

# Bacterial load and drug resistance in sewage from industrially polluted regions of South Gujarat region

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**Abstract.** Wastewater of anthropogenic origin is known to harbor various bacteria that are known to be of potential risk to human health and environment. It is of utmost importance to monitor such water sources. Coliforms present in the sewage water samples of municipal sewage treatment plants located at three different places in the South Gujarat region (Surat, Navsari and Vapi) of India were analyzed for their coliforms load as well as tested for their drug resistance. Using cultivation-based techniques microbial load and drug resistance (Amoxicillin, Tetracycline, Ciprofloxacin, Erythromycin, Trimethoprim and Sulphamethoxazole) were analyzed. Water treatment statistically significantly decreased the bacterial load in Vapi and Navsari samples. The optical density of with and without antibiotics of all the three locations was shown to increase significantly after 72 hours. Of all the isolates tested, except isolate 'VA5' (resisted up to 90 µg of Ampicillin) all other isolates resisted 256 µg concentration of antibiotics tested. This study indicates that the sewage water is being contaminated with drugs and/or antibiotics due to industrial and/or anthropogenic activities. Regular monitoring of the water quality is required followed by implementation of environmental laws for reducing the pollutants, that are of human health and environment concern.

**Keywords:** antibiotics; coliforms; MDR; sewage treatment

## 1. Introduction

Rapid industrialization and urbanization are making water sources contaminated chemically and biologically. Due to such contaminated water, there were many outbreaks of diseases around the world (Stevens *et al.* 2003). Since last few decades, indiscriminate usage of antibiotics for human, animal husbandry and agricultural purposes led widespread emergence of multi-drug resistance (MDR) bacteria (Kardos 2017, Manyi-Loh *et al.* 2018). These changes instigated a major therapeutic challenge to understand their ecology and drug resistance mechanisms (Arias and Murray 2009). Eutrophication of rivers and overuse of antibiotics facilitate growth and survival of MDR bacteria (Verma and Rawat 2014, Klein *et al.* 2018, Singh *et al.* 2019). Such bacteria and their resistance genes were identified from various sources (drinking sources, hospitals, and other environments) including the fresh water (Schwartz *et al.* 2003, Nazaret and

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Aminov 2015).

A surge in antibiotic resistance bacteria and drug resistance genes in the environment were shown to cause by waste water from the effluent (Karkman *et al.* 2018, Manaia *et al.* 2018). Fecal bacteria and antibiotic residues were shown to be discharged from sewage treatment plants into the environment (Hendricks and Pool 2012).

Coliforms are a group of fecal bacteria belonging to *Enterobacteriaceae*. In a study involving coliforms as an indicator for diarrheal risk, *Escherichia coli* (a member of *Enterobacteriaceae*) was shown to be an indicator for fecal contaminations (Gruber *et al.* 2014). Coliforms are known to be part of intestinal micro-flora of humans and as well as warm blooded animals, they are also being used as an index for potential presence of entero-pathogens (Stevens *et al.* 2011). Traditionally microbially safe drinking water has been determined by measuring the coliforms and their members were known to exhibit multi drug resistance (Cooke 1979, Czekalski *et al.* 2012, Odonkor and Addo 2018). Such resistance to drugs is known to be correlated with heavy metal resistance, which can be attributed to co-resistance and cross resistance (Baker-Austin *et al.* 2006, Knapp *et al.* 2017). Usage of heavy metals in agricultural and veterinary applications rendered bacteria resistant to metals (Nicholson *et al.* 2003, Medardus *et al.* 2014). Previous studies have also reported the presence of metals in the aquatic sources/bodies (Shah *et al.* 2012, Sharma *et al.* 2014, Dubey and Ujjania 2015). Studies have been reported that members of *Enterobacteriaceae* were shown to be drug and heavy metal resistant (Cenci *et al.* 1982, Atieno *et al.* 2013, De La Rosa-Acosta *et al.* 2015). Coliforms detection and their defense using antibiotics became increasingly tough (Rompré *et al.* 2002, Andersson and Hughes 2014, Jassim and Limoges 2014).

