	S	13
<i>Proteus mirabilis</i>			35	S	29	S	26	S	31	S	31	S
HEBBAL		<i>Enterobacter amnigenus</i>	16	R	1	R	20	R	13	R	25	S
		<i>Citrobacter freundii</i>	25	S	14	R	13	R	15	R	25	S
		<i>Vibrio furnissis</i>										

NAGAVARA	<i>Pantoea dispersa</i>	35	S	30	S	28	S	30	S	27	S
	<i>Shigella</i>	50	S	50	S	50	S	50	S	50	S
	<i>Pseudomonas pseudoalcaligenes</i>										
HBR LAYOUT	<i>Escherichia coli</i>	50	S	50	S	50	S	50	S	50	S
	<i>Proteus vulgaris</i>	23	S	22	S	20	R	24	S	19	S
	<i>Aeromonas craviae</i>										
BANASWADI	<i>Escherichia hermannii</i>	40	S	29	S	27	IR	24	S	35	S
	<i>Shigella flexneri</i>	16	R	0	R	25	IR	19	IR	13	R
	<i>Vibrio hollisae</i>										
KAUSHALANAGAR	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	24	S	22	S	42	S	15	R	8	R
GANGEHALLI	<i>Salmonella choleraesuis subspecies choleraesuis</i>	23	S	17	R	29	S	21	IR	21	S
	<i>Yersinia pseudotuberculosis</i>	30	S	23	S	30	S	25	S	21	S
	<i>Citrobacter freundii</i>	50	S	50	S	50	S	50	S	50	S
CV RAMAN NAGAR	<i>Klebsiella pneumoniae subspecies ozaenae</i>	35	S	26	S	24	S	26	S	31	S
	<i>Vibrio furnissii</i>	25	S	14	R	22	IR	23	S	19	IR
SK GARDEN	<i>Escherichia coli</i>	18	R	11	R	25	S	24	S	26	S
	<i>Serratia plymuthica</i>	14	R	13	R	26	S	21	S	1	R
RAMASWAMYPA LYA	<i>Enterobacter aerogenes</i>	42	S	43	S	39	S	30	S	40	S
	<i>Salmonella enteritidis</i>	39	S	31	S	20	R	25	S	32	S
SAGAYAPURAM	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	20	IR	13	R	1	R	13	R	6	R

		<i>Shigella species</i>	21	S	15	R	20	R	20	IR	18	IR
	BHARATHINAGAR	<i>Escherichia hermanii</i>	30	S	28	S	24	S	25	S	21	S
		<i>Kluyvera ascorbate</i>	24	S	22	S	21	R	20	IR	20	IR
	SHANTHALA NAGAR	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	50	S	50	S	50	S	50	S	50	S
		<i>Shigella flexneri</i>	20	IR	9	R	18	R	15	R	9	R
		<i>Vibrio hollisae</i>										
	AGARAM	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	2	R	50	S	50	S	50	S	50	S
		<i>Salmonella enteritidis</i>	22	S	22	S	26	S	26	S	10	R
YELAHA NKA ZONE	ATTUR	<i>Escherichia coli</i>	50	S	50	S	50	S	50	S	50	S
		<i>Salmonella enteritidis</i>	30	S	24	S	20	R	23	S	29	S
		<i>Vibrio furnissii</i>										
	THANISANDRA	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	20	IR	16	R	20	R	17	IR	20	IR
		<i>Salmonella enteritidis</i>	20	IR	21	S	16	R	19	IR	15	R
		<i>Pseudomonas aereginosa</i>										
	BYATANARYAN APURA	<i>Enterobacter cloacae</i>	23	S	15	R	12	R	21	S	14	R
		<i>Salmonella enteritidis</i>	20	IR	18	R	19	R	11	R	19	IR
		<i>Pseudomonas fluorescens</i>										
	DODDABOMMAS ANDRA	<i>Escherichia coli</i>	25	S	18	R	10	R	15	R	0	R
		<i>Proteus mirabilis</i>	20	IR	13	R	24	S	21	S	22	S
	HORAMAVU	<i>Enterobacter amnigenus</i>	20	IR	1	R	16	R	13	R	6	R
		<i>Shigella dysenteriae</i>	30	S	29	S	27	S	26	S	29	S

