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RESEARCH ARTICLE

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Temporal dynamics in the bacterial abundances in Indian Sundarbans: unveiling the influence of reverse migration under the effects of the COVID-19 pandemic

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ABSTRACT: The Sundarbans, a vital ecosystem in the Ganges Delta, faces multifaceted challenges exacerbated by climate change and socio-economic factors. This study investigates the impact of COVID-19 pandemic induced reverse migration on the microbial dynamics of the Indian Sundarbans using metagenomics guided wastewater surveillance.Samples were periodically collected once in a year over a span of three years with one-year interval between each sampling event. The sampling years- 2019, 2021 and 2023 were categorised as Pre-COVID-19, Mid-COVID-19 and Post-COVID-19 periods respectively. Following sampling, thorough metagenomic profiling was conducted to evaluate temporal dynamics in bacterial abundances amidst pandemic.16S metagenomic sequencing revealed the dominance of the members of phylum Proteobacteria in all the three samples. Sample collected during the Mid-COVID period exhibited a distinct enrichment of antibiotic resistance pathways, potentially linked to the influx of migrant workers with limited healthcare access and potential reliance on self-medication. Interestingly, during the peak of the pandemic, the abundance of opportunistic pathogens like *Pseudomonas* and *Acinetobacter* in the wastewater decreased compared to pre- and post-pandemic levels. Conversely, *Sphingomonas* associated with respiratory infections, showed a surge during the mid-COVID period. Agricultural practices adopted during the lockdown could explain the observed rise in nitrogen-fixing bacteria like *Methylotenera* and *Rhizobium* in the Mid-COVID sample.As reverse migration reshapes demographic patterns, proactive measures are needed to mitigate health risks and sustain ecosystem health. Additionally, it would highlight the utility of metagenomics-guided surveillance in informing public health policies amidst dynamic socio-economic and environmental changes.

KEYWORDS: Indian Sundarbans, COVID-19 pandemic, Reverse migration, Community genomics, Wastewater surveillance

INTRODUCTION

The Sundarbans, the world's largest mangrove forest in the Ganges Delta, is a critical habitat for coastal protection, climate change mitigation, and biodiversity conservation. However, as per the report of Intergovernmental panel on Climate Change, 2007, it faces threats from natural disasters, human interventions, poverty, inequality, and limited accessibility due to its unique island biogeography. Climate change-related factors like sea-level rise and extreme weather events exacerbate the situation [1,2].

The Sundarbans region, comprising 52 out of 106 islands, has a population of 4.7 million, with 47% of marginalized groups and 40% below the poverty line. A vast area of Sundarbans has experienced both external and internal migration from Bangladesh and neighbouring districts which has increased population, leading to resource competition and poverty. Challenges like coastal erosion and cyclonic surges have moreover impacted agricultural productivity. Approximately 30% of households have at least one family member employed outside West Bengal equating to a migrant workforce of more than two lakhs individuals approximately [3].

In 2020, as the World experienced the fastest-spreading global pandemic in the recent history named COVID-19; the Government of India implemented an unprecedented nationwide lockdown on March 24, 2020, with just 606 reported cases, providing only a four-hour notice, making it one of the most stringent shutdowns globally [4-6].

On this context, the pandemic disrupted various aspects of life in the Indian Sundarbans, impacting the socio-economic conditions, resource management, healthcare

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access, and environmental conservation efforts. Socio-economic damage of this region has been found to be linked to the strict pandemic lockdown measures that have significantly disrupted the livelihoods of more than 80% of the local population across the vast regions of the Sundarbans, particularly those economically vulnerable groups who are dependent on tourism, fishing, and agriculture [7]. A study by the United Nations Development Programme reported that pandemic has caused a 30% decline in tourism revenue in the Sundarbans [8]. Moreover,the lack of effective initiative to provide alternative livelihood measures, facilitated the reverse exodus of migrant workers into the vast regions of Sundarbans from where approximately 500,000 young males have been reported to leave their native in search of employment each year. As per the available report around two lakh fifty thousand to three lakh migrants belonging to 51% of the families of the region underwent massive inflow into the Indian Sundarbans alone within the last 2 weeks once the lockdown was declared. As per the official data available, primary wave of return to Sundarbans was during March to May 2020, coinciding with the sudden nationwide lockdown in India. In March 2020, approximately 50,000 migrants returned, but this number rose dramatically to around 120,000 in April, reflecting mass layoffs and lack of proper livelihood options due to the extended lockdown. In May 2020, although the number of returning workers slightly decreased to 100,000, it remained significantly high, partly influenced by continued job losses. By June 2020, the migration flow had begun to decline sharply to about 30,000, as restrictions eased and transportation options improved. However, a large number of inward migrants did not move out, directly affecting population density of major villages across the Sundarban deltaic islands. In 2021, during the second wave of COVID-19, a smaller but notable return of about 10,000 workers was recorded. As per the last data available number of reverse migrants reduced to 2000 from 10,000, associated with a gradual recovery of pandemic conditions (Supplementary Figure. 1) [7, 8].This led to, reduction in remittances for migrant households, increased strain on natural resources and an increased risk of disease prevalence in the islands. This movement not only had implications on the socio-economic dynamics of the region but also played a major role in elevating the disease burden in the entire island system [9-11].