Such increasing number of multidrug resistant organisms has become a serious concern for human health. Lee *et al.* (2016) and Sajidu *et al.* (2007) suggested that steps have to be taken in order to improve the physiochemical qualities of effluent water from the water treatment plants and as well as they should be regularly monitored in order to reduce the levels of MDR in such systems. According to World Health Organization, *Enterobacteriaceae* members such as *Escherichia Coli* and *Klebsiella Pneumoniae*, followed by other groups of organisms such as *Staphylococcus aureus*, *Mycobacterium Spp.* have become resistance to the current drugs that are used to treat these infections (<https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>). In a research study on methicillin resistant *S. aureus* secondary infection in Pneumonia infected patients, the cost of hospitalization was significantly higher their control group (Uematsu *et al.* 2016). In another study, in USA alone drug resistant bacteria caused a loss of more than 20,000 deaths and 2 million infections followed by an economic impact of 55–70 \$ Billion (Li and Webster 2018). These studies show the financial liabilities of drug resistance bacteria on our economy.

Gujarat Ecology Commission report in 2017-18 shows that South Gujarat region is the one of the most vulnerable regions for water borne diseases and as well as one of the most water abundant regions of Gujarat ([www.gujenvi.nic.in/PDF/soe-water.pdf](http://www.gujenvi.nic.in/PDF/soe-water.pdf)). Valsad and Surat districts of Gujarat were found to have largest number of polluting industries, co-incidentally Vapi is known to be one of the most critically polluted area of Gujarat is in Valsad district ([www.gujenvi.nic.in/PDF/soe-industrial.pdf](http://www.gujenvi.nic.in/PDF/soe-industrial.pdf)). It has also been noted that the disposal of untreated domestic and industrial effluents are the main causatives for water pollution in Gujarat ([www.gujenvi.nic.in/PDF/soe-industrial.pdf](http://www.gujenvi.nic.in/PDF/soe-industrial.pdf)). It is imperative for us to monitor microbial load and drug resistance in samples from such regions. In the present study, coliforms present in the sewage water samples of municipal sewage treatment plants located at three different places in the South Gujarat region (Surat, Navsari and Vapi) of India were analyzed for their microbial load as well as tested for their

Table 1 Antibiotics used in this study

S/No	Antibiotics
1	Amoxicillin (20 mg/L)
2	Tetracycline (30 mg/L)
3	Ciprofloxacin (5 mg/L)
4	Erythromycin (15 mg/L)
5	Trimethoprim and Sulphamethoxazole (2 mg/L and 24 mg/L)

drug resistance.

## 2. Materials and methods

For the growth of bacteria Eosin Methylene Blue (EMB) agar (selective media for Coliforms), Nutrient Agar (NA) and Nutrient Broth (NB) (general media) was procured from Himedia, Mumbai, India. The antibiotics (AB) that were used in this study were procured from the local pharmacy unless or until specified (Table 1). Heavy metal salts in this study were obtained from SD Fine, Mumbai. Bacteria was grown at 37°C until or unless specified.

### 2.1 Collection of water samples and physio-chemical characterization

Sewage water samples were collected from three municipal sewage treatment plants of South Gujarat region namely Surat, Vapi and Navsari. The samples of influent and effluent were collected in a sterile one-liter bottles and were analyzed at Mahuva Sugar Factory (Mahuva, Surat (Dist), India) for various physio-chemical parameters such as pH, Electrical conductivity, total Chlorine, total Calcium and Magnesium followed by total Bicarbonate by standard procedures. Heavy metals (Silver, Copper, Iron and Nickel) in the sample were estimated using Inductively Coupled Plasma Atomic Emission Spectroscopy at Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology, Mumbai, India.

### 2.2 Isolation and characterization of multiple drug resistant coliforms

Bacteria were enumerated and isolated using EMB agar and NA. The antibiotics were added and the isolates were annotated as resistant or intermediate or susceptible based on the CLSI (Clinical and Laboratory Standards Institute, USA) standards (Table 1). Heavy metals (HM) used in this study was of 0.05 mg/L as its final concentration (Table 2). Bacteria were incubated at 37°C.

