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Antidrug resistance in the Indian ambient waters of Ahmedabad during the COVID-19 pandemic

Manish Kumar^{a,*}, Kiran Dhangar^a, Alok Kumar Thakur^a, Bhagwana Ram^b,
Tushara Chaminda^c, Pradeep Sharma^d, Abhay Kumar^e, Nirav Raval^f, Vaibhav Srivastava^f,
Jörg Rinklebe^{g,h}, Keisuke Kurodaⁱ, Christian Sonne^j, Damia Barcelo^{k,l}

^a Discipline of Earth Science, Indian Institute of Technology Gandhinagar, Gujarat 382355, India

^b Department of Civil Engineering, Indian Institute of Technology, Gandhinagar, Gujarat, 382355, India

^c Department of Civil and Environmental Engineering, University of Ruhuna, Galle, Sri Lanka

^d Department of Environmental Science, Graphic Era Deemed to be University, Dehradun, Uttarakhand, India

^e NCERT, Sri Aurobindo Marg, New Delhi- 110016, India

^f Encore Insoltech Pvt. Ltd., Randesan, Gandhinagar, Gujarat 382007, India

^g University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water and Waste Management, Laboratory of Soil and Groundwater Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany

^h Department of Environment, Energy and Geoinformatics, University of Sejong, Seoul, Republic of Korea

ⁱ Department of Environmental and Civil Engineering, Toyama Prefectural University, Imizu 939-9308, Japan

^j Aarhus University, Department of Bioscience, Arctic Research Centre (ARC), Frederiksborgvej 399, PO Box 358, DK-4000 Roskilde, Denmark

^k Institute of Environmental Assessment and Water Research, IDAEA-CSIC, C/ Jordi Girona 18-26, 08034 Barcelona, Spain

^l Catalan Institute for Water Research, ICRA-CERCA, Emili Grahit 101, 17003, Girona, Spain

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ABSTRACT

The ongoing COVID-19 pandemic increases the consumption of antimicrobial substances (ABS) due to the unavailability of approved vaccine(s). To assess the effect of imprudent consumption of ABS during the COVID-19 pandemic, we compare the 2020 prevalence of antidrug resistance (ADR) of *Escherichia coli* (*E. coli*) with a similar survey carried out in 2018 in Ahmedabad, India using SARS-CoV-2 gene detection as a marker of ABS usage. We found a significant ADR increase in 2020 compared to 2018 in ambient water bodies, harbouring a higher incidence of ADR *E. coli* towards non-fluoroquinolone drugs. Effective SARS-CoV-2 genome copies were found to be associated with the ADR prevalence. The prevalence of ADR depends on the efficiency of WWTPs (Wastewater Treatment Plants) and the catchment area in its vicinity. In the year 2018 study, prevalence of ADR was discretely distributed, and the maximum ADR prevalence recorded was ~ 60%; against the current homogenous ADR increase, and up to 85% of maximum ADR among the incubated *E. coli* isolated from the river (Sabarmati) and lake (Chandola and Kankaria) samples. Furthermore, wastewater treatment plants showed less increase in comparison to the ambient waters, which eventually imply that although SARS-CoV-2 genes and faecal pollution may be diluted in the ambient waters, as indicated by low C_t -value and *E. coli* count, the danger of related aftermath like ADR increase cannot be nullified. Also, Non-fluoroquinolone drugs exhibited overall more resistance than quinolone drugs. Overall, this is probably the first-ever study that traces the COVID-19 pandemic imprints on the prevalence of antidrug resistance (ADR) through wastewater surveillance and hints at monitoring escalation of other environmental health parameters. This study will make the public and policyholders concerned about the optimum use of antibiotics during any kind of treatment.

1. Introduction

The exponential rise in the consumption of antimicrobials in various applications such as medical, veterinary, domestic and agricultural and

their leak to aquatic ecosystems has caused the global prevalence of antidrug resistance (ADR), which is being considered a major threat to public health (Rodriguez-Mozaz et al., 2015; Chatterjee et al., 2010; Baker-Austin et al., 2006). The ADR is not only limited to the survival

* Correspondence to: Discipline of Earth Sciences, Indian Institute of Technology Gandhinagar, Room No. 336 A, Block 5, 382355 Gujarat, India.

E-mail addresses: manish.env@gmail.com, manish.kumar@iitgn.ac.in (M. Kumar).

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and infection by any particular type of microorganism, but can lead to life threatening diseases for both animals and human (Singer et al., 2008; Ferreira da Silva et al., 2007; Jiang et al., 2013). Due to lack of regulations on the prescription and non-prescription use of antimicrobials, its consumption rate in, for example, India has been increased by 105% from 2000 to 2015 while worldwide it is estimated to increase by 63% during 2010–2030 (Klein et al., 2018; Global Antibiotic Resistance Partnership GARP-India Working Group, 2011; Van Boeckel et al., 2015). On top of that, the rate of consumption of certain antimicrobials has escalated during the COVID-19 pandemic in an effort to minimise the risk of severe infections and mortality (Miranda et al., 2020; Liu et al., 2020). Around 70% of COVID-19 patients have received antimicrobial treatment along with overuse of various antibiotics despite only 10% on average show microbial infections (Hsu, 2020; Rawson et al., 2020). As most of the consumed drugs and their metabolites are excreted through urine and faeces, their discharge to aquatic environments depends on the removal efficiency of the WWTPs (Singer et al., 2008; Azuma et al., 2012; Takanami et al., 2010; Auerbach et al., 2007; Kumar et al., 2020a). If the WWTP clearing rate is low, microorganisms exposed to antimicrobials and metabolites develops mutations causing ADR (Aali et al. 2014, Alexander et al. 2020, Guo et al. 2018, Kumar et al., 2020a, 2020c) Thus, the increased use of antimicrobials in the current pandemic will probably pose an increased risk in terms of ADR during post COVID-19 as concerned by a number of recent studies (Kuroda et al., 2021; Lucien et al., 2021; Hsu, 2020; Kumar et al., 2020a; Asaduzzaman et al., 2020).