Human migration significantly influences the microbial composition across diverse ecosystems, including soil, water, and gut microbiome. Migrants introduce new microbes through direct contact, contaminated food, water, and improper waste disposal altering the prevalence of existing ones [12,13]. These microbes can interact with existing ones, leading to competition and potential changes in the microbial community. Human activities associated with migration, such as agriculture, deforestation, and urbanization, have been identified to be the major factors [14]. Human migration can alter environmental factors like temperature, pH, and nutrient availability, influencing the growth and survival of specific microbial populations. Adoption of new dietary practices in new environment can alter the microbial environment in the gastrointestinal tract, influencing the overall microbial composition of individuals and potentially the community. Understanding these shifts is crucial for public health planning, disease prevention, and managing microbial diversity.

Recently, sewage surveillance, also popularised as Wastewater Based Epidemiology (WBE) has emerged as a powerful tool for understanding the shifts in wastewater microbial community in different environments [15]. Analyzing the microbial content present in sewage has an immense role in pathogen monitoring, antibiotic resistance mapping as well as tracking the shift in microbial composition of sewage that in turn can reflect shifts in the health status of a community. Wastewater surveillance can also be used to monitor the occurrence of environmental contaminants and assess the impact of environmental changes on the microbial community. Through early detection of specific pathogens in the sewer network, WBE can foretell the outbreaks of various infectious diseases [16-19].

The current study attempts to investigate the potential impact of COVID-19 pandemic lockdown induced reverse migration on the dynamics of microbial community in one of the most densely populated area of the Indian Sundarbans through complete profiling and estimation of pathogenic load in wastewater bacterial community collected from municipal sewage canal situated in the Rangabelia Panchayat area of the Gosaba island, Sundarbans, India.

MATERIALS AND METHODS

Sample collection

Wastewater samples were periodically collected once in a year (in the month of March), over a span of three years with one-year interval between each sampling event. Wastewater samples obtained in the sampling years 2019, 2021, 2023 labelled as "PC" for Pre-COVID-19, "MC" for Mid-COVID-19, and "OC" for Post-COVID-19 samples, respectively. The wastewater canal situated in Rangabalia village under Gosaba subdivision in South Twenty-Four Parganas, Sundarbans, India was selected as the sampling site under this study.From the same sampling site, three distinct

samples were collected, spaced 5 meters apart resulting in three replicates per sampling event. Followed by collection in aseptic collecting jars, water samples were transported within 10 hours of collection and maintained at -20°C until further processing.

Culture independent studies

a. Pre-processing of samples

Followed by collection, each replicate of the samples was subjected to membrane filtration using a sterile cellulose acetate membrane filter with a pore size of 0.2 micrometres, facilitated by a vacuum filtration setup. The retention of the individual replicates on the membrane filter was subsequently utilised for the DNA extraction.

b. Metagenomic sequencing

The initial step was the isolation of DNA from the prepared membrane filtersin accordance with the procedures outlined in Singh*et al.*[20].Followed by extraction, the total eluted DNA fromthe three replicates of individualsamples were pooled together for the further downstream analyses. Followed by quality and quantity assessment of the extracted DNA, 16S Metagenomic Sequencing was performed through library preparation and primer designing against V3 and V4 hypervariable regions of 16S rDNA gene, as per the protocol explained in Singh *et al.*[20]to generate ~150 Mb of data per sample.

16S Metagenomics data are available in NCBI Sequence Read Archive under SRX numbers: SRX17674869 (“PC” sample); SRX17734450 (“MC” sample); SRX22845025 (“OC” sample).

c. Computational analysis of metagenomic data

The FASTQC toolkit (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) was usedto perform the Quality Control (QC) of the raw reads obtained from 16S metagenomic sequencing platform. After QC processing, paired end reads were subjected to Taxon calling and OTU clustering using QIIME2 pipeline and VSEARCH's clustering method[21] for thorough profiling of microbial population present in the samples against SILVA database.In the next step, Percent Abundance values (PRA) were computed from the Genus-level abundance data of bacterial communities to analyse the trend in prevalence of bacterial genera in each of the wastewater samples under study.The calculated percent abundance data of bacterial generaobtained from the three distinctdatasetswere subsequently used for a comparative study using Venny v2.1.0 [22]to identify a collection of core and unique bacterial assemblages throughout the test samples. Following this, metagenome-wide association studies was performed to link microbes in the sample to the host disorders in order to predict pathogenic load in the community and to understand disease networks of microbial members using Taxon Set Enrichment Analysis [23]. Core set of enriched metabolic functions of the microbial community were predicted along with understanding of microbial interaction patterns through function-driven correlation networks.