#### 2.2.1 Effect of water treatment on cultivable bacteria

To check the effects of treatment on bacteria (cultivable), water samples were serially diluted ( $10^{-2}$ ) from both influent and effluent were plated. 100  $\mu$ L of water sample was spread on to EMB agar plate. Plates were incubated at 37°C for 24 hours and bacterial counts were expressed as Colony Forming Unit (CFU).

Table 2 Heavy metals used in this study

No	Metal	Chemical used
1	Silver	AgNO <sub>3</sub>
2	Nickel	NiCl <sub>2</sub>
3	Copper	CuSO <sub>4</sub> .5H <sub>2</sub> O
4	Iron	Fe <sub>3</sub> O <sub>3</sub>

### 2.2.2 Growth pattern along time scale

Serial diluted ( $10^{-2}$ ) 0.5 mL of water sample from both influent and effluent was inoculated into 4.5 mL sterile NB into the test tubes. The tubes were added with antibiotics followed by its control without antibiotics. All tubes were incubated at 37°C and optical density (OD) value (A600) was recorded for every 24 hours for up to three days (72 hours). Water samples of influent were processed for the fourth day also.

### 2.2.3 Growth curve of MDR isolate

Based upon the colony morphology, the isolate that exhibited green metallic sheen on EMB agar was considered as *Escherichia* Spp isolate. As the growth pattern (2.2.2) was different in presence of antibiotics, we have tested whether the growth curve of MDR is same as the non-MDR (control organism was procured from ATCC culture). The MDR isolate (*Escherichia* Spp looking like) was compared with the *Escherichia coli* (IMTECH, Chandigarh, India) as a control. Drug susceptibility of the control *E. coli* was tested and found to be susceptible (Hexa discs (Himedia, Mumbai, India)) to the drugs tested in this study. Both the organisms were grown in NB at 37°C. Growth curve of both the bacteria was plotted. The MDR isolate was later identified as *Escherichia coli* by 16S rRNA sequencing.

### 2.2.4 Relation of colony forming units and optical density

To test whether the CFU count correlates with optical density, simultaneously both agar and broth-based analysis was setup. They both were added with the antibiotics (AB) and heavy metal (HM) tested in the study. Serial dilution ( $10^{-2}$ ) of 0.5 mL of water sample from effluent was inoculated into 4.5 mL of sterile NB test tubes. The tubes were added with antibiotics and heavy metal followed by its control without antibiotics and heavy metal. All tubes were incubated at 37°C and OD value (A600) was recorded after 48 hours. Simultaneously, 100 µL of water samples from the same dilution tube was spread onto EMB plates with antibiotics and heavy metal salt individually and including a combination of both (heavy metal and antibiotics), and as well as their controls (without antibiotics and heavy metals). Plates were also incubated at 37°C for 48 hours and bacterial counts were expressed as Colony Forming Units. Morphologically different colonies were picked up from the plates with antibiotics and heavy metal. The isolated colonies were maintained on NA and NB with antibiotics and heavy metal. Bacterial colonies were isolated from the antibiotics and heavy metal treatment were also tested individually against the heavy metals and antibiotics tested in this study (data not presented). Morphologically different colonies were isolated and tested further.

### 2.2.5 Determination of the antibiotic resistance concentration

The isolates (resistant to drugs and heavy metals) were tested once again using Hexa discs

Table 3 Physicochemical parameters of the water samples

Location	Treatment	pH	EC (Mili moss/Cm)	Cl (Mili equivalent/L)	Ca+Mg (Mili equivalent/L)	HCO <sub>3</sub> (Mili equivalent/L)
Vapi	Influent	5.8	6.76	32.4	32.4	1.2
	Effluent	7.5	9.35	51.2	39.6	22.8
Navsari	Influent	7.24	0.5	2.8	5.2	3.2
	Effluent	7.51	0.38	2.4	6.8	3.6
Surat	Influent	6.9	1.26	6	7.2	9.6
	Effluent	6.7	0.72	4	6.8	10

(Himedia, Mumbai) to further validate their resistance property to the drugs tested, including other drugs and antibiotics (evaluated based on CLSI). These isolates were also tested for their efficacy against the various antibiotic concentrations, tested using Hi-COMB tests (Himedia, Mumbai, India) and the results were interpreted as mentioned in the manufacturer instructions. The antibiotics were of Amoxicillin, Ciprofloxacin, Erythromycin, Tetracycline and Trimethoprim and of concentrations ranging from 0.01 to 256 µg.