The high consumption of antimicrobials causes an increase in the prevalence of ADR in several environmental compartments including drinking, waste and groundwater, sludge, sediments and municipal solid waste leachate (Al-Judaibi, 2014; Ferreira da Silva et al., 2007; Kumar et al., 2020d; 2020e; Ram and Kumar, 2020; Zhang et al., 2015; Storteboom et al., 2010; Threedeach et al., 2012). In the case of for example *E.coli* isolates from the effluent of WWTPs have shown a higher prevalence of antidrug resistance as compared to the influent, which is probably due to poor treatment conditions, prolonged microbial activities, and chemical properties of the antimicrobial drugs (Reinthal et al., 2003; Silva et al., 2006; Miranda and Castillo, 1998; Marcinek et al., 1998). Specifically, the conventional treatment processes at WWTPs do not completely mineralise the parent antimicrobial drugs, and generate some residues, metabolites or transformation products that may have the same biological activity as the parent drugs (Zhang et al., 2015; Kumar et al., 2020c). Thus, WWTPs are considered hotspots for the spreading ADR due to high microbial density, horizontal gene transfer (HGT), nutritional richness and the availability of antimicrobial metabolites (Zhang et al., 2015; Threedeach et al., 2012; Silva et al., 2006). Previous studies have reported a correlation between the prevalence of ADR and inefficiently treated wastewater discharge, having the abundance of *E. coli*, extravasating to river and lake waters (Na et al., 2018; Yang et al., 2017; Honda et al., 2016, 2018; Biswas et al., 2015; Akhter et al., 2014; Ram and Kumar, 2020; Kumar et al., 2020d; 2020e). Thus, a better understanding of the occurrence, distribution and frequency of antidrug resistance in the urban waters is needed to prevent or slower the rate of increase in ADR.

With the same purpose, presumptive actions are needed to study the prevalence of the ADR during wastewater treatment and the water bodies receiving the WWTP effluents. Wastewater based epidemiology (WBE) is an efficient way to trace the prevalence of ADR in highly COVID-19 infected areas, which are potentially major zones of high consumption of drugs, can be identified with the help of the WBE approach for tracing the SARS-CoV-2 genome concentration in wastewaters (Kumar et al., 2020b). Also, with the help of authorised software and apps (for example: Arogya-Setu app in India), the infected population within a certain region can be predicted. Identifying the WWTPs in such infected areas aids in correlating ADR with the elevated cases of COVID-19. Therefore, the impact of such highly contaminated zones on the prevalence of ADR in wastewaters needs to be studied well.

ADR is not included in the water quality standards and guidelines of India mostly due to the lack of proper treatment facilities in many cities where domestic wastewater is directly discharged to aquatic environments (IS10500, 2012). In this study, we select the Ahmedabad City of Gujarat Province in western India with a population of 5.6 million (2011 Census) to assess the prevalence of ADR in WWTP, lake and river locations within various zones of the city. The specific objectives of the present study are: i) to compare and discuss the prevalence of *E. coli* in the surface water and wastewater in Ahmedabad in order to have a prior knowledge of ADR pervasiveness in different compartments, ii) to analyse a comparative status of the antidrug resistance in the *E. coli* isolated from the urban waters of the city and iii) to further understand the imprints of COVID-19 situation on the status of SARS-CoV-2 genome concentration and ADR prevalence at various zones of the city.

2. Material and methods

2.1. Sample collection and ADR analyses

The water samples were collected from 6 different locations of Ahmedabad city on 23rd June 2018, and 16th October 2020 (Fig. 1). Two locations on the stretch of Sabarmati river: Nehru Bridge (NB) and Sardar Bridge (SB); two lakes: Kankariya Lake (KL) and Chandola Lake (CL), and two WWTP locations: Chandkheda (inlet: CI and outlet: CO) and Vasna, also known as Juhapura (inlet: VI and outlet: VO), selected to assess ADR. For SARS-CoV-2 gene detection, a total of 10 locations were selected to represent various zones of the city that comprises all ADR sampling locations. We kept ADR locations low to match the number of locations tested in 2018 (Ram and Kumar, 2020). The geographical details about the selected locations are well described in our previous study by Ram and Kumar (2020) (See Supplementary Information). Sterile bottles (Tarson-546041) of medical grade were used to collect the samples, which were then kept in iceboxes until arrival at the laboratory. For on-site measurement of pH, EC, ORP, TDS and salinity, a multi-parameter probe, HANNA HI9828 was used. The procedure for testing the isolation of *E. coli* for ADR is likewise described in Ram and Kumar (2020) (See Supplementary information). Briefly, the water samples were filtered through membranes with 0.45- μm -pore size, and *E. coli* trapped by the membranes were incubated on Chromocult® Coliform Agar ES (Merck Microbiology, Darmstadt, Germany). Each *E. coli* isolate was tested for susceptibility to six antibiotics (kanamycin, KM; tetracycline, TC; norfloxacin, NFX; ciprofloxacin, CIP; levofloxacin, LVX; and sulfamethoxazole, ST) by Kirby-Bauer method using PERL-CORE® Sensitivity Test (ST) Agar (EIKEN Chemical Co., Ltd, Tokyo).

2.2. SARS-CoV-2 RNA detection

The SARS-CoV-2 RNAs were isolated and detected from 30 mL wastewater samples that were centrifuged at 4000g for 40 min, followed by filtration of supernatant using 0.22-micron syringe filter (Mixed cellulose esters syringe filter, Himedia). After filtration, 25 mL of the supernatant was treated with polyethylene glycol and NaCl at 80 g/L and 17.5 g/L respectively, and incubated at 17 °C, 100 rpm overnight. The mixture was centrifuged for 90 min at 14000g and the supernatant was discarded to collect a pellet containing viruses and their fragmented genes. The pellet was re-suspended in 300 μl RNase-free water and kept in 1.5 mL vials at – 40 °C, until further analyses.

RNA isolation from the pellet with the concentrated virus was performed using NucleoSpin® RNA Virus isolation kit (Macherey-Nagel GmbH & Co. KG, Germany). The samples were spiked with MS2 phage as an internal control prior to the RNA extraction provided by TaqPath™ Covid-19 RT-PCR Kit. The nucleic acid was extracted and a Qubit 4 Fluorometer (Invitrogen) was used for RNA concentrations estimation. The molecular process inhibition control was evaluated through the MS2 phage for QA/QC analyses of nucleic acid extraction and PCR inhibition (Haramoto et al., 2018). We have described the methodologies in Kumar

