Title: Extended ecological restoration of bacterial communities in the Godavari river during the COVID-19 lockdown period - a spatiotemporal meta-analysis

Authors: Kunal Jani[1], Jayshree Bandal[2], Yogesh Shouche[1], Shuja Shafi[3], Esam I. Azhar[4], Alimuddin Zumla[5], Avinash Sharma[1*]

Institutional affiliations:

[1]DBT-National Centre for Cell Science, Pune, India.

[2]Department of Microbiology, KTHM College, Nashik, India

[3]Mass Gatherings and Global Health Network, London, United Kingdom.

[4]Special Infectious Agents Unit, King Fahd Medical Research Center, and Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.

[5]Centre for Clinical Microbiology, Division of Infection and Immunity, University College London, and NIHR Biomedical Research Centre, UCL Hospitals NHS Foundation Trust, London, United Kingdom.

***Correspondence**

Dr. Avinash Sharma
Scientist 'D'
DBT/Wellcome Trust Fellow, National Centre for Cell Science, Pune, Maharashtra 411007, India. Fax No: +91-20-25329001 Telephone No: +91-20-25329028
E-mail: avinash.nccs@gmail.com; avinash@nccs.res.in

Abstract

The unprecedented COVID-19 pandemic has had major impact on human health worldwide. Whilst national and international COVID-19 lockdown and travel restriction measures have had widespread negative impact on economies and mental health, they may have beneficial effect on the environment, reducing air and water pollution. Mass gathering religious bathing (MBE) events such as the Kumbh Mela are known to cause perturbations of the ecosystem affecting resilient bacterial populations within water of rivers in India. Lockdowns and travel restrictions provide a unique opportunity to evaluate the impact of minimum anthropogenic activity on the river water ecosystem and changes in bacterial populations including antibiotic resistant strains. We performed a spatiotemporal meta-analysis of bacterial communities of Godavari river, India. Targeted metagenomics revealed 0.87-fold increase in the bacterial diversity during the restricted activity of lockdown. A significant increase in the resilient phyla viz. *Proteobacteria* (70.6%), *Bacteroidetes* (22.5%), *Verrucomicrobia* (1.8%), *Actinobacteria* (1.2%) and *Cyanobacteria* (1.1%) was observed. There was minimal incorporation of allochthonous bacterial communities of human origin. Functional profiling using imputed metagenomics showed reduction in infection and drug resistance genes by -0.71-fold and -0.64-fold, respectively. These observations may collectively indicate the positive implications of COVID-19 lockdown measures which restrict MBE, allowing restoration of the river ecosystem and minimize the associated public health risk.

Keywords: COVID-19, Lockdown measures, Kumbh Mela, Bacterial populations, Antibiotic resistance, Public health, Targeted metagenomics

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), is a novel beta-CoV group 2B zoonotic infection of humans first identified in December 2019 from a cluster of human Coronavirus disease-19 (COVID-19) cases in Wuhan, China[1, 2]. The ensuing COVID-19 global pandemic has severely impacted the healthcare systems worldwide, causing ~3.39 million deaths as of 19th May, 2021 [2-4]. World leaders responded by imposing restriction on the movement of people, lockdowns and implementation of public health measures including social distancing, hand washing and wearing of face masks[5]. India was not an exception and imposed its nationwide lockdown on March 25th 2020. Considering, India's population and the population density an event of community transmission might have led to a havoc [6]. Most countries have implemented lockdown measures with national and international travel restrictions, which have affected all facets of life globally with major negative social and economic consequences [7]. On the other hand, the reduced movement of humans may have played a role in cleansing and restoration of environment, biodiversity and ecosystems that were under the perpetual anthropogenic stress [8]. Hence, it became important to evaluate the implications and/or beneficial impact of COVID-19 pandemic on the environment [9].

A recent study by Somani et al. [6], detailing the comprehensive analysis of various pollutants suggested, positive implications of restricted anthropogenic activities during the COVID-19 lockdown, particularly, on the quality of water, level of air pollution, noise pollution and waste generation, etc. Restricted movement and lower traffic were also found to aid to drop in the levels of nitrogen dioxide and noise pollution [10]. Similarly, a review by Rume and Islam [9] on environmental implications of COVID-19 underscores the improved quality of air with the reduction in emission of greenhouse gases and decrease in water pollution across the globe. Additionally, the review also highlights the plausibility of ecological restoration especially at the tourist spots. In Indian, the concentrations of pollutant such as $PM_{2.5}$, PM_{10}, O_3, NO_2, SO_2 and CO were consistently noted to be higher than its permissible limits but during the course of lockdown it was found to under the permissible limits (with some occasional spikes) in the various megacities [5, 11-12]. Additionally, the stringent lockdown had its contribution in cleansing the rivers and other aquatic ecosystem which are otherwise under the continuous burden of discharges from untreated sewage and industries [13-15]. Surprisingly, in the matter of weeks of lockdown the water quality of longest river of India i.e., Ganga, was more pronounce than the collective efforts of more than a decade [16-18]. Singhal and Matto [17], highlighted that the sudden decrease in the number of pilgrims and 500% decrease in the introduction of domestic and industrial effluents has led the improvise the water quality. Similarly, in case of rivers Yamuna, Cauvery and Krishna Central Pollution Control Board [19] reported concentrations of various factors like dissolved oxygen (DO), pH and coliform count to be under the permissible limits during the lockdown. Alternatively, the COVID-19 was associated with the inevitable situation of generation of enormous biomedical waste. Ineffective disposal of the biomedical waste was related to the environmental hazard especially affecting the water bodies [9]. The latter was also supported by a case study from Bangladesh indicating responders suffering effective disposal of COVID-19 preventives, leading to contamination of the water bodies [20].

Apart from the factors known to influence the water pollution, in India the recurring pilgrimages that are hosted on the banks of the river are source of unique perturbations to the aquatic ecosystem [21]. One such pilgrimage is the Kumbh Mela, the world's largest religious mass gathering event hosted along the banks of holy rivers in India, and it attracts millions of pilgrims from India and other countries [22-24]. The Kumbh Mela involves the ritual practice of bathing in the river which substantially increases the risk associated with the spread of water infectious diseases among the fellow pilgrims [23], and also causes perturbations of the ecosystems. Our spatiotemporal investigation of the river Godavari during the 2015 Kumbh Mela event by using bacterial communities as a proxy revealed the deleterious impact of the mass bathing event (MBE). It was observed to be associated with the loss of diversity, with invasive establishment of allochthonous bacterial communities, enormous introduction of human faecal microbiota and, increased virulence and drug resistance genes [25].

The COVID-19 lockdown measures, travel restrictions and associated scaling down of mass gathering religious events over the past year provide a unique opportunity to evaluate the impact of minimum anthropogenic activity on the river water ecosystem. Recent studies on the river Ganga found reduction in the biological oxygen demand (BOD), faecal coliform count, nitrate (NO3) concentration and increase in dissolved oxygen during the lockdown [26, 27]. However, both the studies primarily focus on the quality of the river water and adopted a classical approach of assessment of physiochemical factors and microbial contaminants, and investigation was restricted to the faecal coliform and total coliform counts [26]. However, it fails to provide insight into the bacterial communities thriving in the environment at given time and space. As the bacterial communities are sensitive to subtle variations in their environments, it can servers a proxy to delineated the extend of ecological disturbances or restoration, if any

[28]. In the recent year, the next generation sequencing has widely been used and have proven its competency in determining such anomalies associated with changing environment or in human health [29].

Therefore, the current study attempts to elucidate the implication of COVID-19 lockdown on the environment by analysing change in the river bacterial communities. Using targeted metagenomics, we performed a spatiotemporal and meta-analysis of bacterial community structure and composition of Godavari river, India during the COVID-19 lockdown period 1) to understand the impact of COVID-19 mediated restrictions on the river ecosystem, and 2) to examine the extent of ecosystem restoration, if any.

2. Material and methods

2.1. Sampling location

The current study is an extension of the spatiotemporal investigation of the river bacterial communities under the influence of the mass bathing event at Nashik Kumbh Mela 2015 [25], and it involved sample collection from same sites to reduce experimental heterogeneity. Five different sampling sites were utilised during the 2015 event [25] representing upstream sites (low human interference) and mass bathing sites (higher human activity). Water samples were collected in triplicate from the mass bathing sites located in the Nashik city (Anandvalli, Gharpure ghat, and Tapovan) during the lockdown period (June 2020) implemented as a measure of containment of COVID-19 spread. However, due to travel restrictions imposed due to COVID-19 pandemic, samples were not collected from the upstream sites located in '*Trimbakeshwar'* near the '*Anjaneri'* forest. The samples collected during the lockdown period were termed as LS3, LS4 and LS5 corresponding to sampling site Anandvalli, Gharpure ghat, and Tapovan, respectively (Figure 1).