### 2.3 Data and statistical analysis

All the experiments were performed in triplicates. The data given in the graphs were the average with their standard errors (when represented) using MS-Excel 2013. Statistical significance (Analysis of Variance (ANOVA)), Non-metric dimensional Scaling analysis (NMDS) and Analysis of similarities (ANOSIM) was performed using PAleontological STatistics (PAST, Ver 3.26).

## 3. Results

### 3.1 Physicochemical characterization

Water samples of influent and effluent from municipal water systems from three different places Surat, Vapi and Navsari were analysed for their physicochemical properties (Table 3).

The heavy metals tested were shown to exceed the permissible limits (refer to table S1) in the samples. There was not much significant difference in most of the parameters except bicarbonate, followed by Chloride content and 'Ca+Mg' content. Vapi sample showed a significant increase in the bicarbonate content (from 1.2 to 22.8 Milli eq/L) effluent, but Surat and Navsari did not show much difference. Vapi sample physicochemical properties was slightly different than the other two samples.

### 3.2 Effect of water treatment on cultivable bacteria

In all the samples tested the water treatment has reduced the number of cultivable bacteria indicating the effective treatment (Fig. 1).

Navsari sample showed the highest number of bacterial CFUs ( $175 \pm 14$ ) followed by least in the

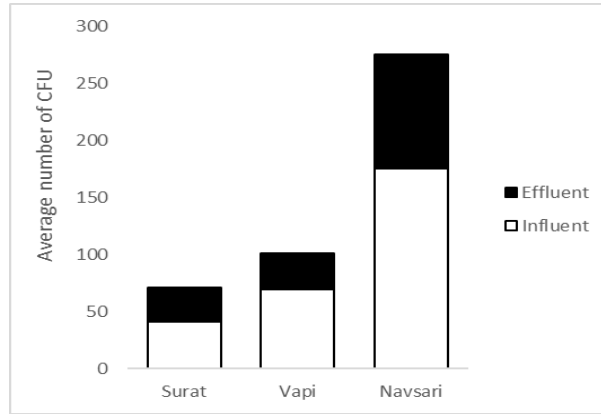


Fig. 1 Effect of water treatment on the CFU count of bacteria as observed from Surat, Vapi and Navsari sewage treatment plants

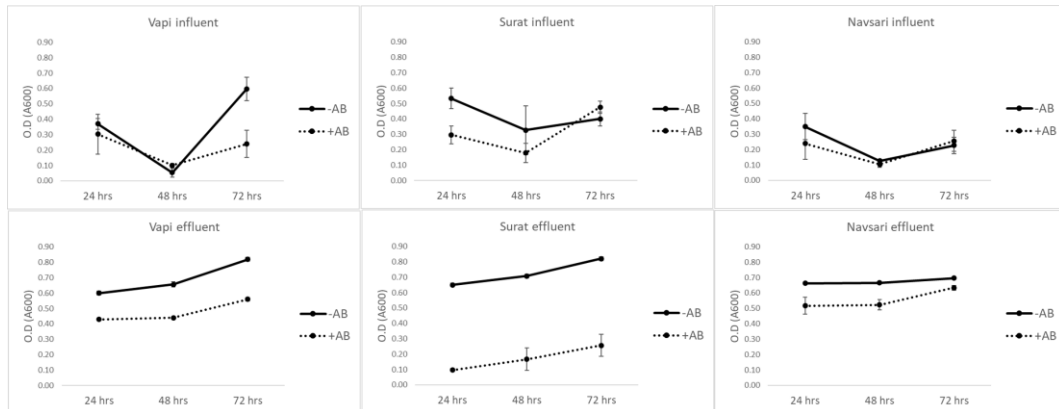


Fig. 2 Optical densities (A600) along the time scale, as observed in presence of antibiotics (+AB, dotted) and in absence of antibiotics (-AB, non-dotted) in the samples from Surat, Vapi and Navsari. The graphs above are influent and the graphs below are effluent

Surat sample ( $41 \pm 3.5$ ). There was a statistically significant difference of the treatment in the CFU counts of Vapi ( $p=0.01$ ) and Navsari ( $p=0.041$ ) but non-significant in the Surat sample.