MAHADE VPURA ZONE	BASAVANAPURA	<i>Escherichia hermanii</i>	31	S	27	IR	26	S	26	S	26	S
		<i>Vibrio hollisae</i>	15	R	13	R	30	S	30	S	25	S
		<i>Serratia entomophila</i>										
	HOODI	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	20	IR	13	R	17	R	22	S	15	R
		<i>Proteus mirabilis</i>	15	R	14	R	16	R	18	IR	29	S
		<i>Vibrio furnissii</i>										
	VIGNANANAGAR	<i>Yersinia pseudotuberculosis</i>	20	IR	10	R	19	R	19	IR	28	S
		<i>Pantoea dispersa</i>	22		20	IR	16	R	20	IR	16	IR
		<i>Pseudomonas fluorescens</i>										
	GARUDACHARPA LYA	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	20	IR	22	S	18	R	18	IR	19	S
		<i>Serratia entomophila</i>	16	R	11	R	18	R	19	IR	12	R
	HAGADURU	<i>Klebsiella pneumoniae subspecies ozaenae</i>	30	S	26	S	25	S	28	S	26	S
		<i>Khuyvera ascorbate</i>	30	S	23	S	35	S	25	S	15	R
		<i>Vibrio hollisae</i>										
	DODDANEKUNDI	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	18	R	18	R	15	R	17	IR	13	R
		<i>Salmonella choleraesuis subspecies choleraesuis</i>	18	R	8	R	18	R	22	S	18	IR
		<i>Vibrio furnissii</i>										
	VARTHUR	<i>Enterobacter amnigenus</i>	27	S	19	IR	24	IR	30	S	23	S
		<i>Serratia plymuthica</i>	16	R	50	S	17	R	19	IR	10	R
		<i>Vibrio furnissii</i>										

	BELLANDUR	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	31	S	23	IR	30	S	25	S	30	S
		<i>Khuyvera ascorbate</i>	25	S	19	IR	24	S	20	IR	11	R
		<i>Aeromonas craviae</i>										
RAJARAJ ESHWAR I NAGAR	HMT	<i>Pantoea dispersa</i>	21	S	18	R	19	R	7	R	25	S
		<i>Proteus mirabilis</i>	25	S	19	R	1	R	15	R	17	IR
		<i>Vibrio hollisae</i>										
	DOODABIDARAK ALLU	<i>Klebsiella pneumoniae subspecies ozaenae</i>	31	S	25	S	30	IR	19	IR	20	S
		<i>Proteus mirabilis</i>	25	S	2	R	11	R	26	S	9	R
		<i>Vibrio furnissii</i>										
	RAJGOPALNAGAR	<i>Pantoea dispersa</i>	20	IR	15	R	15	R	17	R	14	R
		<i>Pseudomonas fluorescens</i>	24	S	18	R	25	S	20	IR	18	IR
	RAJARAJESHWARI NAGAR	<i>Enterobacter amnigenus</i>	20	IR	15	R	17	R	21	S	1	R
		<i>Salmonella enteritidis</i>	15	R	8	R	25	IR	20	IR	13	R
		<i>Vibrio furnissii</i>										
	HEMMIGEPURA	<i>Pantoea dispersa</i>	17	R	16	R	15	R	13	R	17	IR
		<i>Proteus mirabilis</i>	15	R	15	R	22	IR	16	IR	1	R
		<i>Vibrio hollisae</i>										
BOMMANAHALLI	HSR LAYOUT	<i>Yersinia pseudotuberculosis</i>	25	S	33	S	30	S	26	S	33	S
		<i>Citrobacter freundii</i>	26	S	16	R	24	S	24	S	14	R
	BOMMANAHALLI	<i>Escherichia hermanii</i>	20	IR	20	IR	16	R	15	R	25	S
		<i>Buttiauxella agrestis</i>	25	S	24	S	26	S	25	S	16	IR
		<i>Vibrio fluvialis</i>										

	HONGASANDRA	<i>Klebsiella pneumoniae subspecies pneumoniae</i>	23	S	17	R	20	R	25	S	26	S	
		<i>Citrobacter freundii</i>	1	R	1	R	1	R	1	R	1	R	
	SINGASANDRA	<i>Escherichia coli</i>	17	R	15	R	25	S	14	R	15	R	
		<i>Salmonella enteritidis</i>	20	IR	15	R	20	R	20	IR	1	R	
	AREKERE	<i>Escherichia coli</i>	27	S	21	IR	11	R	22	S	1	R	
		<i>Salmonella choleraesuis subspecies choleraesuis</i>	23	S	20	IR	9	R	15	R	1	R	
		<i>Pseudomonas fluorescens</i>											
	ANJANAPURA	<i>Enterobacter amnigenus</i>	30	S	39	S	40	S	45	S	39	S	
			<i>Shigella species</i>	19	IR	15	R	1	R	14	R	1	R
	DASARA HALLI	SHETTYHALLI	<i>Escherichia coli</i>	20	IR	18	R	24	S	23	S	30	S
<i>Serratia plymuthica</i>			25	S	19	IR	23	IR	20	S	18	IR	
<i>Vibrio furnissis</i>													
CHOKKASANDRA		<i>Klebsiella pneumoniae subspecies pneumoniae</i>	14	R	1	R	21	R	17	IR	28	S	
		<i>Serratia sp</i>	24	S	19	R	16	R	14	R	15	R	
		<i>Vibrio furnissis</i>											
PEENYA		<i>Klebsiella pneumoniae subspecies pneumoniae</i>	14	R	11	R	13	R	15	R	1	R	
		<i>Escherichia coli</i>	15	R	9	R	16	R	13	R	25	S	
		<i>Vibrio hollisae</i>											