RESULTS AND DISCUSSION

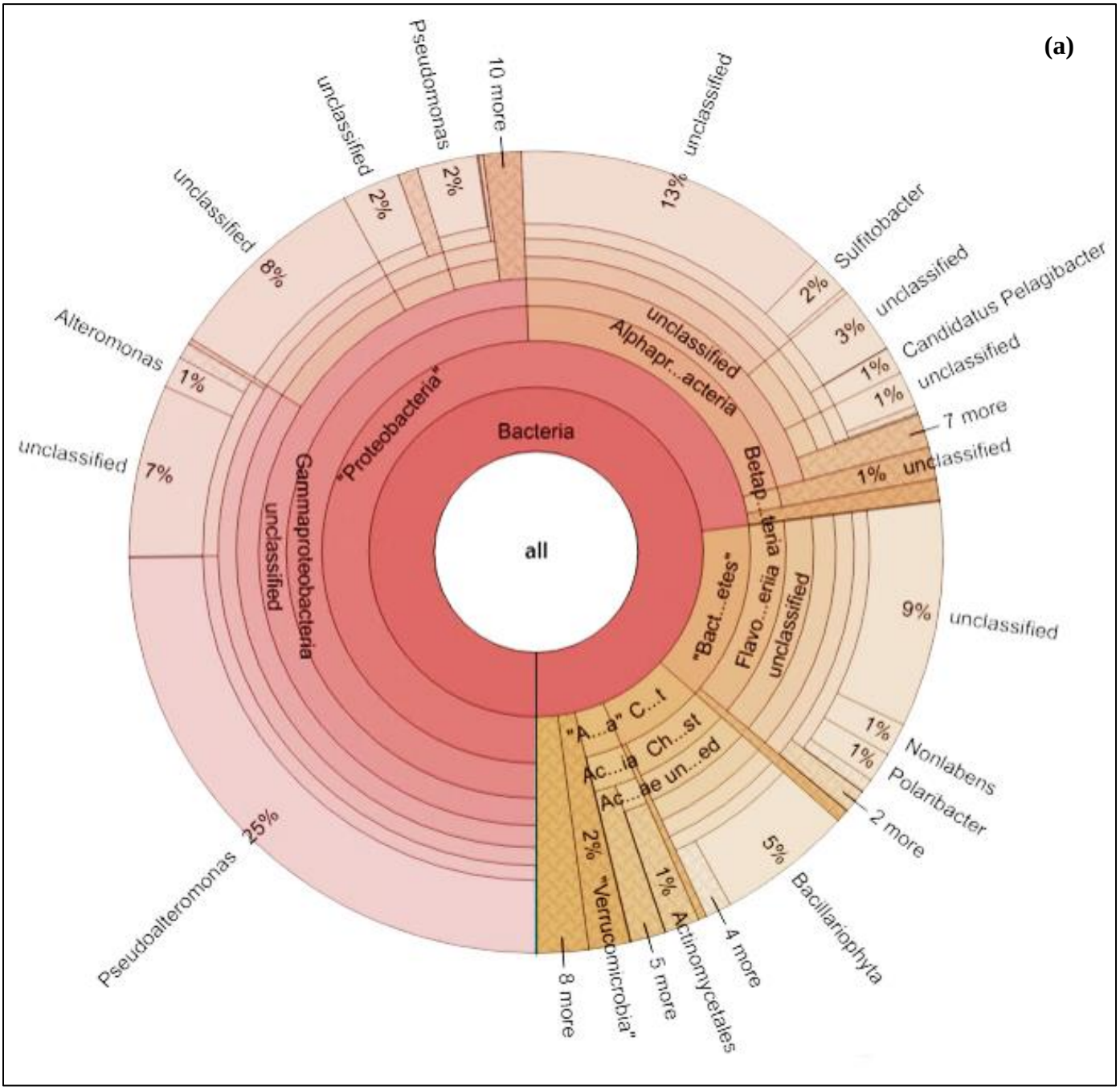
Metagenomic mapping of wastewater bacterial profile