### 3.3 Growth pattern test

There was a clear contrast between test and control treatments optical density of effluent samples, but such contrast was absent in influent (Fig. 2). Most of the influent samples showed a decrease in their optical density after 24 hrs. Vapi sample showed statistically significant decrease OD in test treatments ( $p=0.01$ ) at 72 hours.

Such difference was not evident in any other time period (24 and 48 hours) nor in 'Surat' and 'Navsari' sample. In influent samples both test and control treatments showed an increase in the OD at 96 hours from all the sample places (Surat, Vapi and Navsari) (refer to Fig. S1). This increase was a statistically significant ( $p \leq 0.0001$ ) increase when compared with other time periods (24, 48 and 72 hours). But whereas in effluent samples most of them showed an increasing trend in

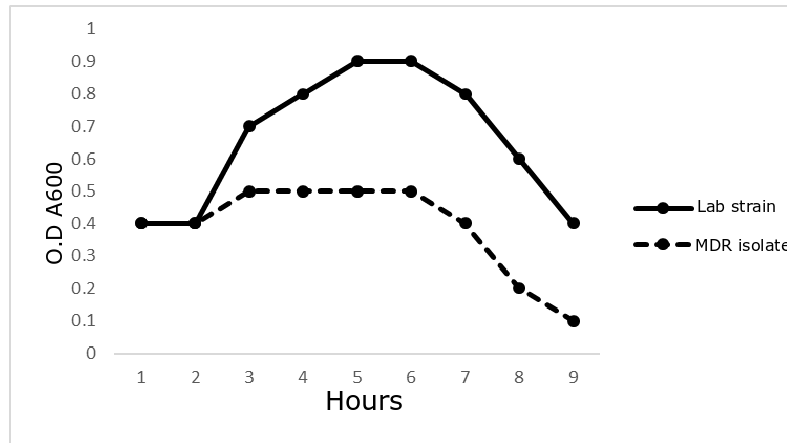


Fig. 3 Growth curve of MDR isolate as compared to the lab strain (Control, *E. Coli*)

their optical density after 24 hours which contrasts with influent samples. Surat effluent sample was found to show a statistically significant difference in between test and control samples ( $p=0.0001$ ) in all the three time periods (24, 48, 72 hours).

### 3.4 Growth curve of MDR isolates

The growth curves of the MDR isolate and lab strain differed significantly long the time scale (Fig. 3).

MDR isolate was shown to have a typical stationary phase after a short log phase pattern. Both the cultures behaved in the same manner during the lag phase but differed in the remaining three phases of growth (logarithmic, stationery and decline).

### 3.5 Correlation between colony forming units and optical density

Effluent samples were tested for Optical density and CFUs in both control and test (in presence of AB, HM and AB+HM) treatments (Fig. 4).

The number of CFUs were statistically significantly decreased in test treatment than in the control treatment ( $p\leq 0.0001$ ). Similarly, in the 'control' samples there was a statistically significant difference ( $p\leq 0.0001$ ) in CFU enumeration of all the three samples. There was a significant ( $p\leq 0.01$ ) decrease in the CFU of 'Navsari' sample in comparison with 'Surat' and 'Vapi' both in 'Test (AB)' and 'Control' treatments. But whereas no such clear significance was evident in other 'Test' treatment (HM and AB+HM) CFU. Optical density was statistically significantly decreased in 'Test' treatment (AB, HM, and AB+HM) than in 'Control' treatment ( $p\leq 0.0001$ ), but whereas no such statistical significance was observed in between the control treatments of three samples. The optical density of 'Navsari' test samples (AB, HM, and AB+HM) were shown to be statistically significantly decreased ( $p\leq 0.0001$ ) in comparison with other samples (Surat and Vapi).