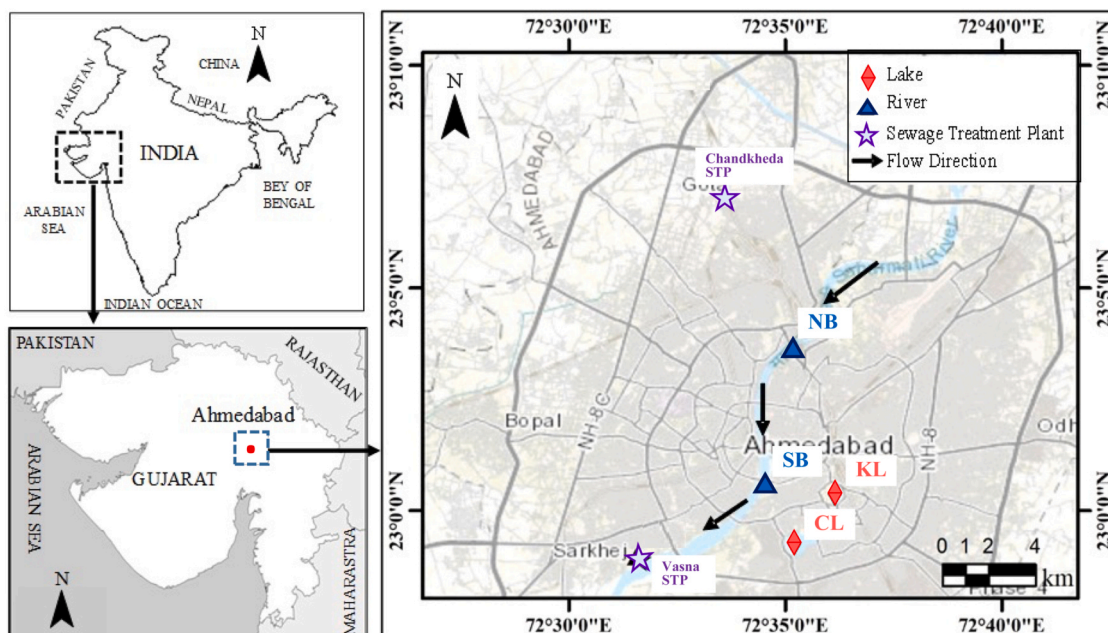


Fig. 1. Map showing the sampling locations in Ahmedabad, Gujarat (i) locations at Sabarmati River (Nehru Bridge: NB; Sardar Bridge: SB), (ii) two lakes (Kankaria Lake: KL; Chandola Lake: CL) and (iii) two different Sewage Treatment Plants (STPs) (Chandkheda STP; Vasna STP).

et al. (2021, 2020b).

Briefly, steps were carried out as per the guideline provided with the product manual of Macherey-Nagel GmbH & Co. KG and RNAs were detected using real-time PCR (RT-PCR). An Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (version 2.19 software) was used for SARS-CoV-2 gene detection. A template of 7 μ l of extracted RNA was used in each reaction with TaqPath™ 1-Step Multiplex Master Mix (ThermoFischer Scientific, USA). Three controls were included: positive control (TaqPath™ COVID-19 Control); negative control (from extraction run spiked with MS2); and a no template control (NTC). Finally, results were interpreted using Applied Biosystems Interpretive Software, and Ct values for three target genes, i.e., ORF1ab, N Protein, and S Protein of SARS-CoV-2, were detected along with MS2 as an internal control.

The samples were considered as positive if at least two genes showed amplification. The average Ct-value of a given sample was then converted to gene copy numbers considering the equivalence of 500 copies of SARS-CoV-2 genes as 26 Ct-value (provided with the kit), and the same was extrapolated to derive approximate copies of each gene. The average effective genome concentration present in a given sample was calculated by multiplying the RNA amount used as a template with the enrichment factor for each sample.

2.3. Quality assurance/quality control (QA/QC) and statistical analysis

To determine the contamination occurred during transport, blanks in the same type of bottle were analysed prior to sampling. Duplicate analysis of samples was conducted to check accuracy and precision. To ensure instrument sensitivity and check cross-contamination, blanks were run for each batch of five samples. Signals were considered significant if the signal-to-noise ratio was more than three. The limit of quantification (LOQ) of the overall method was defined as sample concentration equivalent to 1 copy per reaction tube, which was 1.7×10^2 copies/L. We have calculated the gene copy numbers based on the positive control provided with kit i.e., 10^4 copies/ μ l and the final concentration of 25 copies per reaction. Based on our experience, the same positive control is providing the same Ct values for all 3 genes analysed in this study. Hence, it is evident that primer efficiency is more or less same. Relative to the Ct values of genes of positive controls, copy

numbers have been calculated in test samples of different sources.

ADR analyses were carried out in triplicate for the accuracy and precision of the data generated. Tests were repeated if the standard deviation between the triplicate was higher than 10%. Statistical analysis by Student *t*-test was done to compare the antidrug resistance caused by all six antibiotics in year 2018 and 2020, and the results were represented by Pearson's correlation coefficient (*p*), whose value ranges between zero to unity. The change in percentage resistance of more than 90% (*p* = 0.10) was considered significant.

3. Results and discussions

3.1. Comparison of prevalence of *E. coli*

The prevalence of *E. coli* and environmental parameters is summarised in Table 1. In 2018, the *E. coli* count was highest in river sampling locations, with maximum count of 76,600 cfu (colonies forming unit) mL^{-1} , which was the highest among the lake and WWTP locations except for the Vasana STP. This critically high prevalence is due to the river-human interactions at the riverfront, wastewater discharge or the stagnant flow conditions near the sampling locations (Pormohammad et al., 2019). This reported prevalence in the Sabarmati River was higher than the reported prevalence in rivers of tropical countries like India and Thailand (Chatterjee et al., 2010; Kumar and Sharma, 2014; Honda et al., 2016, 2018; Hamner et al., 2007; Hu et al., 2008). The higher recreational activities at KL location as compared to the CL location are the main cause of higher *E. coli* prevalence at KL (15,600 cfu mL^{-1}) than CL (3467 cfu mL^{-1}) (Kumar and Sharma, 2014; Ram and Kumar, 2020). The varying *E. coli* prevalence at STP locations (inlet and outlet) in 2018 indicates the varying amount of incoming faecal contamination and reduction ratios in the STP.

In the year 2020, the *E. coli* prevalence at STP locations was higher than in 2018 samples ranging from 950,000 to 400,000 cfu mL^{-1} at inlet locations and 19,500–32,500 cfu mL^{-1} at outlet locations. This is attributed to the increased domestic wastewater discharge from the Covid-19 lockdown which also increased the burden on municipal WWTPs resulting in less removal of *E. coli* in WWTPs. It is worth noting that, this critical *E. coli* prevalence alarms the municipal authorities to advance the disinfection processes in WWTPs, and therefore potential

Table 1Sampling locations along with *in-situ* water quality (pH, EC, TDS, ORP and salinity) and prevalence of *E. coli* in 2018 and 2020.

Sampling Location	Year	pH	EC	TDS	ORP	Salinity	<i>E. coli</i>
Nehru Bridge (NB)	2018	8.4	1320	1090	-16	691	24,267
	2020	7.67	554	343	123.5	0.25	1400
Sardar Bridge (SB)	2018	8.00	1541	1100	2	691	76,600
	2020	7.30	533	352	115.7	0.27	5200
Kankaria Lake (KL)	2018	8.70	3015	2050	13	1350	15,333
	2020	8.58	5934	3323	30.9	2.71	13,100
Chandola Lake (CL)	2018	8.10	3240	2300	29	1510	3467
	2020	7.86	1014	590	43	0.44	ND
Chandkheda Inlet (CI)	2018	6.70	2100	1480	-274	972	4220
	2020	6.85	3745	2324	-238.6	1.87	950,000
Chandkheda Outlet (CO)	2018	7.30	1620	1400	-57	911	2893
	2020	7.52	3624	2249	118.6	1.81	32,500
Vasna Inlet (VI)	2018	6.60	1500	1060	-117	674	96,393
	2020	6.97	3254	2017	-231.7	1.61	4,000,000
Vasna Outlet (VO)	2018	6.90	1506	1070	-193	670	9467
	2020	7.34	2767	1715	90.3	1.36	19,500
ND: Not Detected	Unit	-	$\mu\text{S cm}^{-1}$	mg L^{-1}	mV	ppt	cfu mL^{-1}

human health effects could be reduced (Pormohammad et al., 2019). Whereas, the reduced *E. coli* prevalence in the Sabarmati river, can be attributed to the improved water quality and attenuation capacity of the river due to less human and industrial interaction. Another reason can be the dilution level of the samples collected from the river than that of the lake and WWTPs (Pormohammad et al., 2019).