2.2. Sample collection and processing

Surface water samples were collected in triplicates from the defined bathing areas located at the river bank. The samples were stored in the sterile containers and transported to the laboratory by maintaining the cold chain. Each sample aliquot was subjected to physicochemical analysis at the Department of Microbiology, KTHM College, Nashik. Environmental parameters such as turbidity (OD), total dissolved solid (TDS), total solids (TS), total suspended solids (TSS), dissolved oxygen (DO), biological oxygen demand (BOD), Fecal coliforms, and MPN of total coliforms were assessed as per the procedures of APHA (1998) whilst pH (Pocket digital pH meter, Milwaukee pH 600) and temperature (Vintage USA E8000) were recorded onsite. The remaining water sample was filtered through 0.22µ Millipore filters (Merck Millipore, USA) and stored at -20°C until further processing.

2.3. High-throughput sequencing and data analysis

Community DNA extraction was carried out from the stored 0.22µ Millipore filters using DNeasy Power Water Kit (Qiagen, The Netherlands). The qualitative and quantitative assessment of the DNA was performed in accordance with the Sharma et al. [30]. The DNA was processed for the targeted amplicon sequencing by amplification of the V4-hyper variable region of the 16S-rRNA gene using primers 515F-806R [31]. Library preparation was performed by following the protocols of Illumina Inc (USA). The obtained libraries were sequenced on Illumina Miseq using 2x250bp v2 chemistry. The sequences are available on the NCBI SRA portal under BioProject Id PRJNA698474.

In order to maintain the uniformity, the amplicon sequences generated during the Kumbh Mela 2015 [25] study were retrieved prior to analysing the current sequencing data (BioProject Id PRJNA383664). The bioinformatics analysis was in accordance with Jani et al. [25] which involved analysis using QIIME (v1.9) (Quantitative Insights into Microbial Ecology) [32]. Assembly of the paired-end reads using FLASH tool (Fast Length Adjustment of Short reads), followed by adaptor and quality-filtering using Cutadapt (v3.2) and MOTHUR (v1.32), respectively [33-35]. The OUT-picking (operational taxonomic units) was performed using closed reference approach against the Greengenes database with UCLUST algorithm with sequence similarity of 97% [36, 37]. Taxonomic assignment of the OTUs was performed by adopting RDP naïve Bayesian classifier against the Greengenes database [38]. Further, downstream analysis precludes sequence rarefaction to the lowest number of reads per sample. Estimates of the alpha diversity indices viz. OTU richness, Shannon, Chao1, and Good's coverage was carried out using QIIME (v1.9). Statistical analysis and data visualization was performed in R using the packages cowplot, corrplot, Hmisc, PerformanceAnalytics, phyloseq, vegan and ggplot2 [39- 45].

2.4. Tracking the source of bacterial communities

The source and establishment of the human microbiota was assessed by employing an approach of Bayesian mixing model [46]. The model aid identification and quantification of potential microbial communities of human origin. Microbiome data (examining various body sites i.e., skin, oral and stool) of Indian sub-population representing both healthy and disease state was collected and compiled. The human microbiome data was used as a potential source (a reference dataset) of microbial communities whereas the mass bathing data (generated in this study and BioProject Id PRJNA383664) was used as a sink (target dataset) to deduce the origin of microbiota observed during the mass bathing event [47, 48].

2.5. Metagenomic imputations

Functional profiling of these bacterial communities was performed by an approach of imputed metagenomics using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) [49]. Specifically, the computational argument of "metagenomic contributions" was applied to decipher the functional potential of bacterial communities observed before and during the mass bathing event as well as during the minimal state of perturbation to the river i.e., COVID-19 lockdown [50].

3. Results and Discussion

3.1. Estimates of environmental factors

Assessment of the environmental parameters along mass bathing sites across three points viz. before MBE, during MBE and lockdown indicate substantial variability; especially when compared between the groups i.e., before vs during MBE, and during MBE vs lockdown (Table 1). The estimates of the total solids (TS), total dissolved solids (TDS), and total suspended solids (TSS) were found to increase during course of MBE (by 2.08, 1.72, and 2.45-fold, respectively) and decrease during the minimal anthropogenic activity i.e., the period of lockdown (-0.74, -0.65, and -0.81-fold, respectively). The significant increase in the TS ($p \leq 0.01$), TDS ($p = 0.055$) and TSS ($p \leq 0.005$) lead to surge in the turbidity of water. A significant variation in turbidity was noted under the temporal gradient ($p \leq 0.02$). The turbidity increased by 2.26-fold during the course of mass bathing event. In contrast, the turbidity was found to degrease by -0.79-fold during the lockdown period. Increase in the turbidity and solute concentration during MBE corroborated the fact that the event attracts participation by millions of pilgrims at the event. Additionally, the untreated human solid and sewer waste along with the industrial and households waste being routinely discharged into the river leads to deterioration of water quality [22, 23]. Contamination of freshwater ecosystem consequently have deleterious impact on the aquatic biota, including microorganisms [15, 51]. In contrast, the imposed restriction due the COVID-19 pandemic has been found to improvise the water quality and ecosystem, which was also noted in the case of river Damodar [27]. The observation was further supported by the study on river Ganga, suggesting positive implications of the lockdown towards ecosystem restoration [26]. Further, a study by Cooke et al. [7] highlighted both the benefits and risk due to pandemic on the freshwater fishes. In contrast, pH and temperature of the water wasn't found to differ significantly (p= 0.65, p= 0.62, respectively).

Further, evaluation of the oxygen relationship depicted significant variation in the biological oxygen demand (BOD) ($p \leq 0.009$) under temporal variation across mass bathing sites. The BOD was increased by 1.13-fold during the mass bathing event whereas found to decrease by -0.94-fold during the lockdown. The variations in BOD was the consequence of substantial variation in the concentration of the dissolved oxygen (DO) (p= 0.11). The concentration of the DO was decreased by -0.6-fold but raised by 2.5-fold during the lockdown. Similarly, the microbiological assessment of the river water indicated 67.7-fold increase in the MPN count (p= 0.34) which was found to reduce to -0.99-folds during the lockdown. Similarly, the fecal coliform count (p= 0.052) raised by 0.98-fold and lowered to -0.87 during the lockdown. The increase in the BOD is suggestive of the enormous influx of solutes due to bathing in the river, sediment resuspension, discharge from industries and leaching from unattended solid wates during the MBE [51-53]. These changes in the river physiochemistry collectively have deleterious impact on the river microbial communities. Perturbation to the river bacterial communities and augmentation of specific bacterial groups like fecal coliforms poses a risk of spread of gastrointestinal diseases during the event [54, 55]. However, the lockdown was also found to be beneficial concerning the oxygen relationship of the river water which also coincides with observations of Dutta et al. [26] depicting the increased level of DO and reduction in BOD and fecal coliform counts in the river Ganga.

3.2. Structure and composition of bacterial community

Analysis of high-quality paired end reads using QIIME yielded 7469 OTUs. The reads were normalized to 105246 prior to performing further downstream analysis. The estimates of alpha diversity index revealed substantial variability under the spatiotemporal realm however it was not be supported by the statistical significance (Table 1). The estimates of non-parametric Shannon index depicted variance with respect to the time, i.e., it was found to decrease by -0.53-fold during the mass bathing event while it was found to recover during the lockdown by 0.87-fold. This observation suggested potential reestablishment of diversity as minimal divergence of -0.13-fold was noted between before MBE and lockdown samples. It was further supported by the assessment of Chao1 index and observed OTUs (Table 1). In summary, the species richness was found to follow an order of During MBE< Lockdown< Before MBE samples (Figure S1). Loss of bacterial diversity found during the MBE might be supported by deteriorated quality of the river water and enormous influx of organic and inorganic waste having adverse impact on the microbial communities and river ecosystem [52-53, 56]. Alternatively, 0.87-fold increase in the Shannon index implied the events of ecosystem restoration under the improvised water quality during the imposed lockdown period [9, 26].

The most abundant bacterial phyla found under the spatiotemporal constraint consists of *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, Planctomycetes, Cyanobacteria,* [*Thermi*], *Chlorobi* and *Chloroflexi* contributing for 99.9% of bacterial community (Figure 2). The river bacterial community composition before the MBE was comprised of varied bacterial taxa having relative abundance ≥1% viz. *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Planctomycetes, Verrucomicrobia,* and *Cyanobacteria*. These phyla are known as the major constituents of river autochthonous communities driving key functions of primary production in river ecosystem [57, 58]. Conversely, members of bacterial phylum *Actinobacteria* are effective players in degradation of variety of compounds [59]. However, these phyla were found to be replaced by the selective augmentation of phylum *Firmicutes* (94.6%) along the mass bathing sites during the course of mass bathing event. It indicated the potential of members of phylum *Firmicutes* to withstand and flourish in the oxygen limiting and high solute conditions [60]. Minimal human activity during the period of lockdown sites featured reestablishment of various bacterial phyla *Proteobacteria* (70.6%), *Bacteroidetes* (22.5%), *Verrucomicrobia, Actinobacteria* and *Cyanobacteria* having relative abundance of ≥1%. It further supported the observed increase in the Shannon index by 0.87-fold during the lockdown period.