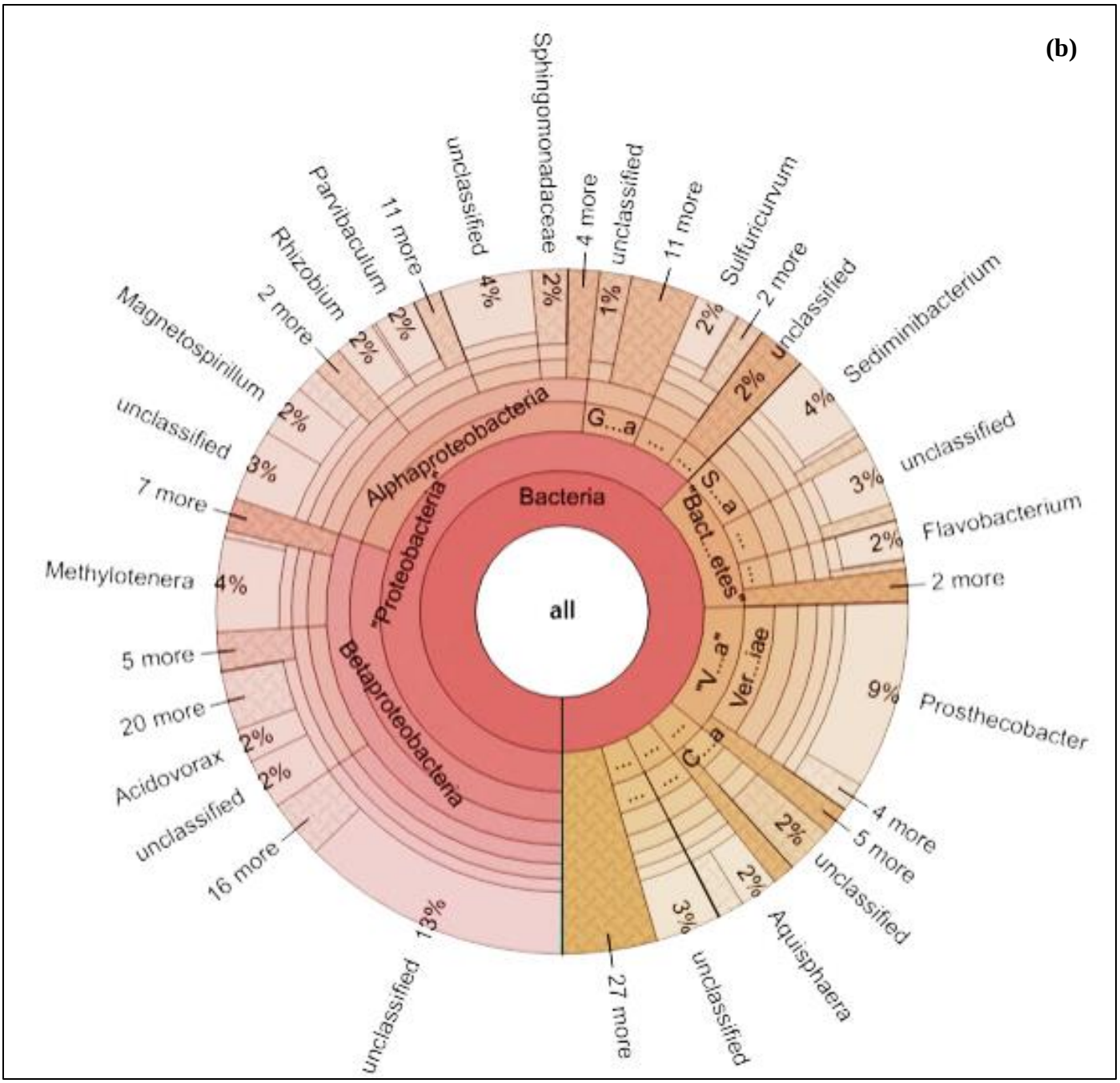
Metagenomic Data sets obtained from 16S Metagenomic sequencing of the wastewater samples were found to contain 140140, 193357 and 136573 reads for PC, MC and OC samples respectively with average sequence length of 300 bp. Shannon Diversity Index values of the samples were recorded to be 6.009, 7.450 and 7.731 respectively. Community study of the respective samples, represented through Krona charts revealed dominance of the members of phylum Proteobacteria in all the three samples with the abundance of 73%, 62 %, 51% respectively in PC, MC and OC. Proteobacteria was observed to be accompanied by prominent representatives of phylum Bacteroidetes in all the samples, placed in the second position in terms of abundance. Unlike the remarkable presence of cyanobacterial members in both PC and OC samples, prevalence of the bacterial members belonging to phylum Verrucomicrobia and Firmicutes was notable in wastewater samples MC, collected during the mid COVID period (Figure.1).Genus level data exhibited predominance of *Pseudoalteromonas* (61.279%) in the PC sample followed by *Pseudomonas, Sulfitobacter, Nonlabens, Alteromonas, Candidatus*etc. Bacterial genera like *Prosthecobacter, Methylotenera, Sediminibacterium, Magnetospirillum, Sulfuricurvum*were found to top the list of 20 most abundant bacterial members in MC sample. On the other hand, *Wandonia*was in higher titre with the abundance of 8.502 % in OC sample followed by *Flavobacterium, Hydrogenophaga, Acinetobacter, Rheinheimera, Pseudomonas* etc (Figure.2). Opportunistic pathogen *Pseudomonas* was predominant in PC sample with PRA value of 62.979%. An exception was observed in case of our OC sample, where another candidate from the nosocomial pathogenic candidate, *Acinetobacter* topped the list with PRA- 34.780% followed by *Pseudomonas* (PRA- 28.628%). *Vibrio, Escherichia, Burkholderia, Staphylococcus, Streptococcus, Serratia, Enterococcus* are among the other

pathogens that were ubiquitously present in all the three samples under study (Figure.3).