3.2. Mechanism and pathways of antibiotic resistance

Though antimicrobials and antibiotics are among the essential medical interventions, increased antimicrobial resistance threatens the success of patient treatment. Antibiotic resistance has been listed as one of the three major threats to the public health in 21st century by the world health organisation (WHO) (World Health Organization, 2014). Thus, to understand and reduce the consequences of antibiotic resistance, we need to understand its mechanism. Antimicrobial resistance is expected to be the result of the environmental interactions of several

organisms. As most antimicrobials consists of naturally produced compounds in nature, many of the bacteria have overcoming molecular mechanism to overcome the drugs thereby being intrinsically resistant to antimicrobials (Blair et al., 2015; Munita and Arias, 2016). However, we are here dealing with the acquired resistance by the bacteria which were originally susceptible to the particular antimicrobial.

Summarising the molecular and biochemical mechanisms of antibiotic resistance is shown in Fig. 2 (Munita and Arias, 2016). These mechanisms of antidrug are generally categorised based on genetic and mechanistic basis. In a genetic basis, antidrug resistance can be developed due to mutational resistance, horizontal and vertical gene transfer (HGT and VGT). Whereas, in a mechanistic basis, antidrug resistance can be developed due to changes in the target site, modifications of antibiotic molecule, and decreased antibiotic penetration and efflux. Fig. 2 also shows that how COVID-19 spread may impact the development of antidrug resistance. The increased pharmaceutical pollution during COVID-19 spread can increase environmental stress on bacteria or

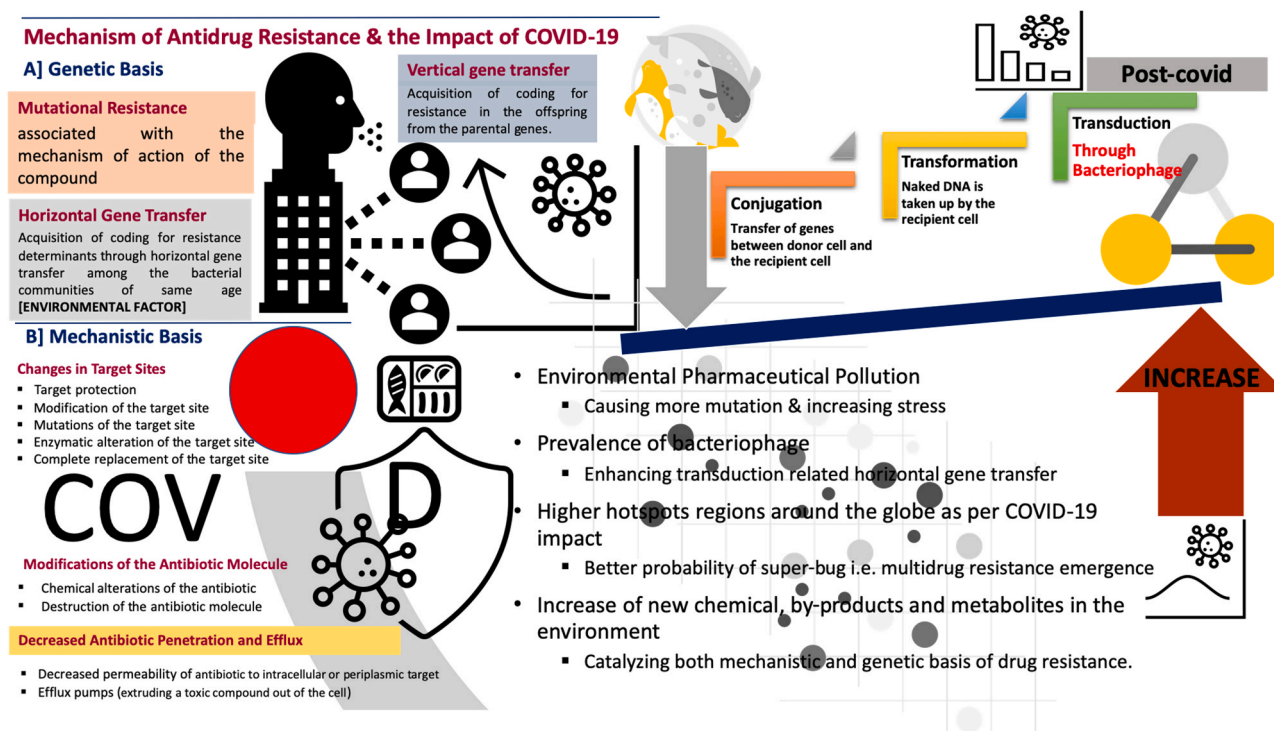


Fig. 2. Mechanism of Antidrug Resistance and the impact of COVID-19: Probable changes in molecular and biochemical triggers of an antidrug resistance.

microbes causing more mutation. This catalyses both the genetic and mechanistic basis of drug resistance. Also, the higher prevalence of bacteriophage may enhance the transduction related to HGT. Thus, the highly infected regions or hotspots of COVID-19 spread around the globe have a greater probability of the emergence of super bugs having multidrug resistance. Drugs like Remdesivir, Ivermectin, Azithromycin, Favipiravir, Chloroquine, Umiferovir, Ritonavir, Aspirin, and Hydroxycoloroquinine are going to remain under the scanner.

3.3. Comparison of occurrence of ADR

Fig. 3 and Fig. 4 represents the comparative sensitivity of *E.coli* towards six antibiotics including the fluoroquinolone drugs NFX (norfloxacin), CIP (ciprofloxacin), LVX (levofloxacin) as well as TCE (tetracycline drugs), KM (kanamycin monosulphate), and ST (sulfa-methoxazole), at various sampling locations (CI, VI, CO, VO, NB, SB, CL, and KL) in 2018 and 2020. In 2018, the river location NB had 0% resistance for all antibiotics, whereas SB location had 40% resistance towards all antibiotics except 60% resistance for KM. SB is the central urban location. This indicates that the ADR on the urbanisation and the discharge conditions. However, in 2020, this resistance increased at both river locations for all antibiotics, except for KM at SB. For all Quinolone drugs, the antidrug resistance increased to 50% at both river locations in 2020, whereas it was varying for TCE, KM and ST. At location NB, resistance was observed to be increased for TCE, KM and

ST. Whereas, at location SB, resistance increased for TCE, ST, but decreased for KM. This indicates inflow or generation of antidrug resistant *E.coli* in the river water from urbanised sources which reflect increased use of antimicrobials, due to the unavailability of COVID-19 specific drugs (Abelenda-Alonso et al., 2020; Getahun et al., 2020; Hsu, 2020). Though the prevalence of *E. coli* was highest in 2018, more antidrug resistant *E.coli* are generated in the year 2020 due to heavy usage of antimicrobials.