Similarly, localisation of bacterial communities with respect to the time depicted, predominance of *Planococcaceae,* followed by *Comamonadaceae, Bacillaceae, Flavobacteriaceae,* ACK-M1, *Moraxellaceae, Verrucomicrobiaceae, Rhodocyclaceae* and *Oxalobacteraceae* before the MBE (relative abundance ≥1%). In contrast, enrichment of *Planococcaceae* (53.1%) (Figure S2), *Bacillaceae* (41.1%) and *Sphingomonadaceae* (2%) was noted during the course of mass bathing due to its virtue of sustaining the extreme conditions of high solute concentration and low oxygen conditions [60, 61]. Furthermore, due to minimal human interference during the lockdown period, augmentation of diverse families viz.

Moraxellaceae, Flavobacteriaceae, [*Chromatiaceae*], *Comamonadaceae, Sphingomonadaceae, Rhodobacteraceae, Pseudomonadaceae, Verrucomicrobiaceae, Oxalobacteraceae* and *Rhodocyclaceae* (relative abundance ≥1%) was observed.

At genera level (relative abundance ≥1%), predominance of *Bacillus, Lysinibacillus, Flavobacterium, Acinetobacter, Limnohabitans* and *Planomicrobium* was observed as a constituents of autochthonous river microbiota before MBE (Figure S3). Conversely, *Lysinibacillus* (43.8%), *Bacillus* (41%) and *Planomicrobium* (4.9%) were found to flourish during the MBE. Moreover, the correlation between the changing environmental factors and bacterial community at the level of genus was carried which revealed distinct influence of environmental factors on the bacterial communities (Figure S4). The variations in the optical density of the water along with the TS, TDS, TSS and BOD was found to favour the bacterial genera *Lysinibacillus, Bacillus* and *Planomicrobium* ($p \leq 0.05$). However, the dissolved oxygen was found to cast negative impact on the *Lysinibacillus, Bacillus* and *Planomicrobium* ($p \leq 0.05$). Additionally, the substantial variation in the dissolved oxygen was found to be positively correlated with the *Rhodobacter* and *Novosphingobium* ($p \leq 0.05$). Whereas, changes in the estimates of fecal coliforms was found to favour the *Lysinibacillus* ($p \leq 0.05$). Interestingly, the *Lysinibacillus, Bacillus* and *Planomicrobium* showed positive correlation between themselves ($p \leq 0.001$), in contrast they cast negative impact on the bacterial genera viz. *Flavobacterium* ($p \leq 0.05$), *Acinetobacter* ($p \leq 0.05$), *Rheinheimera, Rhodobacter* ($p \leq 0.05$), *Novosphingobium* and *Limnohabitans.* Conversely, *Acinetobacter* shares positive correlation between *Rhodobacter* ($p \leq 0.001$) and *Novosphingobium* ($p \leq 0.01$). Similarly, *Rhodobacter* and *Novosphingobium* depict significant positive correlation ($p \leq 0.01$). Enumeration of members of *Lysinibacillus* and *Bacillus* indicates the potential shedding of human skin associated microbiota during the MBE. Enrichment of *Planomicrobium* corroborated it facts of ability of various *Planomicrobium* species to thrive in variety of halophilic environments and their association with recreational waters [28, 60]. Although species of *Lysinibacillus* represents common members of human skin microbiota but some species of *Lysinibacillus* have also been associated with the sepsis in immunocompromised patients [61, 62]. Thus, increase in abundance of *Lysinibacillus* may pose a serious health risk for the fellow pilgrims bathing in the river. Interestingly, during the lockdown period reestablishment of few bacterial genera that were observed before the MBE viz. *Acinetobacter, Flavobacterium* and *Limnohabitans* (relative abundance ≥1%) was observed.

3.3. Impact of lockdown on the bacterial communities

The relationship between the bacterial communities and the changing physiochemical conditions over the period investigation was derived by applying the linear regression model. The analysis revealed significant influence of varying environmental factors such as optical density of water, TS, TDS, TSS followed by dissolved oxygen, and BOD ($R^2 = 0.61$, $p = 0.013$; $R^2 = 0.73$, $p = 0.0031$; $R^2 = 0.7$, $p = 0.0049$; $R^2 = 0.72$, $p = 0.004$; $R^2 = 0.41$, $p = 0.065$; $R^2 = 0.49$ and, $p = 0.037$, respectively). The environmental factors were found to cast negative impact on the bacterial communities during the course of mass bathing [25]. However, the current analysis depicts the positive correlation between above-mentioned factors and the bacterial communities which support composite increase (based on the Shannon index) in river bacterial diversity during the period of lockdown (Figure 3, Figure S1).

The beta diversity analysis was carried out to determine the divergence in the bacterial communities, under the differential anthropogenic impact across the studied time points. Principal Component Analysis (PCA) analysis demonstrated three distinct groups based on the temporal variations (i.e., before MGE, during MGE and lockdown) indicating significant impact of mass bathing on the river bacterial communities. The samples belonging to the before MGE and lockdown period showed lower intra-group variability (Figure S5). However, the during MGE samples showed higher intra-group variability which corroborates the fact of samples DS4 and DS5 being sink to other mass bathing sites. Although, the Shannon index demonstrates the increase in bacterial diversity during the lockdown period while, the distinctive clustering observed during the beta diversity analysis indicates the variation in the bacterial community structure and composition across the studied time points. The latter is supported by the observed changes in bacterial community structure and composition at the level of family and genera (Fig. S2, S3). The composite observation of distinct clustering, lower intra group variability during the lockdown period, significant increase in bacteria diversity (0.87-fold increase in Shannon index) and enumeration of resilient bacterial communities implies positive impact of lockdown on the river ecosystem and consequently on the river bacterial communities [9, 26]

3.4. Microbial source tracking

The attempt to identify the source of diverse bacterial communities using Source tracker (a Bayesian mixing model) with microbiome profile of healthy Indian sub-population as a reference dataset revealed significant increase in skin and stool associated microbiota in the river during the course of mass bathing event ($p \leq 0.01$). A 3.37-fold ($p = 0.003$) increase in skin associated bacterial communities was recorded across the mass bathing sites during the course of Kumbh Mela 2015. Similarly, the proportion of the human stool associated microbiota also found to increase by 1.56-fold (p= 0.009) during the MBE, which was supported by the elevated levels of MPN and fecal coliform counts across mass bathing sites. Interestingly, during the lockdown period -0.99-fold decrease in both human skin and stool associated microbiota was observed, when compared with mass bathing period (p= 0.004) (Figure 4a, 4b, 4c). It suggestive of the fact that the lockdown had a positive impact, as it indirectly led to cleaning of the river ecosystem. Although health risk associated with such alien communities remains largely unknown however, assessing the source of these microbial communities might aid to devise the mitigation strategies for potential infections related to the eye, skin and bowel [63, 64]. As observed earlier prevalence of *Lysinibacillus* during the MBE might have an unfavourable outcome of sepsis [61]. It is in conjunction with the observation from the previous study on sea bathing where bather itself act a source of non-point pollution [65].