Prediction of pathogenic load

Taxon-set enrichment analysis-based prediction of pathogenic load could successfully establishthe correlation between bacterial load in the samples and the likelihood of disease in the population in the future through generation of the disease network graph. Liver cirrhosis, respiratory infections, ulcerative colitis, Crohn's disease, diarrhoea, and enterocolitis are among the diseases associated with wastewater, as evident in the networks derived from the bacterial load of the samples under study (Figure.4).




(b)
all
Bacteria
Proteobacteria
Alphaproteobacteria
Betaproteobacteria
Bact...etes
Ver...iae
Sphingomonadaceae
4 more
unclassified
11 more
Sulfuricurvum
2 more
unclassified
Sediminibacterium
unclassified
Flavobacterium
2 more
Prosthecobacter
4 more
5 more
unclassified
Aquisphaera
unclassified
27 more
unclassified
16 more
Acidovorax
20 more
5 more
Methylotenera
7 more
unclassified
Magnetospirillum
2 more
Rhizobium
Parvibaculum
11 more
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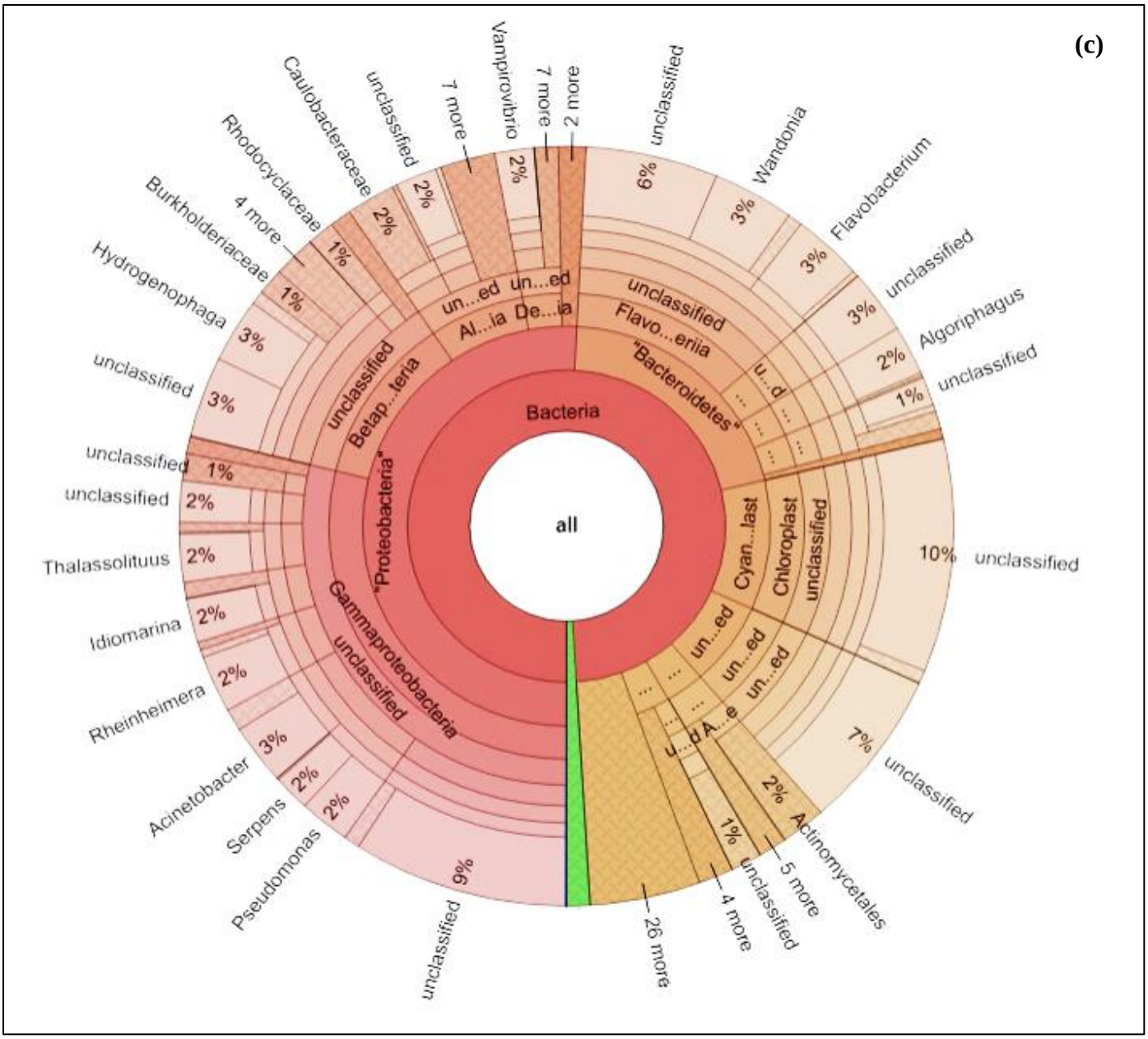