In 2018, no ADR was observed for any of the antibiotics at location CL and KL, except for NFX, TCE and ST at location KL. (Fig. 3 and Fig. 4). However, significant resistance was observed for all antibiotics, except KM, at both lake locations with higher values at CL than KL. This indicates more urbanised discharge carrying antidrug resistant *E.coli* accumulates at the location CL. One of the major reasons for the generated resistance at CL is the occasional discharge to the CL from nearby open Pirana solid waste dumping site (Singh et al. 2008). This call for a monitoring of urban wastewater flows being discharged to the lake ecosystem.

Among the sampled WWTP locations in the year 2018, at locations VI and VO, no resistance was observed for any of the antibiotics except TCE (20% in influent) (Fig. 3 and Fig. 4). Whereas, at CI location resistance for NFX, LVX, TCE, KM, was observed but only found to be increasing towards CIP and KM at location CO. These results show the increase in antidrug resistance after WWTP treatment, which was consistent as reported in the studies from Sweden and Austria (Reinthalder et al., 2003;

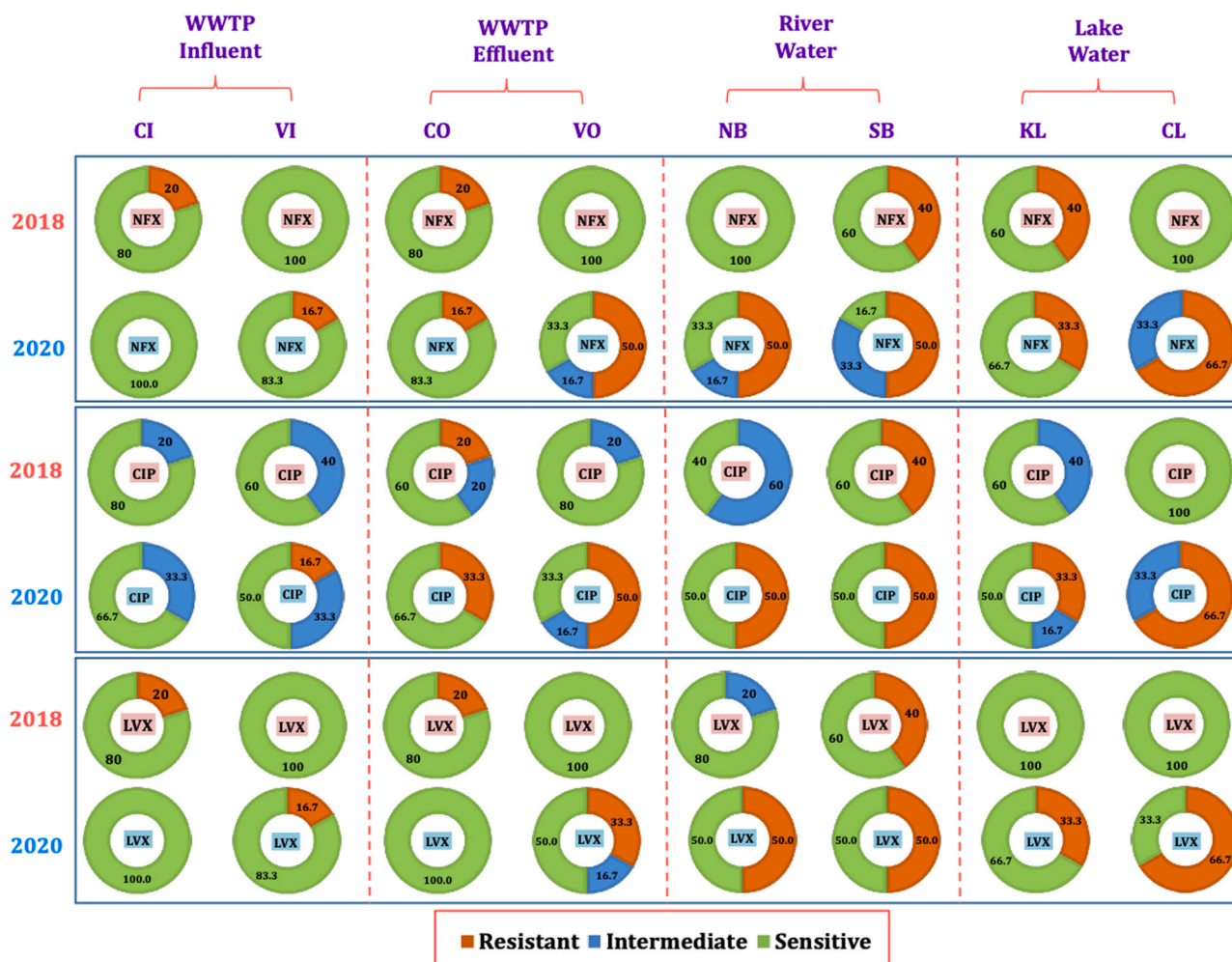


Fig. 3. Percentage of antibiotic resistance in the influents of different water compartments in years 2018 and 2020 against fluoroquinolone drugs i.e. NFX (Norfloxacin), CIP (Ciprofloxacin), LVX (Levofloxacin) for locations including WWTPs CI (Chandkheda Inlet), CO (Chandkheda Outlet), VI (Vasna Inlet) and VO (Vasna Outlet); Rivers, NB (Nehru Bridge) and SB (Sardar Bridge), and Lakes, KL (Kankaria Lake) and CL (Chandola Lake).