3.5. Infectious diseases and drug resistance genes

Assessment of the functional potential of the river bacterial communities across temporal divergence using an approach of metagenomics imputation depicted substantial variations in abundance of the gene families involved in the metabolism and genetic information processing. However, gene families related to the infectious diseases and drug resistance were targeted to evaluate the impact of changes in the bacterial communities on the public health. Elevated levels (represented as fold change) in the abundance of gene families belonging to infectious diseases, particularly, *Helicobacter pylori* infection (1.34-fold), *Salmonella* infection (2.87-fold), Legionellosis (1.59-fold), *Staphylococcus aureus* infection (5.96-fold) and *Tuberculosis* (2.1-fold) were noted during the mass bathing event. However, during the lockdown substantial reduction in the levels of gene families viz. *Helicobacter pylori* infection (-0.49-fold), *Salmonella* infection (-0.74-fold), Legionellosis (-0.54-fold), *Staphylococcus aureus* infection (-0.96-fold) and *Tuberculosis* (-0.73-fold) was recorded, when compared to during MBE (Figure 5a). Likewise, gene families driving the drug resistance in the bacterial communities were also found to rise with the MBE. Specifically, increase in beta-lactam resistance (1.04-fold), vancomycin resistance (2.49-fold), cationic antimicrobial peptide (CAMP) resistance (2.86-fold), and platinum drug resistance (1.75-fold) was observed during the MBE. The plausible reestablishment of the bacterial communities during the lockdown appeared to have lower prevalence of the gene families associated with drug resistance. A reduction in the levels of beta-Lactam resistance (-0.39-fold), vancomycin resistance (-0.73-fold), cationic antimicrobial peptide (CAMP) resistance (-0.76-fold), and platinum drug resistance (-0.59-fold) was noted during the lockdown period (Figure 5b). Such elevated levels of gene families involved in the infectious diseases and drug resistance during the mass bathing event might pose a serious threat to the attendees which may have catastrophic outcomes like epidemics. Many recent studies on the river ecosystem and/or the religious pilgrimage supports the observed increase in the abundance of gene families involved in infectious diseases and drug resistance. Specifically, the study on Ganga targeting bla_{NDM-1} gene revealed increase in antibiotic resistance during the period of pilgrimage [21]. Similarly, in our previous study we also noted increase in antibiotic resistance in bacteria isolated across the MBE and described a novel cefotaxime resistant strain of *Corynebacterium godavarianum* from the Godavari river, India [66, 67]. The recent study by Yadav et al. [68] involving shotgun metagenomic analysis based on the MinION sequencing supports the observed elevated levels of antibiotic resistance genes during mass bathing.

Conclusion

In summary, a meta-analysis of the river bacterial communities under the extensive anthropogenic impact of mass bathing to minimal impact during the COVID-19 lockdown revealed significant impact of MBE driving reduction in the bacterial diversity and increase in potential risk to the human health. Alternatively, the lockdown provides a plausibility of natural restoration of ecosystem as noted by reestablishment of resilient bacterial communities. However, the extent of reestablishment of resilient bacterial communities from the end of previous mass bathing event (year 2015) and before the lockdown period could not be evaluated and could not be ignored. Our study represents a unique set of baseline data during the lockdown period which will serve as reference for comparative analysis in future studies on Godavari river.

Funding

This work was supported by the 'Department of Biotechnology (DBT), Government of India' (by Grant No. BT/Coord.II/01/03/2016), under the project Establishment of Centre for excellence National Centre for Microbial Resource (NCMR). Avinash Sharma acknowledges the support by the DBT/Wellcome Trust India Alliance under the project grant (IA/E/17/1/503700). Professor Sir Ali Zumla acknowledges support from the European and Developing Countries Clinical Trials Partnership (EDCTP2) Programme, Horizon 2020 , the European Union's Framework Programme for Research and Innovation, grants PANDORA-

ID-NET, TESA-2 and CANTAM-2. We are grateful to Ms. Vaishali Tile for her help in collecting samples during the lockdown.

Conflicts of interest

All authors have an interest in Mass Gatherings Medicine. All authors declare that there are no conflicts of interest.

Ethics approval

No applicable

Consent to participate

Not applicable

Consent for publication

All authors have read and approved the publication of the current manuscript.

Availability of data and material

The sequences generated during this study are available on the NCBI SRA portal under BioProject Id PRJNA698474.

Code availability

Open-source software are used during this study and detailed description is provided in the Materials and methods.

Author Contributions

K.J., Y.S., and A.S. were involved in the study design; J.B. was involved in the sample collection and analysis of environmental parameters; K.J., and A.S. were involved in the experimental work, bioinformatics and statistical analysis; K.J., S.S., E.I.A., S.A., Y.S., and A.S. were involved in the interpretation of the data and conceptualization; All the authors were involved in drafting of the manuscript.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P (2019) A novel coronavirus from patients with pneumonia in China. NEJM. 2020 Jan 24.
2. Coronavirus disease (COVID-19) pandemic World Health Organization, Geneva (2020) https://www.who.int/emergencies/diseases/novel-coronavirus-2019, Accessed on April 14 2021.
3. Wang C, Pan R, Wan X, Tan Y, Xu L, Ho CS, Ho RC (2020) Immediate psychological responses and associated factors during the initial stage of the 2019 coronavirus disease (COVID-19) epidemic among the general population in China. Int J Environ Res Public Health 17:1729.
4. WHO Coronavirus Disease (COVID-19) Dashboard. https://covid19.who.int/ Accessed on April 14 2021.
5. Amanat F, Krammer F (2020) SARS-CoV-2 vaccines: status report. Immunity 52:583-589.
6. Somani M, Srivastava AN, Gummadivalli SK, Sharma A (2020) Indirect implications of COVID-19 towards sustainable environment: an investigation in Indian context. Bioresource Technology Reports 11:100491.
7. Cooke SJ, Twardek WM, Lynch AJ, Cowx IG, Olden JD, Funge-Smith S, Lorenzen K, Arlinghaus R, Chen Y, Weyl OL, Nyboer EA (2020) A Global Perspective on the Influence of the COVID-19 Pandemic on Freshwater Fish Biodiversity. Biol Conserv 9:108932.
8. Bates AE, Primack RB, Moraga P, Duarte CM (2020) COVID-19 pandemic and associated lockdown as a "Global Human Confinement Experiment" to investigate biodiversity conservation. Biol Conserv 248:108665.
9. Rume T, Islam SD (2020) Environmental effects of COVID-19 pandemic and potential strategies of sustainability. Heliyon e04965.

10. National Aeronautics and Space Administration (NASA) (2020). Airborne Particle Levels Plummet in Northern India. Retrieved on April 14, 2021 from https://earthobservatory.nasa.gov/images/146596/airborne-particle-levels-plummet-in-northern-india.
11. Sharma S, Zhang M, Gao J, Zhang H, Kota SH (2020) Effect of restricted emissions during COVID-19 on air quality in India. Sci Total Environ 728:138878.
12. Venter ZS, Aunan K, Chowdhury S, Lelieveld J (2020) COVID-19 lockdowns cause global air pollution declines. PNAS 117:18984-90.
13. Vaseem H, Banerjee TK (2016) Evaluation of pollution of Ganga River water using fish as bioindicator. Environ Monit Assess 188:1-9.
14. Yadav A, Pandey J (2017) Water quality interaction with alkaline phosphatase in the Ganga River: implications for river health. B Environ Contam Tox 99:75-82.
15. Jani K, Ghattargi V, Pawar S, Inamdar M, Shouche Y, Sharma A (2018a) Anthropogenic activities induce depletion in microbial communities at urban sites of the river Ganges. Curr Microbiol 75:79-83.
16. Central Pollution Control Board (CPCB) (2020a). Impact of Lockdown on Water Quality of River Ganga. Retrieved from: https://www.cpcb.nic.in/latest-cpcb.php
17. Singhal, S., Matto, M. (2020) COVID-19 lockdown: A ventilator for rivers. DownToEarth, Retrieved from https://www.downtoearth.org.in/blog/covid-19-lockdown-a-ventilator-for-rivers-70771 Accessed on April 14, 2021.
18. Katariya, M. (2020) 10 Things That Have Happened For The First Time In Years During The Coronavirus Lockdown. Scoopwhoop, Retrieved from https://www.scoopwhoop.com/news/things-that-have-happened-for-the-first-time-in-years-during-coronavirus-lockdown/ Accessed on April 14, 2021.
19. Central Pollution Control Board (CPCBb) (2020) Report on Assessment of Impact of Lockdown on Water Quality of River Yamuna- Delhi Stretch Retrieved from https://www.cpcb.nic.in/latest-cpcb.php
20. Islam SD, Mondal PK, Ojong N, Bodrud-Doza M, Siddique MA, Hossain M, Mamun MA (2021) Water, sanitation, hygiene and waste disposal practices as COVID-19 response strategy: insights from Bangladesh. Environment, development and sustainability 1-22.
21. Ahammad ZS, Sreekrishnan TR, Hands CL, Knapp CW, Graham DW (2014) Increased waterborne bla NDM-1 resistance gene abundances associated with seasonal human pilgrimages to the Upper Ganges River. Environ Sci Technol 48:3014-20.