The OD in 'AB test' treatment of Surat showed a statistically significant ( $p\leq 0.008$ ) decrease in comparison with the other two samples (Navsari and Vapi). But such significance was absent in

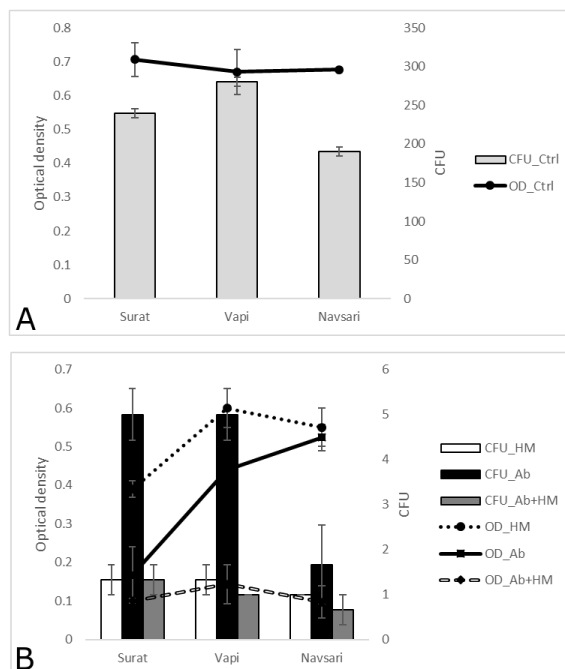


Fig. 4 Relation of Colony Forming Units (CFU) and Optical Density (OD) in control (a) and test (b) samples. The test samples include treatments, with Antibiotics (AB) and Heavy metals (HM)

‘HM test’ and ‘AB+HM test’ treatment samples. The morphologically different isolates that were observed in both antibiotics and heavy metal inoculated plates (AB+HM) were further screened for resistance against various concentrations of the antibiotics and were sent to sequencing for further identification.

### 3.6 Determination of the antibiotic resistance concentration

A total of five isolates (morphologically different) from each sample (Surat, Vapi and Navsari) of antibiotics and heavy metal added (AB+HM) test treatments were tested using Hi-COMB tests. Except isolate ‘VA5’ (resisted up to 90  $\mu\text{g}$  of Ampicillin) all other isolates resisted 256  $\mu\text{g}$  concentration of antibiotics tested.

### 3.7 Heavy metal and drug/antibiotic resistance effect on samples

Non-metric dimensional scaling was performed to see the overall effect on the samples based upon the various variables that were tested in this study (Fig. 5). There was more effect of treatments tested in comparison with geographical location of the samples (ANOSIM  $p=0.0002$ ).

### 3.8 Identification of the bacteria by 16S RNA sequencing

Out of 15 isolates that were sent to GSBTM only nine were identified and remaining isolates could not be amplified. The isolates were identified as in Table 4.



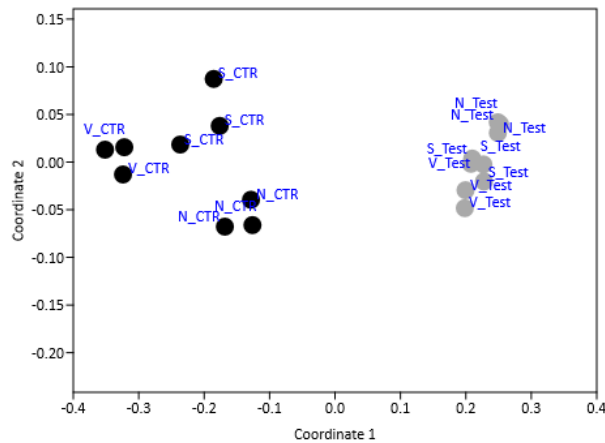


Fig. 5 Effect of water treatment on the CFU count of bacteria as observed from Surat, Vapi and Navsari sewage treatment plants