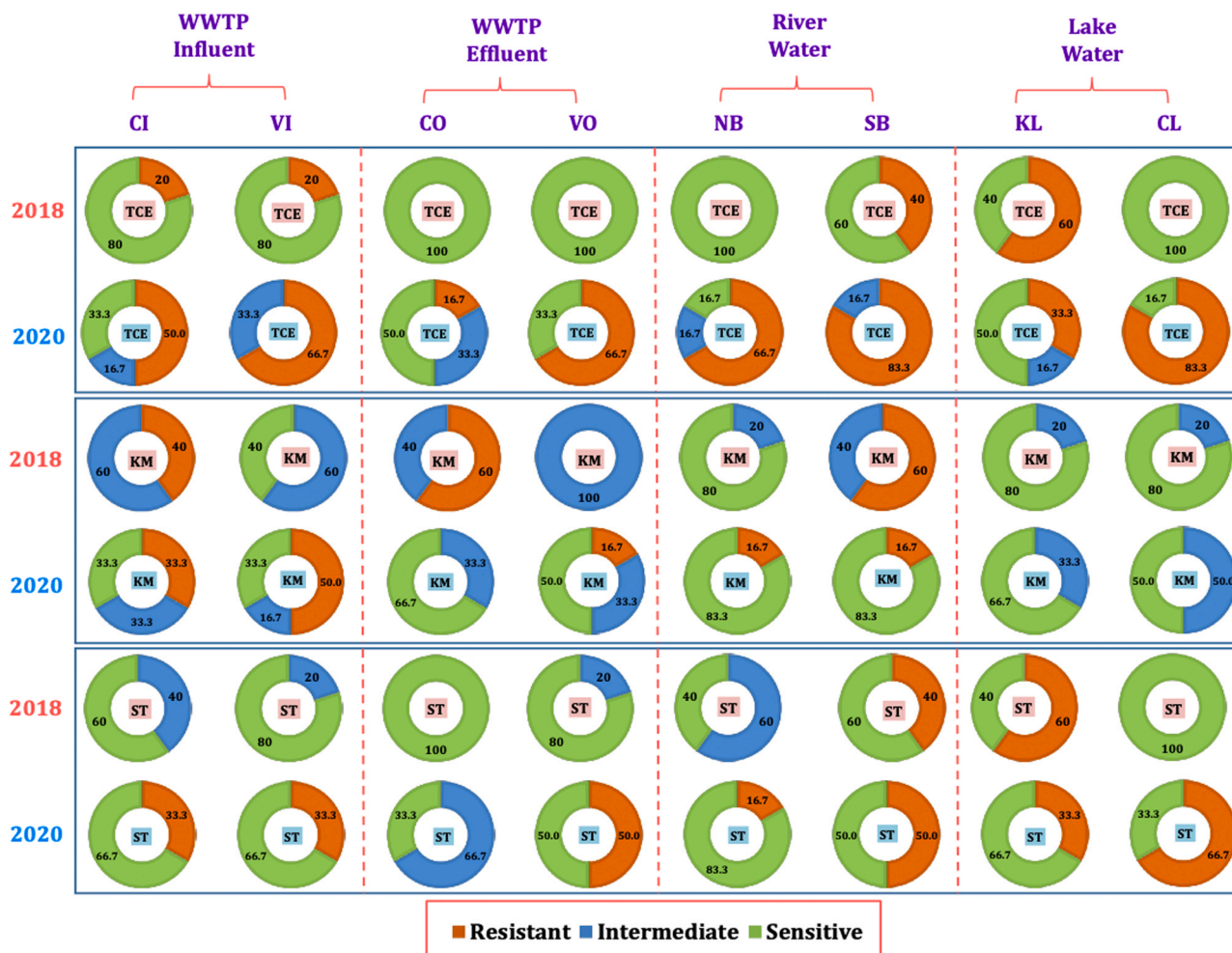


Fig. 4. Percentage of antibiotic resistance in the influents of different water compartments in years 2018 and 2020 against tetracycline drugs (TCE), aminoglycosides i.e. KM (kanamycin), and others i.e. ST (sulfamethoxazole) for locations including WWTPs CI (Chandkheda Inlet), CO (Chandkheda Outlet), VI (Vasna Inlet) and VO (Vasna Outlet); Rivers, NB (Nehru Bridge) and SB (Sardar Bridge), and Lakes KL (Kankaria Lake) and CL (Chandola Lake).

Flach et al., 2018). Interestingly, ADR increased significantly for all antibiotics in the year 2020 at the VI and VO locations when compared to year 2018. In the year 2020, ADR was observed for all antibiotics at VI and these resistances were observed to be increasing or being constant at VO locations for all antibiotics except KM (decreased by 35%) (Figs. 3 and 4). Such a high increase in the resistance in treated effluent can be attributed to a long residence time of wastewater in WWTP, where *E. coli* is in contact with the antibiotics or antibiotic residues for a long time (Honda et al., 2018). In the case of CI in the year 2020, no resistance was observed towards the quinolone drugs, whereas the observed ADR for KM, ST, and TCE, was reduced significantly at CO location. However, resistance was observed to be generated for NFX and CIP at CO in year 2020. The high resistance towards quinolone drugs is attributed to the discharge having domestic origin (Threedeach et al., 2012; Auerbach et al., 2007); because these drugs are prescribed for treatments of respiratory and urinary tract infections, their use has increased significantly during the COVID-19 pandemic (Abelenda-Alonso et al., 2020; Getahun et al., 2020; Hsu, 2020).

Overall, domestic municipal wastewater likely possesses higher concentrations of antimicrobials than any other ambient water. Aeration enhances the generation and replication of antidrug resistant *E. coli* if there is a high density and diversity of the microbial population in a given wastewater (Ram and Kumar, 2020; Kumar et al., 2020f). The advanced or hybrid wastewater treatment processes should be adopted to effectively remove the antimicrobials and their residue in order to reduce the possibility of resistance (Dhangar and Kumar, 2020).

Treatment technologies such as MBR-NF/UF, MBR-UV oxidation, AS-gamma radiation was found to be very effective (removal efficiency: ~ 90–100%) for most of the antibiotics and other pharmaceuticals (Dhangar and Kumar, 2020).

The abundance in both antidrug resistance and *E. coli* count in the STPs was found to be statically related. Previously, in case of the Zenne river of Belgium, the abundance of *E. coli* and antidrug resistance increased from upstream to downstream after merging the effluent from Brussel’s WWTP (Proia et al., 2018). Thus, proper and timely monitoring should be done to track such load of *E. coli* and ADR while discharging the treated effluents to the river water. From the current study, it is seen that the antidrug resistance to NFX, CIP, LVX, TCE and ST is found at most sampling locations. Such ADR generated during COVID_19 requires rigorous monitoring at local and international level through wastewater based epidemiology (Kumar et al., 2020b). However, the lack of sanitation and treatment facilities in the undeveloped and developing countries is a big challenge to monitor the spread of ADR in the environmental waters (Pormohammad et al., 2019). Perhaps the current pandemic may accelerate the upgradation of the current status of WWTP processes to tackle the pharmaceuticals and other antimicrobials successfully and to monitor ADR (Kumar et al., 2020a).