22. Vortmann M, Balsari S, Holman SR, Greenough PG (2015) Water, sanitation, and hygiene at the world’s largest mass gathering. Curr Infect Dis Rep 17(2):461. https://doi.org/10.1007/s11908-015-0461-1
23. David S, Roy N (2016) Public health perspectives from the biggest human mass gathering on earth: KumbhMela, India. Int J Infect Dis 47:42–45
24. Memish ZA, Steffen R, White P, Dar O, Azhar EI, Sharma A, Zumla A (2019) Mass gatherings medicine: public health issues arising from mass gathering religious and sporting events. Lancet. 393:2073-84.
25. Jani K, Dhotre D, Bandal J, Shouche Y, Suryavanshi M, Rale V, Sharma A (2018c) World’s largest mass bathing event influences the bacterial communities of Godavari, a holy river of India. Microb Ecol. 76:706-18.
26. Dutta V, Dubey D, Kumar S (2020) Cleaning the River Ganga: Impact of lockdown on water quality and future implications on river rejuvenation strategies. Sci Total Environ 743:140756.
27. Chakraborty B, Roy S, Bera A, Adhikary PP, Bera B, Sengupta D, Bhunia GS, Shit PK (2021) Cleaning the river Damodar (India): impact of COVID-19 lockdown on water quality and future rejuvenation strategies. Environ Dev Sustain 3:1-5.
28. Lee CS, Kim M, Lee C, Yu Z, Lee J (2016) The microbiota of recreational freshwaters and the implications for environmental and public health. Front Microbiol 7:1826.
29. Kajale S, Jani K, Sharma A (2020) Contribution of archaea and bacteria in sustaining climate change by oxidizing ammonia and sulfur in an Arctic Fjord. Genomics. https://doi.org/10.1016/j.ygeno.2020.11.005
30. Sharma A, Jani K, Thite V, Dhar SK, Shouche Y (2019) Geochemistry shapes bacterial communities and their metabolic potentials in tertiary coalbed. Geomicrobiol J 36:179-87.
31. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108(Suppl 1):4516–4522
32. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335-6.
33. Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957-63.

34. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet journal, 17:10-12.
35. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-41.
36. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069-72.
37. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-1.
38. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261-7.
39. McMurdie PJ, Holmes S (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 8(4):e61217
40. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020). vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan
41. Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4 https://ggplot2.tidyverse.org
42. Wilke CO (2020). cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'. R package version 1.1.1. https://CRAN.R-project.org/package=cowplot
43. Wei T, Simko V (2017). R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Available from https://github.com/taiyun/corrplot
44. Harrell FE Jr, with contributions from Charles Dupont and many others (2021). Hmisc: Harrell Miscellaneous. R package version 4.5-0. https://CRAN.R-project.org/package=Hmisc
45. Peterson BG, Carl P (2020) PerformanceAnalytics: Econometric Tools for Performance and Risk Analysis. R package version 2.0.4. https://CRAN.R-project.org/package=PerformanceAnalytics

46. Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, Bushman FD, Knight R, Kelley ST (2011) Bayesian community-wide culture-independent microbial source tracking. Nat Methods. 8:761-3.
47. Chaudhari DS, Dhotre DP, Agarwal DM, Gaike AH, Bhalerao D, Jadhav P, Mongad D, Lubree H, Sinkar VP, Patil UK, Salvi S (2020) Gut, oral and skin microbiome of Indian patrilineal families reveal perceptible association with age. Sci Rept 10:1-3.
48. Gaike AH, Paul D, Bhute S, Dhotre DP, Pande P, Upadhyaya S, Reddy Y, Sampath R, Ghosh D, Chandraprabha D, Acharya J (2020) The gut microbial diversity of newly diagnosed diabetics but not of prediabetics is significantly different from that of healthy nondiabetics. Msystems 5.
49. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RL, Knight R, Beiko RG (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotech 31:814-21.
50. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:1-8.
51. Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S (2011) A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14-49.
52. Spietz RL, Williams CM, Rocap G, Horner-Devine MC (2015) A dissolved oxygen threshold for shifts in bacterial community structure in a seasonally hypoxic estuary. PloS one 10:e0135731.
53. Amalfitano S, Corno G, Eckert E, Fazi S, Ninio S, Callieri C, Grossart HP, Eckert W (2017) Tracing particulate matter and associated microorganisms in freshwaters. Hydrobiologia 800:145-54.
54. Fries JS, Characklis GW, Noble RT (2006) Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, NC. J Environ Eng 132:1338-45.
55. Fisher JC, Levican A, Figueras MJ, McLellan SL (2014) Population dynamics and ecology of Arcobacter in sewage. Front Microbiol 5:525.
56. Gibbons SM, Jones E, Bearquiver A, Blackwolf F, Roundstone W, Scott N, Hooker J, Madsen R, Coleman ML, Gilbert JA (2014) Human and environmental impacts on river sediment microbial communities. PloS one. 9:e97435.
57. Warnecke F, Amann R, Pernthaler J (2004) Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environ Microbiol 6:242-53.

58. Newton RJ, McLellan SL (2015) A unique assemblage of cosmopolitan freshwater bacteria and higher community diversity differentiate an urbanized estuary from oligotrophic Lake Michigan. Front Microbiol 6:1028.
59. Van der Gucht K, Vandekerckhove T, Vloemans N, Cousin S, Muylaert K, Sabbe K, Gillis M, Declerk S, De Meester L, Vyverman W (2005) Characterization of bacterial communities in four freshwater lakes differing in nutrient load and food web structure. FEMS Microbiol Ecol 53:205-20.
60. Jung YT, Kang SJ, Oh TK, Yoon JH, Kim BH (2009) *Planomicrobium flavidum* sp. nov., isolated from a marine solar saltern, and transfer of *Planococcus stackebrandtii* Mayilraj *et al.* 2005 to the genus *Planomicrobium* as *Planomicrobium stackebrandtii* comb. nov. Int J Syst Evol Microbiol 59:2929-33.
61. Wenzler E, Kamboj K, Balada-Llasat JM (2015) Severe sepsis secondary to persistent *Lysinibacillus sphaericus, Lysinibacillus fusiformis* and *Paenibacillus amylolyticus* bacteremia. Int J Infectious Diseases 35:93-5.
62. Ndiaye C, Lo CI, Bassene H, Raoult D, Lagier JC, Sokhna C (2019) *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., and *Erwinia mediterraneensis* sp. nov., three new species isolated from the human skin. New microbes and new infect 31:100579.
63. Guidelines for safe recreational water environments Volume 1: Coastal and Fresh Waters. WHO 2003. http://www.who.int/water_sanitation_health/publications/srwe1/en/ Accessed on April 14 2021.
64. Papastergiou P, Mouchtouri V, Pinaka O, Katsiaflaka A, Rachiotis G, Hadjichristodoulou C (2012) Elevated bathing-associated disease risks despite certified water quality: a cohort study. Int J Environ Res Public Health 9:1548-65.
65. Fewtrell L, Kay D (2015) Recreational water and infection: a review of recent findings. Curr Environ Health Rep 2:85-94
66. Jani K, Khare K, Senik S, Karodi P, Vemuluri VR, Bandal J, Shouche Y, Rale V, Sharma A (2018b) *Corynebacterium godavarianum* sp. nov., isolated from the Godavari river, India. Int J Syst Evol Microbiol 68:241-7.
67. Jani K, Bandal J, Rale V, Shouche Y, Sharma A (2019) Antimicrobial resistance pattern of microorganisms isolated and identified from Godavari River across the mass gathering event. J Biosci 44:1-6.

68. Yadav R, Rajput V, Gohil K, Khairnar K, Dharne M (2020) Comprehensive metagenomic insights into a unique mass gathering and bathing event reveals transient influence on a riverine ecosystem. Ecotoxicol Environ Saf 202:110938.

Figures and legend

Figure 1: The map depicts the location of the sampling sites along the bank of the Godavari river, Nashik, India. Samples collected before the event are named as BS3, BS4, and BS5, samples collected during the event are DS3, DS4, and DS5 and samples collected during the COVID lockdown were termed as LS3, LS4 and LS5; that correspond to locations Anandwalli, Gharpure ghat, and Tapovan, respectively.