Table 4 Isolates identified by GSBTM, Gujarat

No.	Sample ID	Organism name	% Identity
1	N(A)1	<i>Shigella sp.</i>	99%
2	N(A)2	<i>Escherichia fergusonii</i>	99%
3	N(B)3	<i>Escherichia coli</i>	99%
4	N(B)5	<i>Escherichia fergusonii</i>	99%
5	S(A)3	<i>Enterobacter cloacae</i>	99%
6	S(A)10	<i>Enterobacter cloacae</i>	100%
7	V(A)5	<i>Enterobacter Spp.</i>	99%
8	V(A)6	<i>Escherichia coli</i>	99%
9	V(A)7	<i>Shigella Spp.</i>	99%

#### 4. Discussion

The present study involves the analysis of water samples for multi drug and heavy metal resistance *Enterobacteriaceae* members in waste water systems. Various studies have been published and multi drug resistance bacteria have been identified in similar environments (Cooke 1979, Akhter *et al.* 2014, Gruber *et al.* 2014). In aquatic systems species belonging to *Enterobacteriaceae* have been focused, as they are known to play an important role in human health (Gruber *et al.* 2014, Maloo *et al.* 2014). Some of these studies have also focused on the physiochemical properties of the water systems including the bacteria.

In this study, physiochemical parameters were shown a decreased trend effluent, especially in bicarbonate parameter. Similar results were obtained when effectiveness of New Delhi (India) sewage plants were analyzed with respect to bicarbonate (Gautam *et al.* 2013). Gujarat Ecology Commission report of 2017-18 of water pollution states that, overall BOD and COD are gradually decreasing and are under the prescribed limit; but the presence of total coliforms and total dissolved solids were found to be exceedingly high in some of the samples tested ([www.gujenvi.nic.in/PDF/soe-industrial.pdf](http://www.gujenvi.nic.in/PDF/soe-industrial.pdf)). Not only abiotic the biotic component was also shown to be affected by the treatment. The total bacterial count was shown to decrease after the

water treatment (Bréchet *et al.* 2014, Osińska *et al.* 2017). In the present study CFU were shown to decrease in effluent from all the three locations, especially in Navsari sample. Water treatment plants are known to reduce the enterococci populations in between 0.5 to 4 log (Martins da Costa *et al.* 2006), but a study on *E. coli* in sewage treatment from Southern Austria that evaluated drug resistance patterns without the evaluation of basic resistance mechanisms, showed an increase in their numbers in effluent samples (Reinthaler *et al.* 2003).

Czekalski *et al.* (2012) has reported that there was an increase in the MDR strains and accumulation of resistance genes after waste water treatment. An increase in the faecal bacteria and antibiotic residues was shown in the treated sewage samples, indicating the need to evaluate the treated samples more than the before treated samples (Hendricks and Pool 2012). Multi drug resistant *Enterobacteriaceae* members were detected in this study. Similarly such organisms were also found when water samples from five different drinking water distribution systems in Mafikeng of South Africa were analyzed for fecal and total coliforms (Mulamattathil *et al.* 2014). An increase in the optical density after 48 hrs of growth of all the three samples (both in influent and effluent samples) tested both in presence and absence of antibiotics was observed. This may be attributed to the nutrient release due to the death of bacterial cells and utilization of such nutrients by the survival cells. Navarro Llorens *et al.* (2010) has also expressed similar idea of survivor cells in the stationary phase which utilize the nutrients that are released into the medium due to the death of the cells. This increase in number of cells can also be due to the appearance of mutants with an improved fitness to scavenge the nutrients better than the parental strain, within the bacterial populations that are derived from multiple population shifts within the same culture (Navarro Llorens *et al.* 2010).