Fig. 5 highlights the statistical comparison of overall ADR in the year 2018 and 2020, whose causes are well described above. It is clearly seen that the mean percentage value of overall ADR was increased for the resistant strains of *E. coli* in the year 2020 than 2018, except in the case of kanamycin (remains nearly same). Whereas, the mean percentage

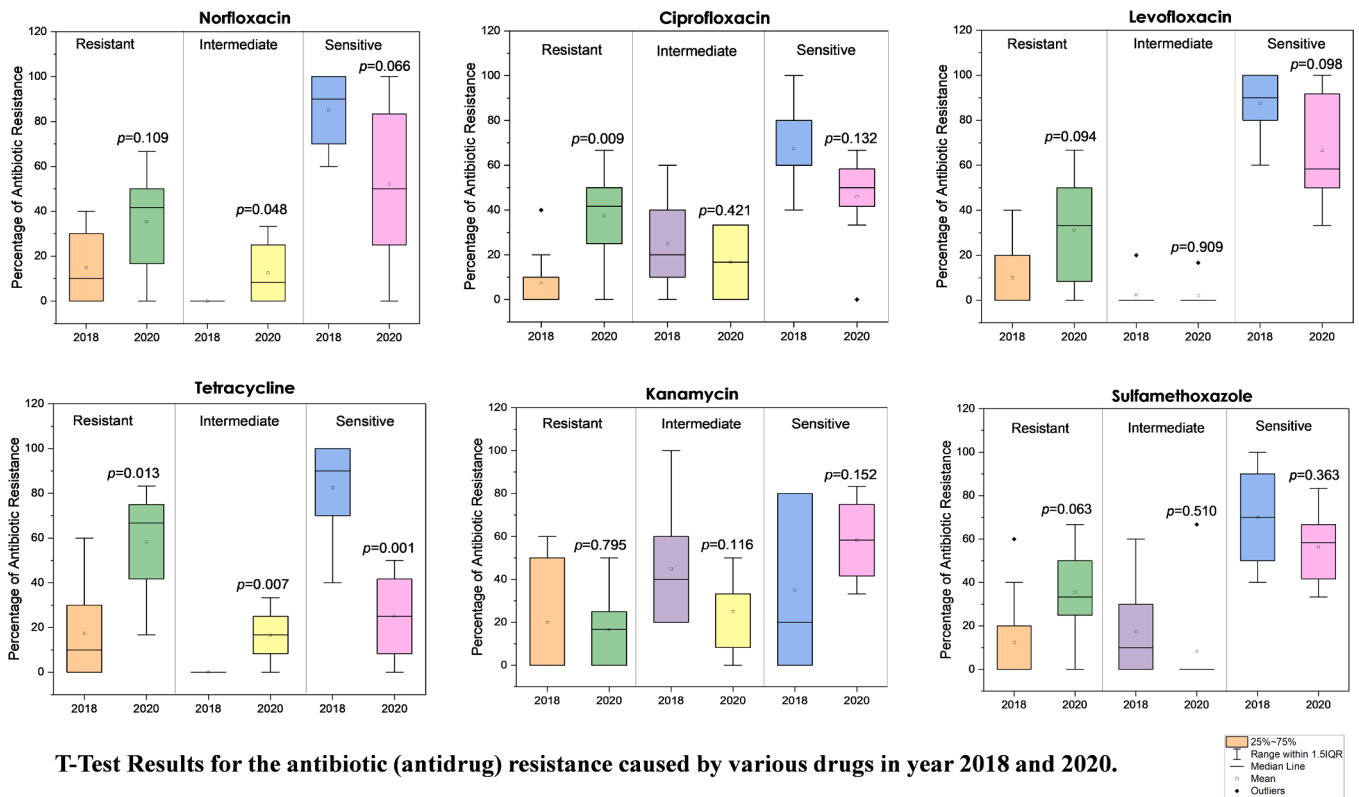


Fig. 5. Comparison of antibiotic (antidrug) resistance against various antibiotics in 2018 and 2020 with the results of a statistical T-test.

value of overall ADR observed to be decreasing for the sensitive strains of *E. coli* in the year 2020 than 2018, except in case of kanamycin (increases). The percentage of ADR (in resistant *E. coli* strains) for almost all antibiotics: CIP, LVX, TC, KM, ST (except NFX: 89.1% change), was

observed to be very significant in the year 2020 than 2018, as $p < 0.10$. This indicates that the significant change is occurring due to increase in the mean value of percentage of ADR. Overall, the comparison of overall ADR shows a significant increase statistically in the year 2020 than

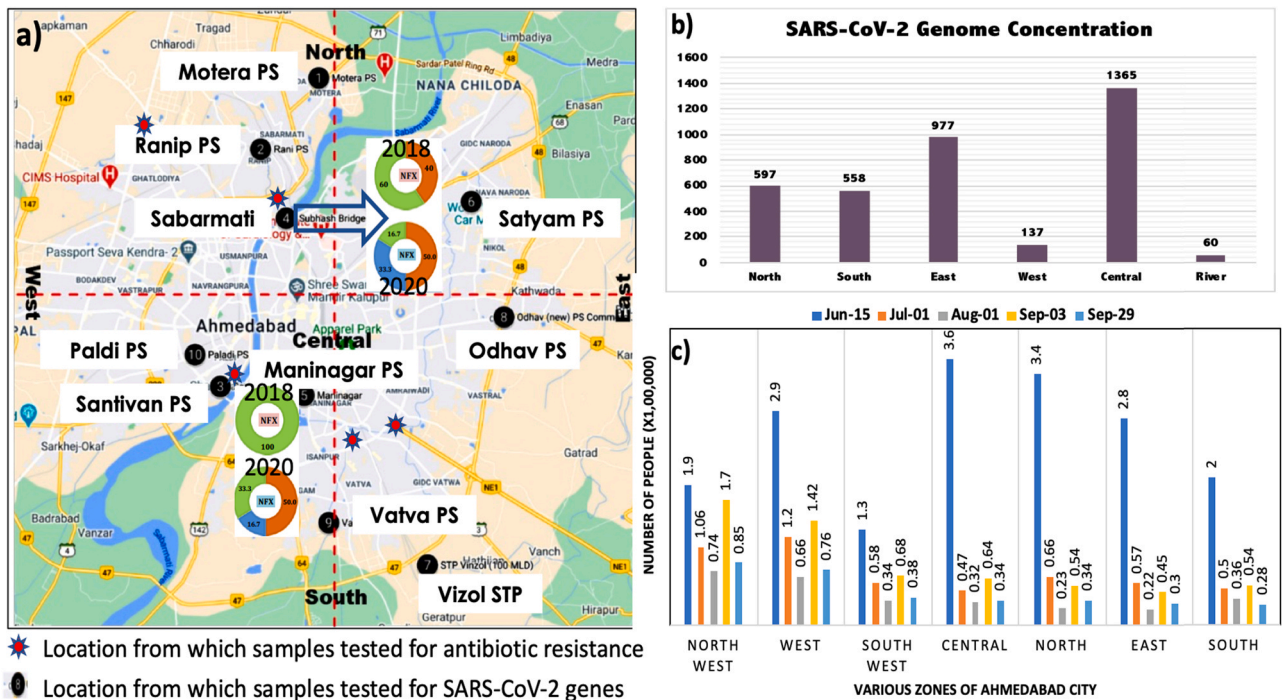


Fig. 6. Illustration depicting: a) zonation of Ahmedabad along with the sampling locations for SARS-CoV-2 RNA analyses, and antibiotic resistance bacteria (ARB) analyses. Two locations of the rivers i.e. SB and NB are shown with their increased resistance against norfloxacin between 2018 and 2020; B) Zone-wise scenario of effective SARS-CoV-2 genome concentrations (copies/L) measured in the samples and C) Number of people under threat of COVID-19 infection as predicted by Aarogya-setu application based on active cases reported and population density of a given area.