Supplementary Table 2: Resistance Patterns of all Identified Isolates in Intracity Sampling by Site and Zone

Resistance Patterns of *Klebsiella Pneumoniae* and *Escherichia Coli*

Klebsiella pneumoniae and *Escherichia coli* were the two most abundantly isolated species. They are also highly clinically relevant with significant resistance being reported in India and worldwide. Of the 21 isolates of *Klebsiella pneumoniae*, 18 were *Klebsiella pneumoniae* subspecies

pneumoniae and the remaining were subspecies *ozaenae*. Of the 18, 7 (~40%) showed complete (n=3) or intermediate (n=4) resistance to all antibiotics tested, and 2 were completely susceptible to all antibiotics and the remaining showed a combination of resistance profiles. Among the 15 isolates of *E. coli*, 3 (20%) showed complete resistance to all 5 antibiotics (Supplementary table 2).

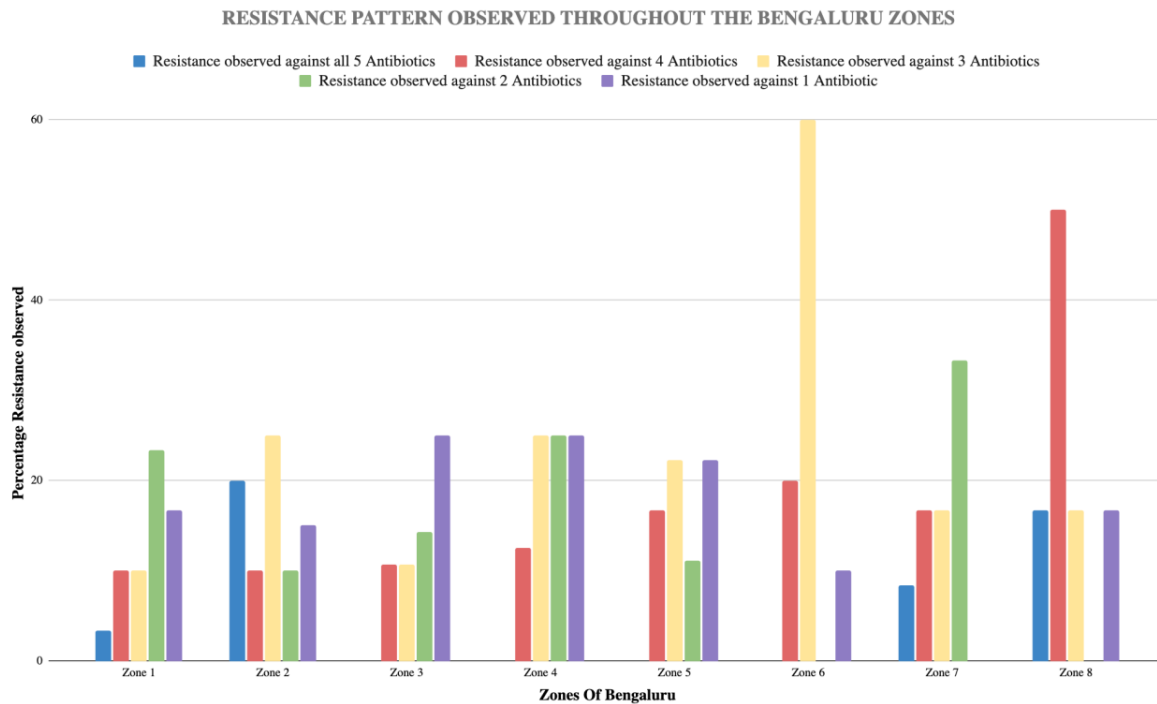


Figure 2: Resistance Pattern Observed throughout the Bengaluru Zones

Amr Profiles of Organisms of Veterinary Importance

Wastewater, especially that in open drains, contains bacterial species that are clinically relevant for humans and animals

and that are found only in the environment. Isolates that are clinically relevant from a veterinary perspective and their AMR profile is shown in the Table 2

Sno	Species	Colonies	Resistance to				
			CIP	CF	TZP	ERT	MER
1	<i>Klebsiella pneumoniae</i>	18	12 (66.7%)	9 (50%)	5 (28%)	11 (61.1)	6 (33.3%)
2	<i>Escherichia coli</i>	15	7 (47%)	8 (53%)	7 (47%)	8 (53%)	7 (47%)
3	<i>Salmonella choleraesuis</i>	9	4(44%)	3(33%)	2(22%)	5(56%)	1(11%)
4	<i>Salmonella enteritidis</i>	7	5(71%)	4(57%)	1(14%)	3(43%)	1(14%)
5	<i>Proteus mirabilis</i>	7	3 (43%)	3 (43%)	1 (14%)	6 (86%)	3 (43%)
6	<i>Citrobacter freundii</i>	6	1 (17%)	3 (50%)	2 (33%)	2 (33%)	2 (33%)
7	<i>Yersinia pseudotuberculosis</i>	3	1(33%)	0	0	1(33%)	0