Figure. 1. Krona Chart visualization of the microbial community composition up to the genus level in wastewater bacterial populations from (a) PC, (b) MC, and (c) OC samples.

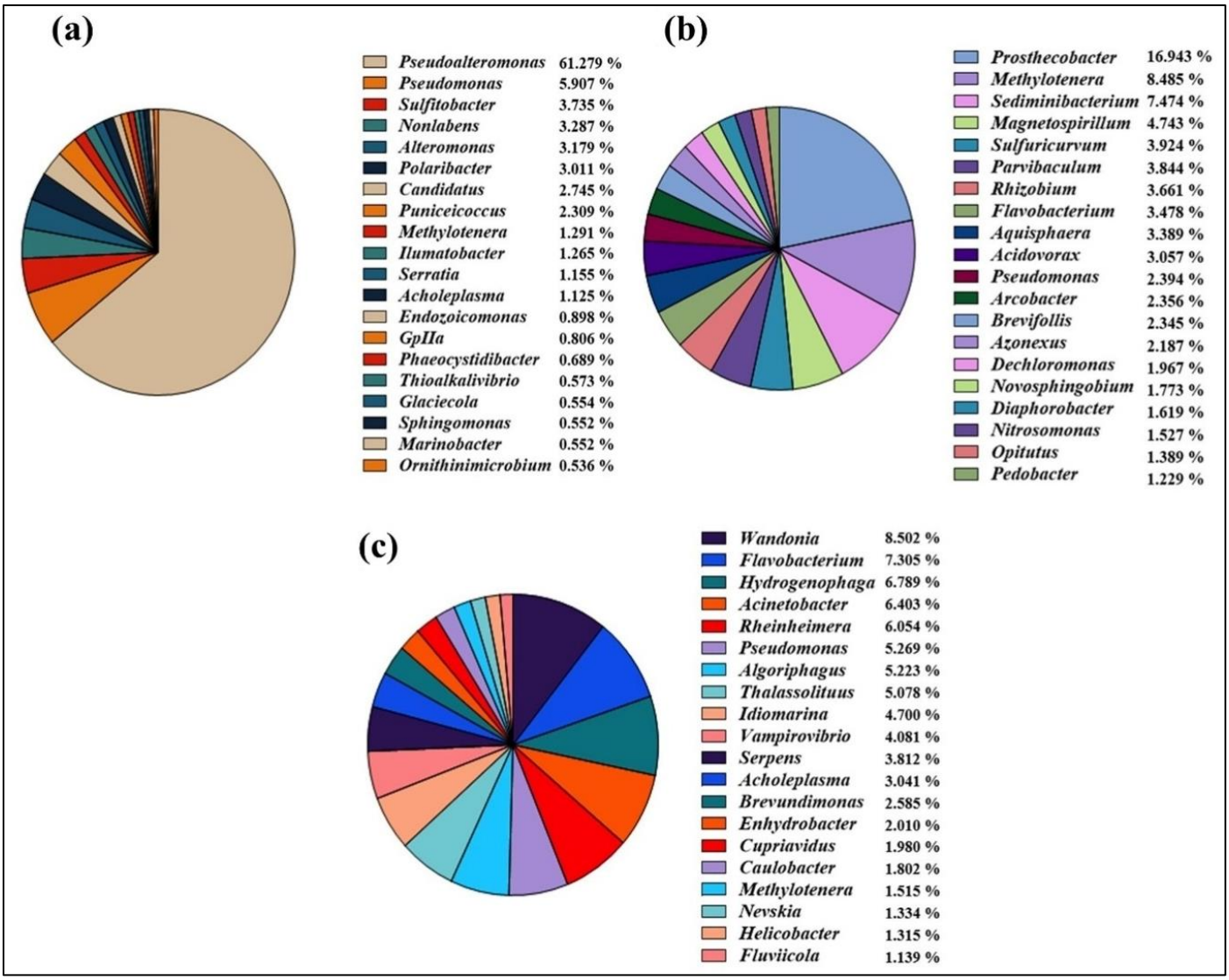
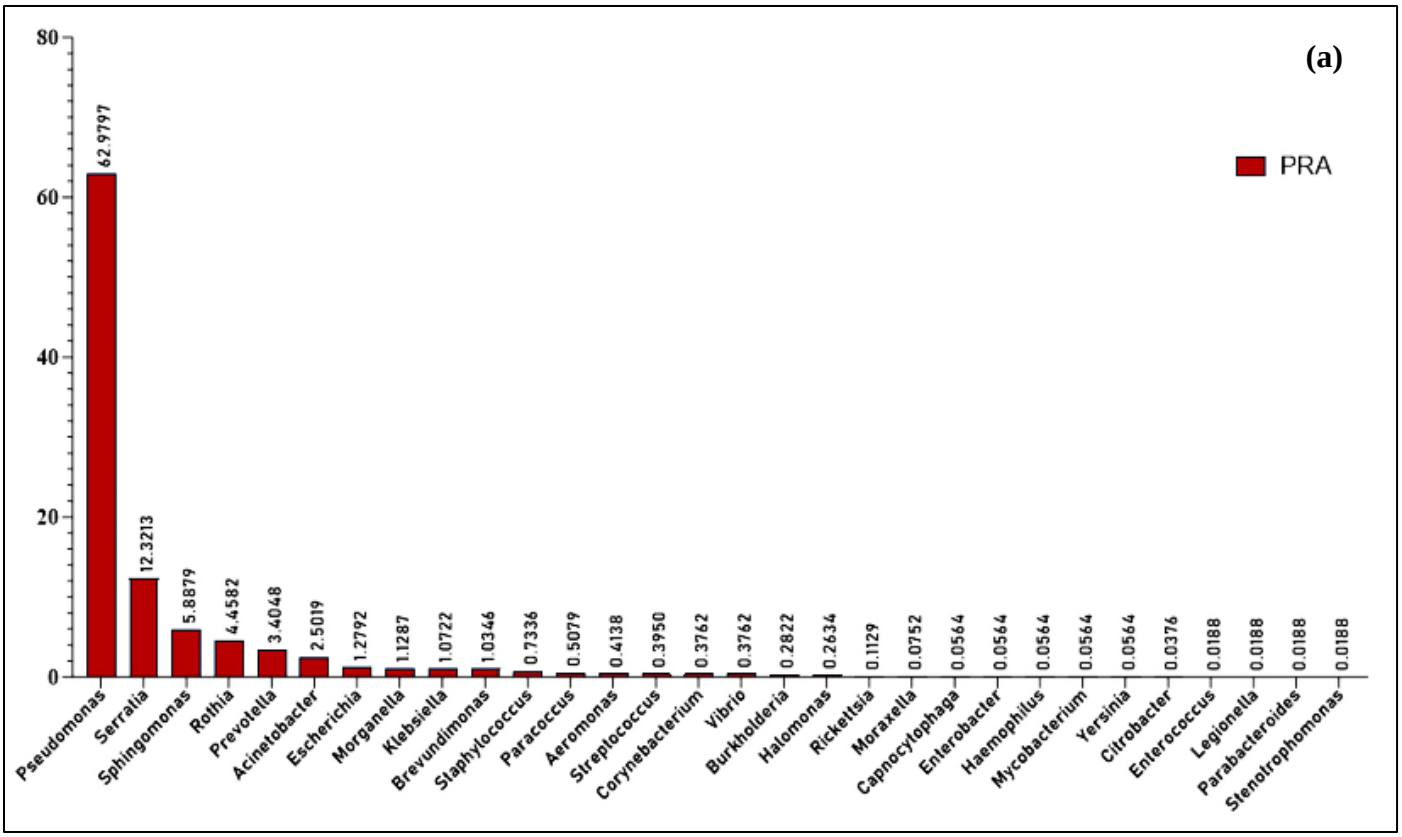
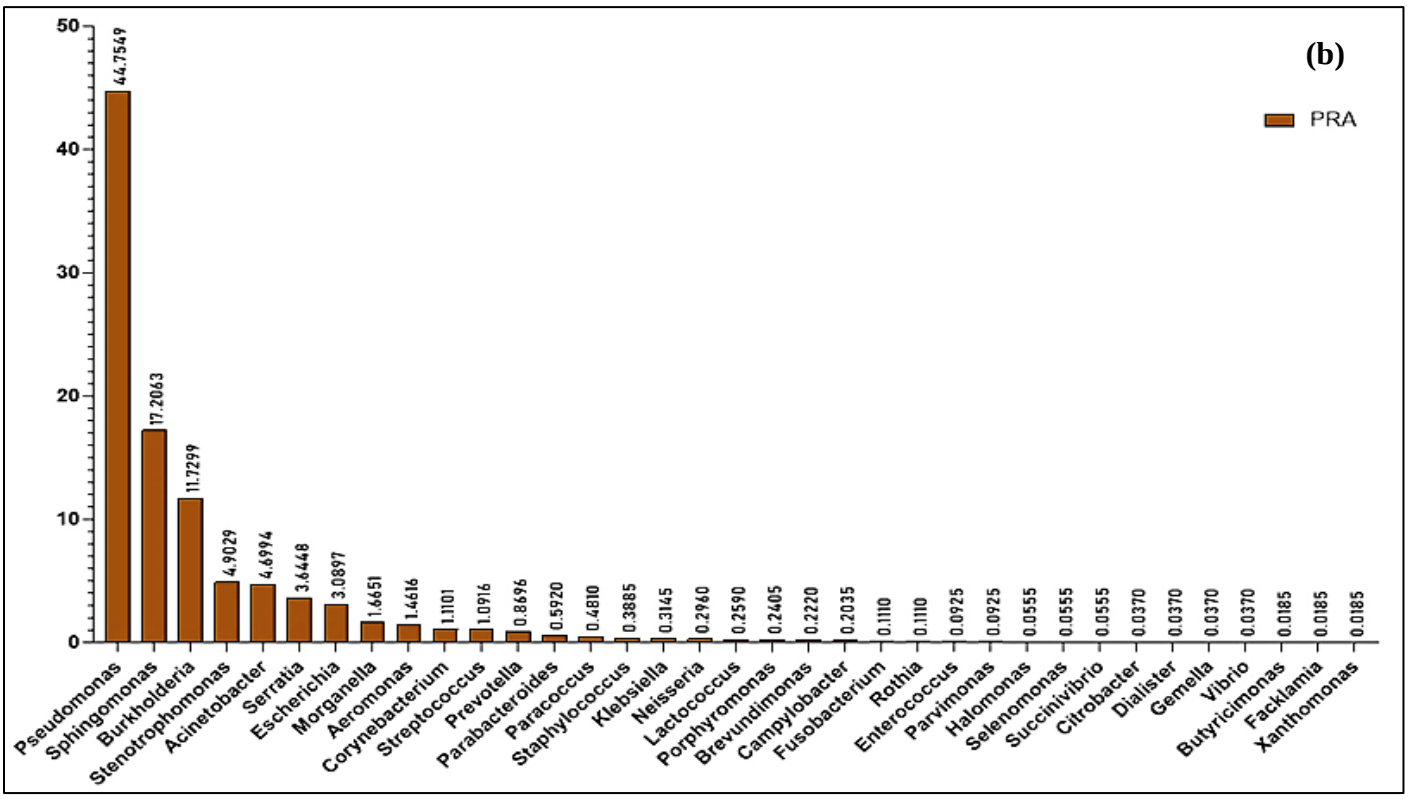