2018.

3.4. Imprints of COVID-19 spread over ADR distribution

The increased cases of COVID-19 is not surprisingly correlated to SARS-CoV-2 genes in waste and natural waters (Medema et al., 2020; Ahmed et al., 2020; Haramoto et al., 2020; La Rosa et al., 2020; Sherchan et al., 2020; Kumar et al., 2020; Nemudryi et al., 2020; Kocamemi et al., 2020). Fig. 6 represents the population under threat of COVID-19 in Ahmedabad city (as predicted by nationally authorised Arogya-Setu app), Zone-wise scenario of effective SARS-CoV-2 genome concentration (copies/L) in Ahmedabad city, sampling locations for SARS-CoV-2 RNA analyses, and ADR analyses. The population under threat of COVID-19 in various zones of the city has been predicted by Arogya-Setu app based on the confirmed cases and the population of the respective zone. Arogya-Setu is an authorised Indian COVID-19 contact tracing, syndromic mapping and self-assessment digital service provided under the Ministry of Electronics and Information Technology (MeitY), India. The sampling locations were chosen so as to cover various parts of the city. Two locations of the river i.e., SB and NB are shown with their resistance increase for Norfloxacin between 2018 and 2020. These two locations fall in the central zone of the city, which was highly affected by COVID-19, as can be seen from Fig. 6a and b. Table 2 summarises the status of the SARS-CoV-2 gene along copies with their corresponding Ct-values in the water samples collected from various parts of Ahmedabad, Gujarat on 15th October 2020. It also provides the effective genome concentration for the sampled locations. The genome concentrations were observed to be high in central, east, south and north zones of the city, which can be observed at Maninagar (1365 copies/L), Odhav PS (1070 copies/L), Satyam PS (885 copies/L), Vinzole STP (815 copies/L), and Ranip PS (714 copies/L). The sampled river location and the lake locations encompass in the same zones of the city. The high SARS-CoV-2 genome copies in these zones hint at the potential high prescription of antimicrobial drugs as a remedy to the symptoms of COVID-19. This can be the probable reason for a significant increase in ADR towards most of the drugs tested at the sampling locations in these zones. This indicates that the highly infected zones of the city, due to excessive consumption of antimicrobial drugs, have significantly impacted the antidrug resistance generated in the microorganisms. Overall, the spread of COVID-19 in the community has a prodigious correlation with the effective genome concentration of SARS-CoV-2 and with the prevalence of ADR in environmental waters.

4. Limitations

The present study compared the anti-drug resistance in *E. coli* in 2018 and 2020 with the latter prevalence of SARS-CoV-2 genes during the sampling period. Despite the correlations between increased ADR and COVID-19 spread, more future studies with rigorous sampling events are needed to conclude about the cause and effects. In addition, the

concentration of pharmaceutical and personal care products (PPCPs) in the ambient environment should be monitored to quantify their increase owing to COVID-19; and then connect back to the corresponding effect on ADR for quantitative evaluation. In this study, we attempted to start a timely discussion about the likely relationships between ADR and COVID-19 spread throughout the globe. Our approach to analyse the ADR prevalence is mostly qualitative and there may be a slight possibility of both false positive and negative results. To obtain the conclusive evidence, the quantification of genetic markers for antimicrobial resistance will be helpful. In addition, one time point data may be argued -inadequate to derive a conclusion especially when samples used for ADR and SARS-CoV-2 genomes studies do not match. Hence we recommend regular monitoring along the consideration of wastewater flow data for presenting gene flux or *E. coli* flux.

5. Conclusion

Non-fluoroquinolone drugs showed overall more resistance as compared to fluoroquinolone drugs. Tetracycline followed by norfloxacin has shown more resistance as compared to the other drugs. Despite a decrease in the prevalence of *E. coli* on the sampled river locations, the percentage resistance had been significantly increased in the year 2020 compared to year 2018. However, the *E. coli* prevalence in STP samples was increased in the order of 10^2 , but the pattern of antidrug resistance was not consistent. Lake locations also exhibited an increase in the antidrug resistance during the duration of pandemic. The river locations and the lake locations have shown a significant increase in the antidrug resistance, and these locations are from the highly COVID-19 infected zones of the city. The COVID-19 spread in various zones of the city has shown corresponding changes in the SARS-CoV-2 genome concentration and ADR in environmental waters. Overall, due to increased consumption of antimicrobials in the pandemic period, the percentage of antidrug resistance has been increased significantly. Wastewater based epidemiology can be the key tool to monitor the antimicrobials prevalence and antidrug resistance in the pandemic situations.

Notes

The authors declare no competing financial interest.

CRediT authorship contribution statement

Manish Kumar: Conceptualization, Visualization, Project supervision, Writing - review & editing; **Kiran Dhangar:** Data curation, First draft, Writing - review & editing; **Alok Kumar Thakur:** Sampling and analyses in 2020, Data curation, First draft, Writing - review & editing; **Bhagwana Ram:** Sampling and analyses in 2018, Writing - review & editing; **Tushara Chaminda:** Writing - review & editing; **Pradeep Sharma:** Writing - review & editing; **Abhay Kumar:** Writing - review &

Table 2

SARS-CoV-2 Ct-values along with their corresponding gene copies in the water samples collected from various parts of Ahmedabad, Gujarat on 15th October 2020. Effective genome concentrations have also been provided in the last column.

Sampling Station	Ct values			Gene copies/ L			Effective gene concentration
	N	ORF	S	N	ORF	S	
Motera PS	35.50	32.18	33.96	123	1002	317	480
Ranip PS	34.57	31.75	32.98	217	1334	591	714
Paldi PS	38.36	36.47	36.53	23	69	66	53
Santivan PS	36.08	33.63	34.80	87	390	187	221
Sanbarmati	38.46	35.67	37.14	22	110	47	60
Maninagar	34.17	30.77	31.89	278	2605	1213	1365
Satyam PS	34.52	31.37	32.70	223	1724	709	885
Vinzole STP	34.98	31.41	32.96	168	1680	598	815
Odhav PS	34.54	31.06	32.41	220	2131	857	1070
Vatva PS	38.51	32.58	35.69	22	770	109	300

editing; **Nirav Raval**: Writing - review & editing; **Vaibhav Srivastava**: Sampling and analyses, Data curation, First draft, Writing - review & editing; **Jörg Rinklebe**: Writing - review & editing; **Keisuke Kuroda**: Writing - review & editing; **Christian Sonne**: Writing - review & editing; **Damia Barcelo**: Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126125.

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