Figure 1

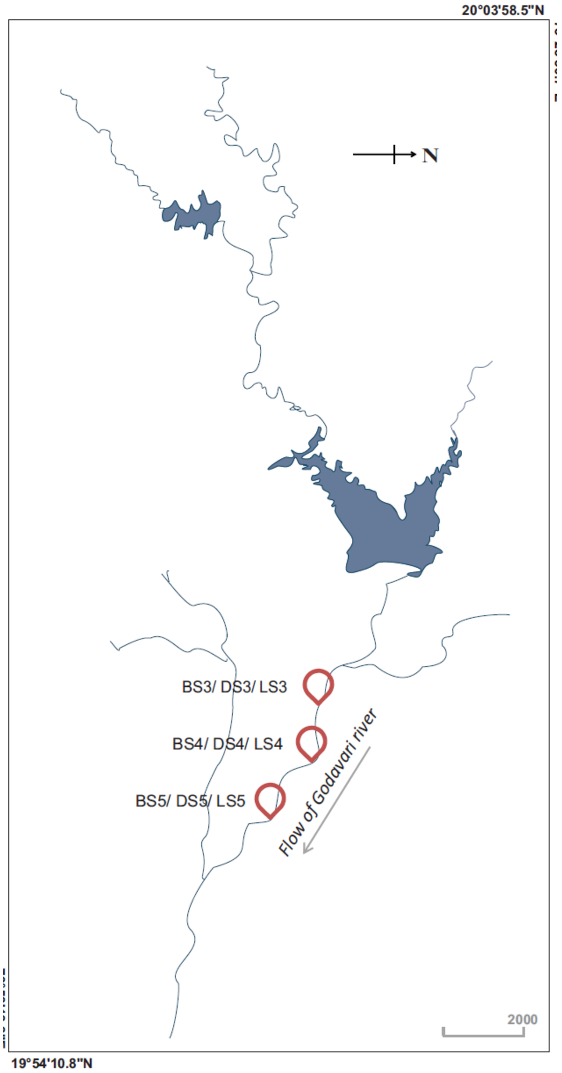

Figure 2: Distribution of most abundant bacterial phyla across the temporal variation of mass bathing event and lockdown period.

Figure 2

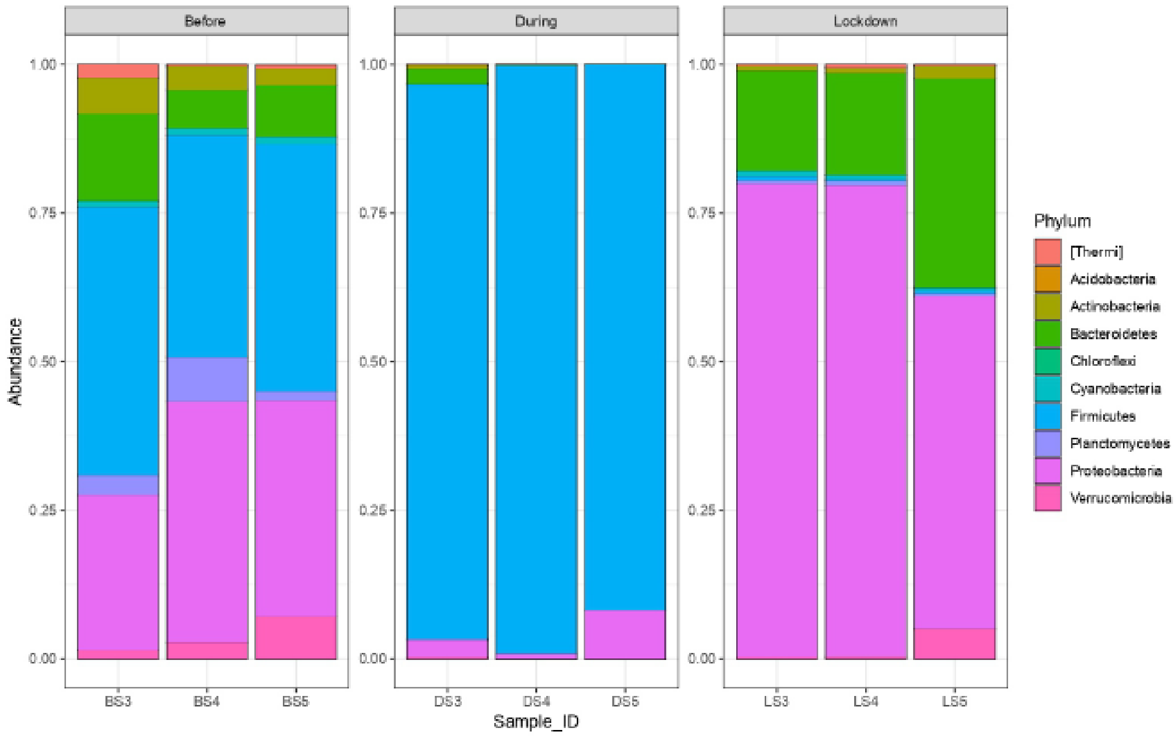

Figure 3: Correlation between the bacterial diversity and the changing environmental parameters across the studied time points. Colour code defines the temporal variations in the samples i.e., before (red) and during (green) the event and COVID-19 lockdown (blue).

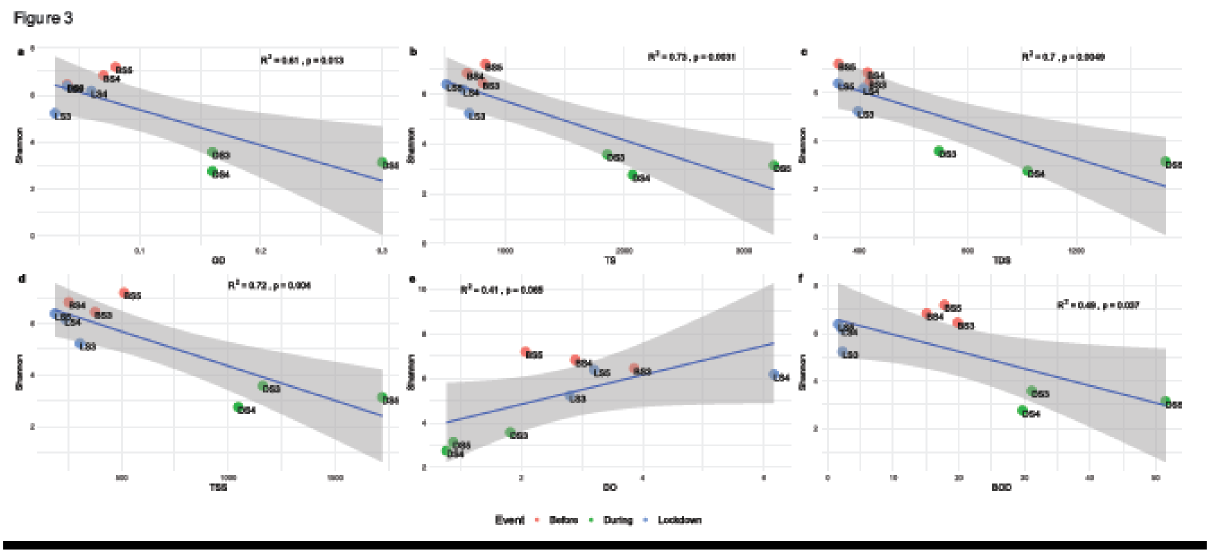

Figure 4: Proportion of human associated microbiota [oral (purple), skin (red) and stool (blue)] determined using Bayesian mixing model across the timepoints understudy. (a) before the mass bathing event; (b) during the mass bathing event; (c) lockdown period.