Table 2: Resistance Profiles of Clinically Important Bacterial Species of Veterinary Importance CIP - Ciprofloxacin; CF - Cefixime; TZP - Piperacillin/Tazobactam; ERT - Ertapenem; MER - Meropenem

3.2. Intercity Samples

Antibiotic Concentrations in Lake and Open Well Samples

19 different pharmaceutical compounds among antibiotics, antifungals and antibiotics were tested in lake and open well

samples. Of these, several antibiotics were found at levels higher than the predicted no-effect concentrations (PNECs) whereas all others were either not detected (Supplementary Table 3A) or detected at very low levels (Table 3).

Drug class	Antibiotic name	STP output	Lake 1 Site 1	Lake 1 Site 2	Lake 1 Open well	Lake 2 Site 1	Lake 2 Site 2	Lake 2 Open well	Lake 3 Site 1	Lake 3 Site 2	Lake 3 Open well	Lake 4 Site 1	Lake 4 Site 2	Lake 4 Open well
Beta lactam	Amoxicillin	0	0	45	0	39	45	0	45	56	141	37	43	112
Macrolide	Azithromycin	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrolide	Clarithromycin	0	0	0	0	0	0	0	0	0	0	0	0	0
betalactamase inhibitor	Clavulanic acid	0	0	0	0	49	51	120	50	44	0	0	0	0
Fluoroquinolone	Ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0
phenazine drugs, anti TB	Clofazimine	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrolide	Erythromycin	0	0	0	0	0	0	0	0	0	0	0	0	0
TB med	Etambutol	0	0	0	0	0	0	0	0	0	0	0	0	0
TB med	Isoniazid	133	152	153	128	87	71	174	52	55	183	254	238	272
Antiviral	Favipiravir	0	0	0	0	0	0	0	0	0	0	0	0	0
Antifungal	Fluconazole	33	28	31	28	0	0	0	28	28	30	0	0	28
Antifungal	Ketoconazole	0	0	0	0	0	0	0	0	0	0	0	0	0
G+ antibiotic	linezolid	0	0	36	0	88	117	84	121	139	39	37	63	34
Fluoroquinolone	Moxifloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0
Beta lactam/carbapenem	Meropenem	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrolide	roxithromycin	0	0	0	0	0	0	0	0	0	0	0	0	0
Antiviral	Remdesivir	0	0	0	0	0	0	0	0	0	0	0	0	0
Ribosome inhibitors	tigecycline	0	0	0	0	0	0	0	0	0	0	0	0	0
Antiviral	Oseltamivir	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table 3A: Antimicrobial Pharmaceutical Compound Levels in All Lake and Well Water Samples in Intercity Samples (June 2023 sampling). September 2023 Data is not Shown for Compounds not Detected

Heavy Metal Concentrations

21 metals and heavy metals were tested by mass spectrometry in the same lake and open well samples

(Supplementary Table 3B). All were found to be less than maximum permissible levels.