Extensively drug resistant and susceptible *Mycobacterium* were shown to have no significant difference in the lag phase of growth (Naidoo and Pillay 2014). Similarly, in the present study, no difference was observed in the optical density during the lag phase; but at the latter growth stages (after 2hours) there was a significant difference in between drug resistance and susceptible Coliforms tested. Naidoo and Pillay (2014) reported that MDR *Mycobacterium tuberculosis* strains showed an increased optical density, but a reduced CFU along the time scale. Similarly, in the present study there was a reduction of CFU and an increase in the OD, especially in the 'Navsari' sample was observed; in contrast to the earlier observation 'Surat' sample showed a lesser OD with a greater CFU. In this study, resistance against the individual antibiotics tested were evaluated and only two were shown (Amoxicillin (individual) Trimethoprim and Sulphamethoxazole (combinational)) to have highest number of resistant coliforms (data not shown). The isolated organisms were also shown to resist antibiotics such as Gentamycin, Tobramycin, and others (data not shown). Not only drug resistance but also heavy metal resistance was shown by the bacterial isolates. Many studies indicated that the heavy metal resistance induces co-resistance to various drugs or antibiotics (Baker-Austin *et al.* 2006, Seiler and Berendonk 2012, Wales and Davies 2015, Nguyen *et al.* 2019).

Studies have also been reported that MDR coliforms exhibited resistance to metals such as mercury, copper, cobalt, zinc and nickel (Atieno *et al.* 2013, Wales and Davies 2015). Bacterial isolates resistant to copper were shown to be resistant to tetracycline, olaquinox, nalidixic acid, ampicillin, sulphonamides and chloramphenicol (Berg *et al.* 2010). Hölzel *et al.* (2012) expressed that exposure to copper co-selects drug resistance in the bacterial community. Similarly, the bacteria isolated in this study showed resistance to copper in addition to other heavy metals and as well as to ampicillin, tetracycline including other antibiotics tested. Isolates in this study resisted to more than 250 µg of tested antibiotic concentrations, except one isolate that resisted up to 90 µg

only. Akhter *et al.* (2014) showed that *E.coli* isolates that are isolated from Gomti river of Gujarat (India) resisted up to 100 µg of antibiotics tested (Amoxicillin and Erythromycin) followed by *Enterobacter* Spp. up to 40 µg of Penicillin.

## 5. Conclusions

The present study indicates the presence of antibiotic resistant coliforms in the sewage treatment facilities, especially in the effluent water samples indicates a significant risk to the public health. It is imperative for us to monitor drug resistance in such systems, especially due to anthropogenic activity. There was an effect of water treatment on the number of CFUs of coliforms of influent and effluent samples. The presence of antibiotics, sample site and sample (influent and effluent) influenced the growth (optical density) of bacteria. The optical density is not directly related to the CFU. The MDR coliform has a different growth pattern in comparison with its control. As suggested by previous studies (Lee *et al.* 2016, Sajidu *et al.* 2007) we need to take concrete steps in order to improve the physiochemical qualities of effluent water from the water treatment plants and as well as they should be regularly monitored for MDR bacteria. Furthermore, such studies should be validated further using molecular studies in monitoring the dynamics of drug resistance in coliforms. This work may be relevant in designing strategies to prevent outbreak of potential disease outbreak and control of spread of multi drug resistance.

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Table S1 Amount of Heavy metals present in the Sewage water of effluent estimated by ICP-AES, IIT Mumbai, India

No	Sample location	Ag (ppm)	Cu (ppm)	Fe (ppm)	Ni (ppm)
1	Surat	75.194	1148.6	1079.8	328.66
2	Vapi	237.83	1147.3	807.41	> 1974.61
3	Navsari	0.242	1168.7	407.65	991.52

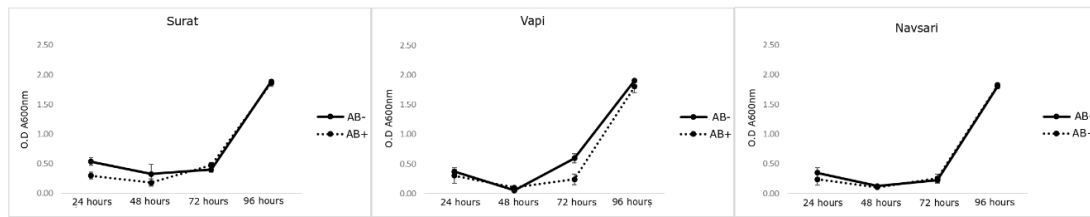


Fig. S1 Optical densities (A600) along the time scale, as observed in presence of antibiotics (+AB) and in absence of antibiotics (-AB) in the samples from Surat, Vapi and Navsari of influent sample