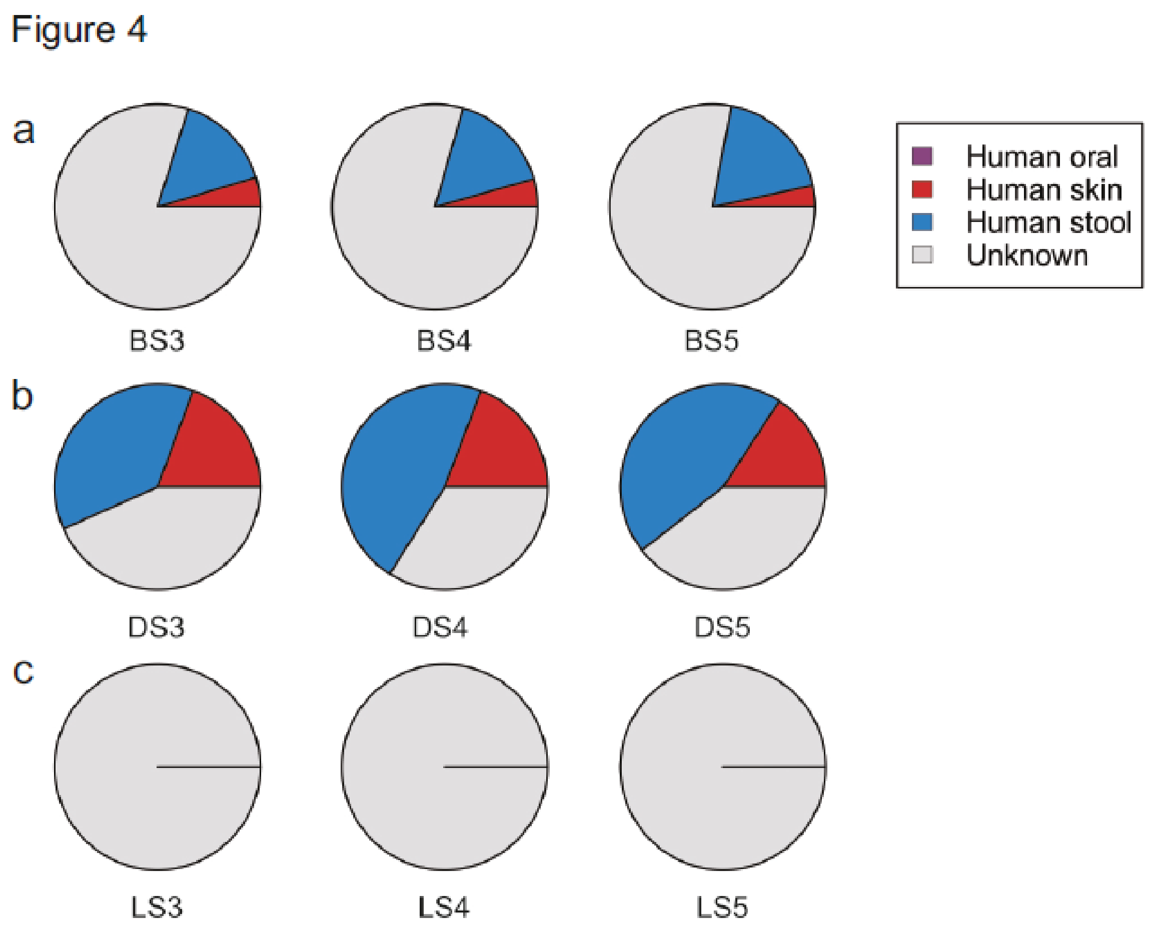

Figure 5: Differential abundance of gene families associated with the (a) infectious diseases and (b) drug resistance across the timepoints understudy.

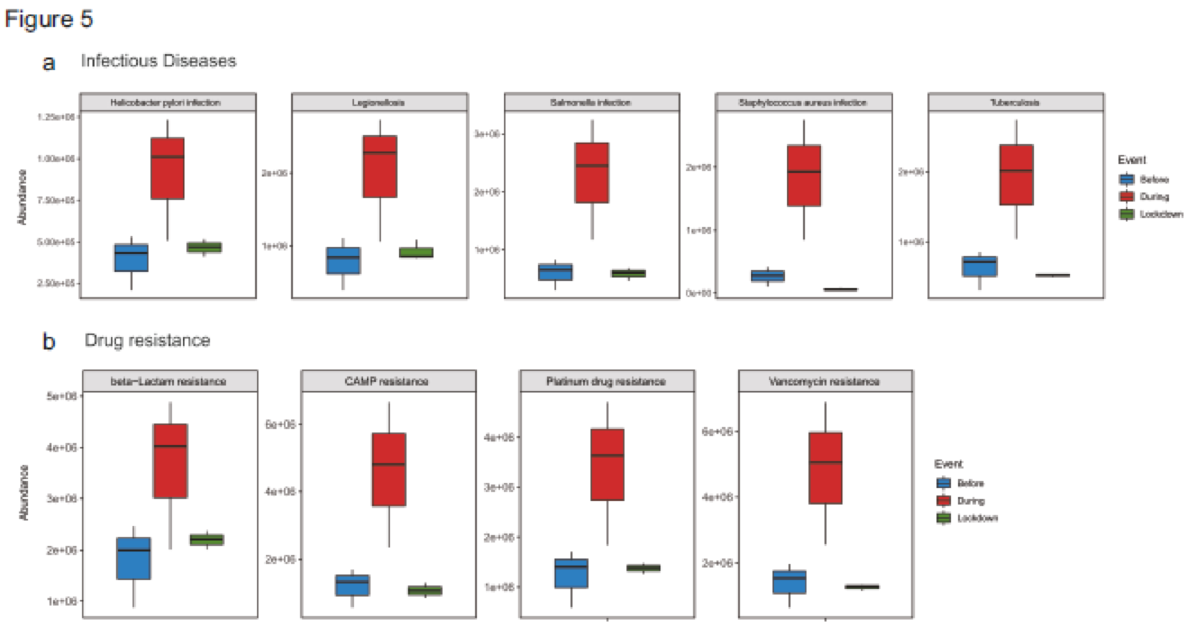

Table 1: Environmental parameters and alpha diversity across the temporal variation of mass bathing event and COVID lockdown.

Sample ID	Event	pH	Temperature (°C)	OD	TS (mg/l)	TDS (mg/l)	TSS (mg/l)	DO (mg/l)	BOD (mg/l)	MPN (per 100ml)	Fecal coliform (per 100ml)	Chao1	Observed OTUs	Shannon
BS3	Before	6.8	25.2	0.04	811	436	375	3.86	19.8	1.40E+04	320	4181.95	2919	6.45
BS4	Before	6.5	23.3	0.07	682	430	252	2.89	15.1	1.10E+04	298	3975.94	2521	6.83
BS5	Before	6.8	23.5	0.08	835	323	512	2.07	17.8	1.40E+04	1007	5528.73	3702	7.19
DS3	During	7.2	23.1	0.16	1856	694	1162	1.82	31.1	1.10E+05	1007	3570.94	2181	3.57
DS4	During	6.8	25.4	0.16	2068	1021	1047	0.77	29.7	1.70E+05	400	1686.41	1062	2.75
DS5	During	6.5	27.7	0.3	3250	1529	1721	0.88	51.5	2.40E+06	1820	1265.5	841	3.13
LS3	Lockdown	7	25.1	0.03	700	395	305	2.8	2.24	1.60E+03	47	1836.44	1337	5.22
LS4	Lockdown	6.7	24.3	0.06	650	415	235	6.16	2.16	1.60E+03	80	2082.19	1609	6.18
LS5	Lockdown	6.8	24.9	0.04	510	322	188	3.2	1.6	1.60E+03	286	2201.25	1651	6.38

SUPPLEMENTARY FILE

Title: Extended ecological restoration of bacterial communities in the Godavari river during the COVID lockdown period - a spatiotemporal meta-analysis

Authors: Kunal Jani[1], Jayshree Bandal[2], Yogesh Shouche[1], Shuja Shafi[3], Esam Azhar[4], Alimuddin Zumla[5], Avinash Sharma[1*]

Institutional affiliations:

[1]National Centre for Cell Science, Pune-411007, India.

[2]Department of Microbiology, KTHM College, Nashik, Maharashtra 422002, India

[3]Mass Gatherings and Global Health Network, London, United Kingdom. Electronic address: msshafi12@gmail.com.

[4]Special Infectious Agents Unit, King Fahd Medical Research Center, Jeddah, Saudi Arabia; Medical Laboratory Sciences Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Electronic address: eazhar@kau.edu.sa.

[5]Division of Infection and Immunity, University College London, and NIHR Biomedical Research Centre, UCL Hospitals NHS Foundation Trust, London, United Kingdom. Electronic address: a.zumla@ucl.ac.uk.

***Correspondence**

Dr. Avinash Sharma

DBT/Wellcome Trust Fellow, National Centre for Cell Science,, Pune, Maharashtra 411007, India. Fax No: +91-20-25329001 Telephone No: +91-20-25329028

E-mail: avinash.nccs@gmail.com; avinash@nccs.res.in

Figure S1: Difference in the estimates of Shannon diversity index.

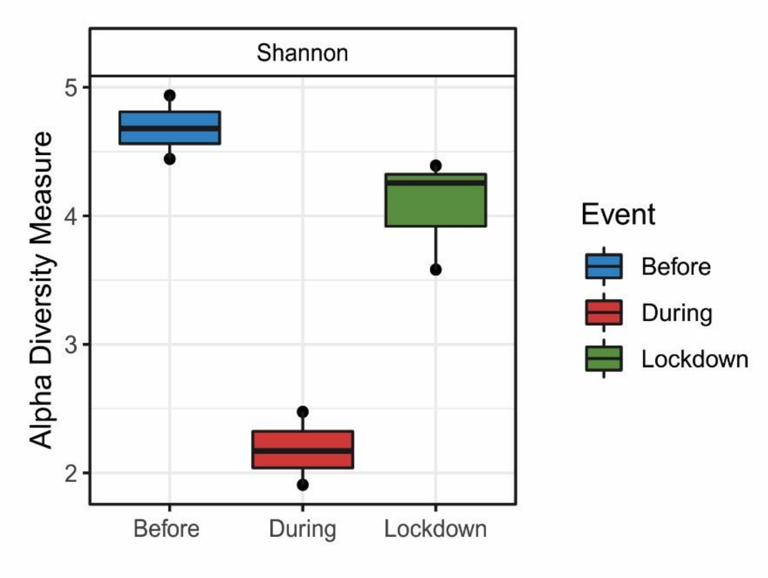

Figure S2: Distribution of bacterial families across the temporal variation of mass bathing event and COVID lockdown.