Metal	Al 396.15 2 nm c/s	B 249.77 2 nm c/s	Ba 455.40 3 nm c/s	Ca 315.88 7 nm c/s	Cd 214.43 9 nm c/s	Co 238.89 2 nm c/s	Cr 267.71 6 nm c/s	Cu 327.39 5 nm c/s	Fe 238.20 4 nm c/s	Ga 294.36 3 nm c/s	In 230.60 6 nm c/s	K 766.49 1 nm c/s	Li 670.78 3 nm c/s	Mg 285.21 3 nm c/s	Mn 257.61 0 nm c/s	Na 589.59 2 nm c/s	Ni 231.60 4 nm c/s	Pb 220.35 3 nm c/s	Sm 359.25 9 nm c/s	Sr 421.55 2 nm c/s	Zn 213.85 7 nm c/s
	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Max acceptable	0.03	0.5	0.7	75	0.005		0.05	0.5	0.3					30	0.1					7	5
Max permissible	0.2	1	0.7	200	0.005	0.01	0.05	1.5	0.3				10	100	0.3			0.05			15
Specimen:																					
Outlet STP	0.017	0.04	0.081	63.132	0	0.001	0	0.001	0.069	-0.001	-0.003	21.263	-0.022	23.389	0.086	70.365	0.003	0.001	-0.002	0.453	0.018
Lake 1 Site 1	0.015	0.039	0.076	58.98	0	0	0	0.001	0.005	0.001	-0.002	20.822	-0.002	22.815	0.001	68.547	0.003	0	0	0.438	0.002
Lake 1 Site 2	0.014	0.038	0.078	59.805	0	0	0	0.001	0.003	-0.001	-0.005	20.381	-0.007	22.388	0	67.142	0.003	0.002	-0.004	0.439	0.002
Lake 1 Well	0.015	0.004	0.179	45.076	0	-0.001	0	0.001	0.006	-0.001	-0.005	4.748	0.028	14.429	0.014	29.183	0.02	0.001	0.015	0.302	0.073
Lake 2 Site 1	0.017	0.039	0.104	31.223	0	0	0	0.001	0.01	-0.001	-0.007	19.367	-0.01	17.311	0.006	60.394	0.003	0.002	-0.005	0.318	0.044
Lake 2 Site 2	0.006	0.038	0.137	30.187	0	0	0	0.001	0.002	-0.002	0	19.155	-0.007	17.192	0	59.86	0.001	0.001	0.003	0.314	0.002
Lake 2 Well	0.024	0.044	0.175	79.088	0	-0.001	0	0.001	0.003	-0.001	0.002	4.75	-0.004	12.239	0.005	43.628	0.001	0.001	-0.005	0.454	0.036
Lake 3 Site 1	0.018	0.041	0.062	26.619	0	0	0	0.002	0.009	-0.001	-0.003	21.515	-0.005	22.403	0.011	75.959	0.003	0.002	0.001	0.319	0.078
Lake 3 Site 2	0.005	0.04	0.067	27.484	0	0	0	0.001	0.004	-0.001	-0.008	21.667	-0.002	22.422	0.004	76.944	0.003	0	0.003	0.323	0.002
Lake 3 Well	0.034	0.02	0.265	125.223	0	0	0	0.001	0.004	-0.002	-0.001	49.608	-0.016	21.99	0.001	97.232	0.001	0	-0.005	0.823	0.019
Lake 4 Site 1	0.014	0.027	0.132	54.205	0	0	0	0.001	0.002	-0.001	-0.009	19.035	-0.005	19.757	0	69.938	0.002	0	-0.005	0.487	0.019
Lake 4 Site 2	0.016	0.028	0.136	55.66	0	0	0	0.001	0.005	-0.003	-0.01	18.938	-0.008	19.693	0	70.684	0.002	-0.002	-0.005	0.492	0.008
Lake 4 Well	0.021	0.017	0.338	84.985	0	0	0	0.001	0.001	-0.001	-0.004	38.427	-0.013	19.652	0.001	62.414	0.001	0	-0.007	0.695	0.028

Supplementary Material Table 3B: Heavy Metal and Metal Levels in all Lake and Well Water Samples in Intercity Samples (June 2023 Sampling). References for Acceptable and Permissible Limits were Taken from Refs 32-33

Antimicrobial Resistance Patterns

AMR patterns of two microorganism species of clinical relevance, *K.pneumoniae* and *E.cloacae*, were studied across the gravitational flow chain (Table 3)

	Amoxicillin (ng/mL) PNEC: 0.250 ng/mL		Clavulanic acid (ng/mL) PNEC: 64 ng/mL		Isoniazid (ng/mL) PNEC: 0.125 ng/mL		Fluconazole (ng/mL) PNEC: 0.25ng/mL		Linezolid (ng/mL) PNEC: 8 ng/mL	
Location/ Time	June 23	Sept 23	June 23	Sept 23	June 23	Sept 23	June 23	Sept 23	June 23	Sept 23
STP output	0	-	0	-	133	-	33	-	0	-
Lake 1 Site 1	0	81.5	0	32.7	152	35.7	28	0	0	0
Lake 1 Site 2	45	0	0	0	153	18	31	0	36	11
Lake 1 Open well	0	0	0	0	128	0	28	-	0	-
Lake 2 Site 1	39	0	49	0	87	29	0	0	88	0
Lake 2 Site 2	45	54.5	51	0	71	16	0	0	117	0
Lake 2 Open well	0	0	120	162	174	84	0	0	84	0
Lake 3 Site 1	45	-	50	-	52	-	28	0	121	-
Lake 3 Site 2	56	0	44	0	55	-	28	0	139	13
Lake 3 Open well	141	-	0	-	183	-	30	0	39	-
Lake 4 Site 1	37	-	0	-	254	-	0	-	37	-
Lake 4 Site 2	43	-	0	-	238	-	0	-	63	-
Lake 4 Open well	112	-	0	-	272	-	28	-	34	-

Table 3: Antibiotic Concentrations in Various Lakes and Open Well Samples. PNEC: Predicted No-Effect Concentrations (31). in Red: Concentrations above PNEC; Dash: Not Tested

<i>K pneumoniae</i>	Outlet STP		Lake 1		Lake 2		Lake 3	
	MIC value	Result	MIC value	Result	MIC value	Result	MIC value	Result
Amoxicillin/Clavulanic Acid	<= 2	S	4	S	<= 2	S	<= 2	S
Piperacillin/Tazobactam	<=4	S	<= 4	S	<= 4	S	<= 4	S
Cefuroxime	2	S	<= 1	S	<= 1	S	<= 1	S
Cefuroxime Axetil	2	S	<= 1	S	<= 1	S	<= 1	S
Ceftriaxone	<0.25	S	<= 0.25	S	<= 0.25	S	<= 0.25	S
Cefoperazone/Sulbactam	<8	S	<= 8	S	<= 8	S	<= 8	S
Cefepime	<=0.12	S	<= 0.12	S	<= 0.12	S	<= 0.12	S
Ertapenem	<=0.12	S	<= 0.12	S	<= 0.12	S	<= 0.12	S
Imipenem	<=0.25	S	<= 0.25	S	<= 0.25	S	<= 0.25	S
Meropenem	<=0.25	S	<= 0.25	S	<= 0.25	S	<= 0.25	S
Amikacin	<=1	S	<= 1	S	<= 1	S	<= 1	S
Gentamicin	<=1	S	<= 1	S	<= 1	S	<= 1	S
Ciprofloxacin	<=0.06	S	<= 0.06	S	<= 0.06	S	<= 0.06	S

Tigecycline	<= 0.5	S	<= 0.5	S	<= 0.5	S	<= 0.5	S
Fosfomycin	32	S	<= 16	S	<= 16	S	64	S
Trimethoprim/ Sulfamethoxazole	<= 20	S	<= 20	S	<= 20	S	<= 20	S

Table 4A: Resistance Profile of K.Pneumoniae in Outlet STP and Downstream Lakes

	Lake 1		Lake 3		Lake 4	
<i>Enterobacter cloacae</i>	MIC value	Result	MIC value	Result	MIC value	Result
Amoxicillin/Clavulanic Acid	>= 32	R	4	R	>= 32	R
Piperacillin/Tazobactam	<= 4	S	<= 4	S	<= 4	S
Cefuroxime	2	S	<= 1	S	4	S
Cefuroxime Axetil	2	S	<= 1	S	4	S
Ceftriaxone	<= 0.25	S	<= 0.25	S	<= 0.25	S
Cefoperazone/Sulbactam	<= 8	S	<= 8	S	<= 8	S
Cefepime	<= 0.12	S	<= 0.12	S	<= 0.12	S
Ertapenem	<= 0.12	S	<= 0.12	S	<= 0.12	S
Imipenem	<= 0.25	S	0.5	S	8	R
Meropenem	<= 0.25	S	2*	I	0.5	S
Amikacin	<= 1	S	<= 1	S	<= 1	S
Gentamicin	<= 1	S	<= 1	S	<= 1	S
Ciprofloxacin	<= 0.06	S	<= 0.06	S	<= 0.06	S
Tigecycline	<= 0.5	S	<= 0.5	S	<= 0.5	S
Fosfomycin	<= 16	S	>= 256	R	64	S
Trimethoprim/ Sulfamethoxazole	<= 20	S	<= 20	S	<= 20	S

Table 4B: Resistance Profile of E.Cloacae in Lakes 1 and Its Downstream Lakes. Pink: Resistant as per CLSI Guidelines on MIC. Although Vitek Tests Colonies for the Listed Antibiotics, Not All are Recommended for Use

Costing Estimates

Without including manpower or sample collection costs, since these are locality specific, estimates for antimicrobial resistance testing can range from a few hundred dollars to many thousands, depending on the number of

samples tested and the type of tests conducted. Bacterial identification and sensitivity testing, if done by techniques such as mass spectrometry or automated machines, can be expensive, albeit more accurate, than those done by manual biochemistry tests. Table 4 gives more details

Purpose	Manual testing	Automated testing	Comments
Plating	Agar powders (MCC, SS, MH)	Agar powders (MCC, SS, MH)	While pre-made plates are possible to purchase, for environmental samples, the sheer number of plates needed (for serial dilutions of samples, subculturing, and sensitivity assays) makes it cost-effective to purchase the powders and make the plates in-house. Costs can be reduced further by purchasing the individual ingredients instead of the pre-made powders
Liquid culturing	Broth powders	Broth powders	For sub-culturing, for biochemical tests for manual identification, to make long term stocks
Identification	Biochemical reagents for bacterial identification (strip tests)	Vitek TM for identification and antibiotic sensitivity	Manual biochemical testing can be tricky- the quality of the test strips (if using test strips) or test performance (if using test tubes for biochemical tests) significantly affects the identification of the microorganism. Automated techniques are easier and more accurate, but can be significantly more expensive.
Antibiotic sensitivity	Antibiotic discs for KB method	BD or Vitek systems	Antibiotic discs can be hard to obtain for uncommon antibiotics and can tend to expire rapidly, especially in hot or humid climates.
Approx costs	INR 3000 (~ USD 33) per sample	INR 6000 (~ USD 63) per sample	A hybrid strategy of using antibiotic-supplemented plates and automated systems could enable identification, counting and sensitivity testing of pathogens of desired resistance (for instance, if one is only interested in examining carbapenem-resistant pathogens) without increasing costs too much.
Additional tests and costs	Antibiotic concentrations & Heavy Metal concentrations: cost Rs. 20,000 (USD 220) PCR for specific AMR genes: costs depend on number of gene targets and type of PCR NGS for all AMR and AMR-related genes: costs depend on number of samples and depth of sequencing.		