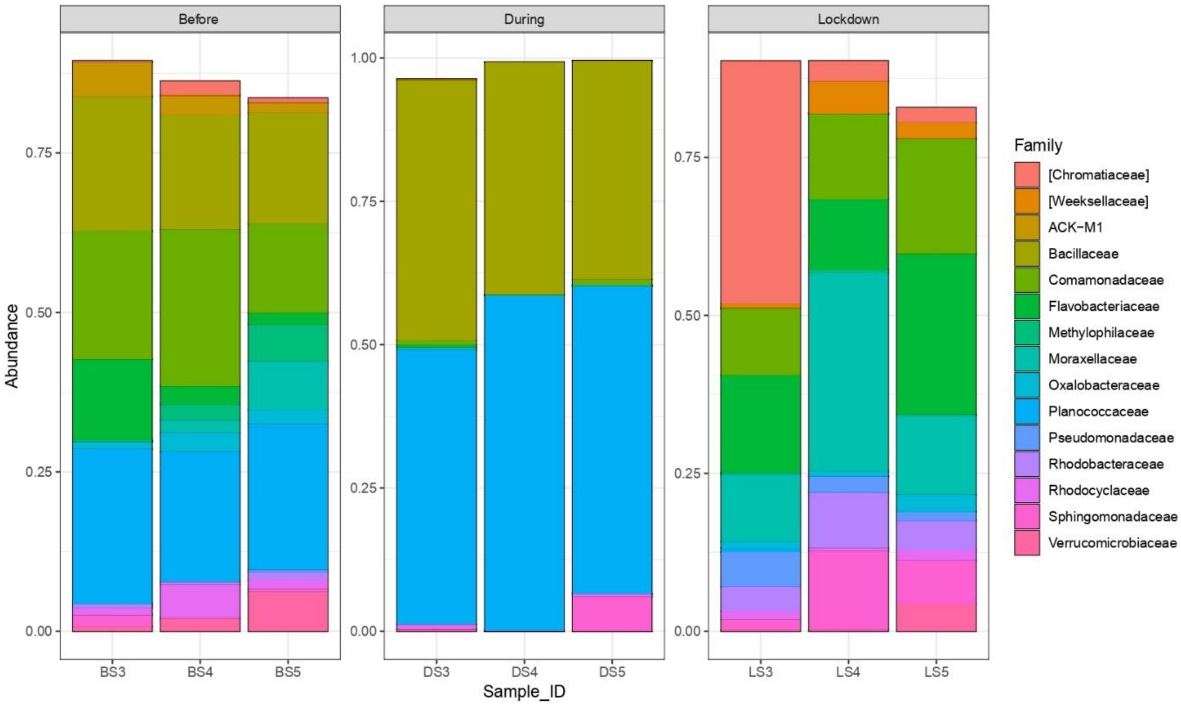

Figure S3: Distribution of bacterial genera across the temporal variation of mass bathing event and COVID lockdown.

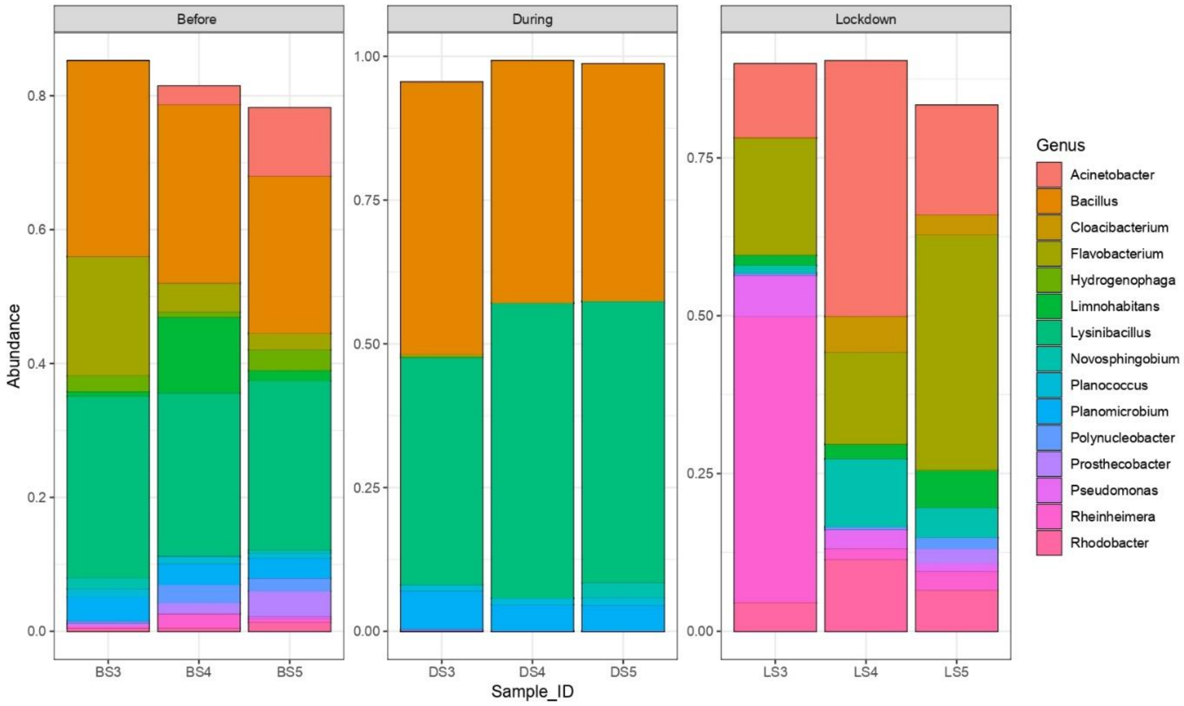

Figure S4: Correlation between the changes in the environmental parameters and the bacterial genera across the temporal variation of mass bathing event and COVID lockdown.

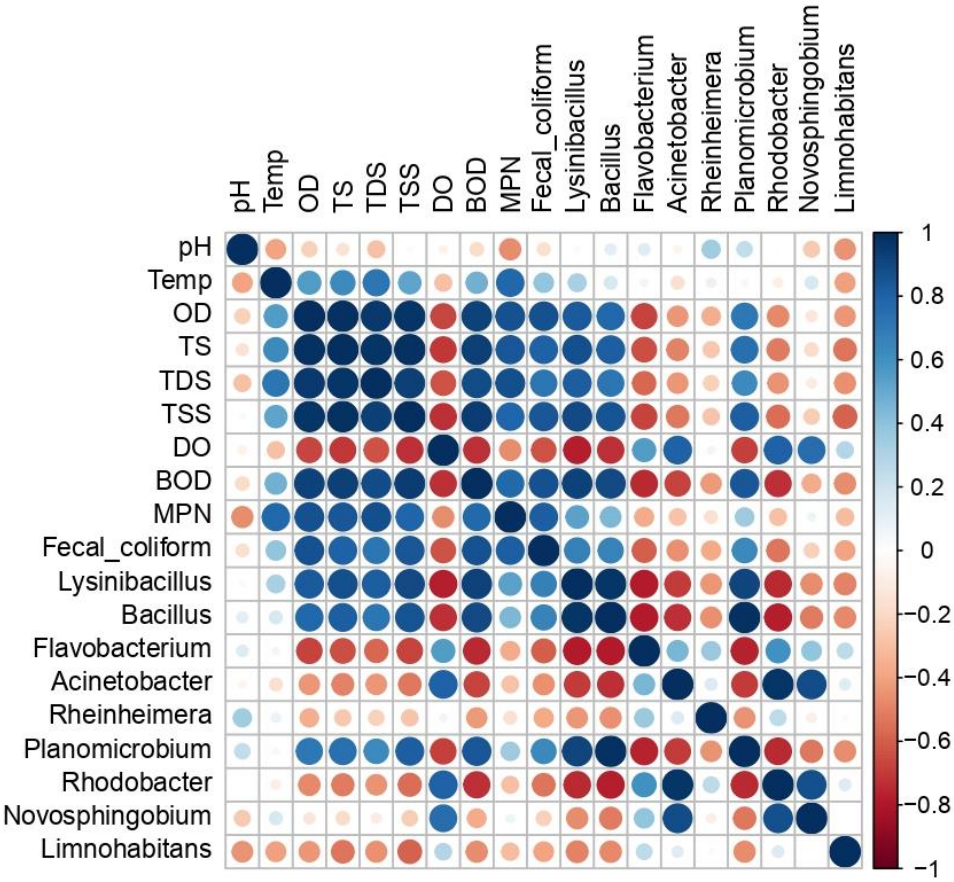

Figure S5: Beta diversity analysis. Principal component analysis demonstrates the spatiotemporal variation in the bacterial communities. Colour code depicts the temporal variations in before (red) and during (green) the event and COVID lockdown (blue).