Table 5: Cost Estimates for AMR Testing in Water Samples. Does not Include Manpower or Sample Collection/Transportation Costs. These Numbers Hold True at this Point of Time and will Change with Inflation, Local Market Availability, Locally Produced Alternatives etc. PCR: Polymerase Chain Reaction. NGS: Next Generation Sequencing

4. Discussion

In this pilot study we investigated the pattern of AMR in the untreated wastewater flowing through open drains collected across the 8 administrative zones of Bengaluru city, Karnataka, India to five antibiotics belonging to four different classes - Meropenem and Ertapenem (Carbapenems), Piperacillin - Tazobactam (Penicillin + Beta lactamase inhibitor), Cefixime (Cephalosporin) and Ciprofloxacin (Fluoroquinolones). Additionally, we also tested the same in treated wastewater flowing from Bengaluru city to peri-urban lakes for their rejuvenation.

4.1. Wastewater Surveillance is Highly Equitable

In hundreds of developing and very rapidly expanding cities like Bengaluru, home to about 13.3 million people and having among the largest urban growth rates globally, across the world, not all wastewater is underground. In fact, it is estimated that more than 80% of cities in the world flow

their wastewater through open drains. Typically, open drains run closest through socioeconomically poorer sections, increasing their risk of disease. As cities grow, their need for water is fulfilled by lakes around them. Unregulated and unplanned urban growth and climate change have together caused several periurban lakes to dry, and for groundwater levels to plummet. As a remediation method, governments are now beginning to flip the idea of the city as consumer, and think of the city as producer- the wastewater generated by cities will be treated and used to rejuvenate dried lakes and increase shallow aquifers. However, rural and peri-urban areas then are at the mercy of the city's wastewater treatment plants and can be affected by dynamic disease systems that they may not have faced otherwise. Hence, studies like ours are important to do on a longitudinal basis to monitor and better prepare for potential health risks.

4.2. Intracity Open Drain Wastewater Findings

Our study indicates a significant presence of multi drug resistant bacteria in the wastewater system. In Bengaluru, about 70% of the water in 2021 was estimated to flow in underground sewage pipes which are part of the formal sewerage network which undergoes treatment before being released into lakes and other reservoirs. Unlike other studies done on influent or effluent water from sewage treatment plants (STPs), our study is unique in that the samples were collected from the residual 30% of wastewater flowing in open storm drains [11,16]. This wastewater comes from slums and illegal pipelines from establishments that are not connected to the formal STP network. As such this water could provide a window into populations that may not access formal healthcare systems and are typically hidden from the mainstream. The presence of carbapenem resistance in this water could indicate the incorrect disposal of antibiotics, the non-linkage of hospitals to the formal sewerage network, or the inappropriate usage and disposal of higher-level antibiotics in non-hospital settings. Water from open storm drains usually empty into water bodies such as lakes without treatment. This water is then used for fishing, agricultural

irrigation or pumped for use as drinking water within the city, or pumped to peri-urban and rural areas for the same purposes. Thus, open drains represent a path by which AMR circulates and escalates within the city and from the city to its surroundings. They may indicate routes by which individuals who have not been exposed to high levels of antibiotics end up with resistant bacteria.

4.3. Antibiotic Resistance Profiles of Enterobacteriaceae Isolates Suggest Multiple Resistance Mechanisms at Play

Enterobacteriaceae such as *Klebsiella pneumoniae* and *Escherichia coli* are among the most common pathogens associated with severe multi drug resistant nosocomial infections in India [17]. They were also the most commonly isolated organisms in our study. Thus, they merited a closer examination of their resistance profiles.

Some unusual patterns noticed among these isolates and their possible reasons are described below. Since resistance to ciprofloxacin is conferred by a separate set of genes encoding DNA gyrase, topoisomerase or the PMQR family, it is not discussed in further detail below [18].

Pattern	Possible reasons
Carbapenem Resistant. TZP Susceptible Cefixime Susceptible	Resistance mutations in efflux pumps or porins (for instance, mutations that change the charge of porins can affect piperacillin differently from meropenem or ertapenem (19)
Carbapenem Resistant. TZP Resistant Cefixime Susceptible	Presence of OXA-48 gene (20) preserves susceptibility to cephalosporins while compromising carbapenem and other beta lactam/lactamase activities
Carbapenem Resistant TZP Susceptible Cefixime Resistant	Mutations in porins could affect carbapenems without affecting TZP (19). Presence of ESBLs affect 3rd generation cephalosporins like cefixime, but can be overcome by the presence of tazobactam in TZP which is a beta lactamase inhibitor
Carbapenem Sensitive TZP Resistant Cefixime Resistant	No presence of genes coding for carbapenemases. This phenotype could also be due to genes such as TEM 3-7, SHV or CTX-M family of genes which confer resistance to penicillins and cephalosporins (21).
Differential Resistance Profiles between Meropenem and Ertapenem	Nearly 20% of the isolates show differential resistance profiles between these two carbapenems. This discrepancy could be due to three reasons: a) ertapenem is known to have a lower threshold for resistance than meropenem (22) b) Ertapenem resistance is known to be enhanced by the presence of ESBL and point mutations in the porin genes to a much larger extent than meropenem (23) c) The half life of ertapenem is 4 hours compared to meropenem's 1 hour (22), making it likely that the wastewater environment potentially contains more ertapenem than meropenem.

Table 5: Some Possible Resistance Combinations and the Molecular Mechanisms behind them thus, our Data Indicates that Resistance to One Member of a Class of Antibiotics does not Necessarily Remove the Entire Class of Antibiotics nor the Classes Below the Antibiotic Level from the Armamentarium among Isolates Present in Wastewater. It also Provides a Glimpse into the Various types of Horizontal Gene Transfers that could Happen in the Milieu of Wastewater.

4.4. Intercity Wastewater Findings

STPs and their treatment systems differ by city and in fact within the city as well. Our findings suggest that the STP-treated water flowing into lakes does not contain significant levels of heavy metals. However, levels of some antibiotics are still higher than PNEC and tend to vary by season. For the most part, the fact suggests that the treatments followed in the STPs are reasonably effective in preventing the transmission of these compounds to peri-urban or rural areas with the water although seasonal increases suggest the need for further monitoring and changes in treatment protocols, if necessary.

4.5. The Need for Longitudinal Cost-Effective Surveillance and Monitoring

The observed spatial variation in AMR profiles across zones also raises important stewardship implications. Aggregated city-level or national antibiograms may inadequately capture the heterogeneity of resistance patterns experienced by frontline clinicians and communities. Hyperlocal AMR intelligence may therefore become increasingly important for future antimicrobial stewardship strategies, particularly in rapidly urbanizing settings where prescribing behaviors, healthcare access, environmental exposure, and wastewater circulation differ substantially across neighborhoods. Such findings support the need for more geographically contextualized and community-embedded stewardship approaches. While it is curious that isoniazid, an anti-tuberculosis drug, should be present in high concentrations (above PNEC), it had reduced by Sept 2023. This could be due to the increased rainfall during this time (due to the monsoon season), diluting any compounds. Similarly, while resistant bacteria are seen in the waters, they are not consistently found across all samples, as seen in the case of *K.pneumoniae* and *E.cloacae*. Both these findings underscore the dynamic nature of these ecosystems and the need for longitudinal, cost-effective surveillance. Our cost calculations indicate that these efforts do not need to be very expensive to yield sufficient information for public health action. While most efforts in wastewater-based AMR have focussed on in-depth sequencing studies to determine AMR genes from a research perspective, for routine health and safety monitoring, government budgets do not need to extend too far to include these basic tests.

4.6. Limitations

This study did not delve into the antibiotic resistance genes causing these phenotypes. We also were unable to test for the levels of active antibiotics in the intracity wastewater samples, which would have given an indication of whether the AMR bacteria were being shed from certain super-generators (such as hospitals or poultry farms or animal husbandry units) or actively being selected for in an environment that had persistently high levels of antibiotics, as shown elsewhere. Another limitation is that this study does not test samples longitudinally (except for the antibiotic testing assays that were conducted in June 23 and Sept 23). The role or impact of bacteriophages was not determined in this study [19-24].

5. Conclusions

Our results indicate: a) wastewater could be a novel and cost-effective method of determining hyperlocal AMR patterns and burden in communities b) drug resistant bacteria are found in significant numbers in open drains c) there is geographical variation within a city of the patterns and burden of AMR, opening up the possibility of community-based and community-relevant antimicrobial stewardship practices. Wastewater surveillance also allows for community-participation in surveillance and for grassroots or participatory initiatives towards all 6 pillars of the National Action Plan for Antimicrobial Resistance, contributing to increased awareness, strengthening knowledge/evidence base, promoting AMR control measures & optimized use of antimicrobials, facilitating novel avenues for investments in AMR research and innovations.

However, surveillance alone is insufficient to improve antimicrobial use [25-27]. The central challenge is not only generating AMR data, but developing implementation architectures capable of translating local resistance intelligence into everyday prescribing decisions within community care settings. Future efforts may therefore need to integrate wastewater surveillance with primary care networks, community laboratories, stewardship feedback systems, and continuous learning approaches such as audit-and-feedback and Plan-Do-Study-Act (PDSA) cycles. Such integration could help move AMR efforts beyond episodic surveillance toward adaptive, community-based antimicrobial stewardship learning systems [28-33].

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