Figure.2. Pie chart showing top 20 bacterial genera along with their corresponding percent abundance value across wastewater collected from (a) PC; (b) MC and (c) OC samples


(a)
PRA
80
60
40
20
0
62.9797
12.3213
5.8879
4.4582
3.4048
2.5019
1.2792
1.1287
1.0722
1.0346
0.7336
0.5079
0.4138
0.3950
0.3762
0.3762
0.2822
0.2634
0.1129
0.0752
0.0564
0.0564
0.0564
0.0564
0.0564
0.0376
0.0188
0.0188
0.0188
0.0188
Pseudomonas
Serratia
Sphingomonas
Rothia
Prevotella
Acinetobacter
Escherichia
Morganella
Klebsiella
Brevundimonas
Staphylococcus
Paracoccus
Aeromonas
Streptococcus
Corynebacterium
Vibrio
Burkholderia
Halomonas
Ricketsia
Moraxella
Capnocylophaga
Enterobacter
Haemophilus
Mycobacterium
Yersinia
Citrobacter
Enterococcus
Legionella
Parabacteroides
Stenotrophomonas


(b)
PRA
50
40
30
20
10
0
44.7549
17.2063
11.7299
4.9029
4.6994
3.6448
3.0897
1.6651
1.4616
1.1101
1.0916
0.8696
0.5920
0.4810
0.3885
0.3145
0.2960
0.2590
0.2405
0.2220
0.2035
0.1110
0.1110
0.0925
0.0925
0.0555
0.0555
0.0555
0.0370
0.0370
0.0370
0.0370
0.0185
0.0185
0.0185
Pseudomonas
Sphingomonas
Burkholderia
Stenotrophomonas
Acinetobacter
Serratia
Escherichia
Morganella
Aeromonas
Corynebacterium
Streptococcus
Prevotella
Parabacteroides
Paracoccus
Staphylococcus
Klebsiella
Neisseria
Lactococcus
Porphyromonas
Brevundimonas
Campylobacter
Fusobacterium
Rothia
Enterococcus
Parvimonas
Halomonas
Selenomonas
Succinivibrio
Citrobacter
Dialister
Gemella
Vibrio
Butyricimonas
Facklamia
Xanthomonas

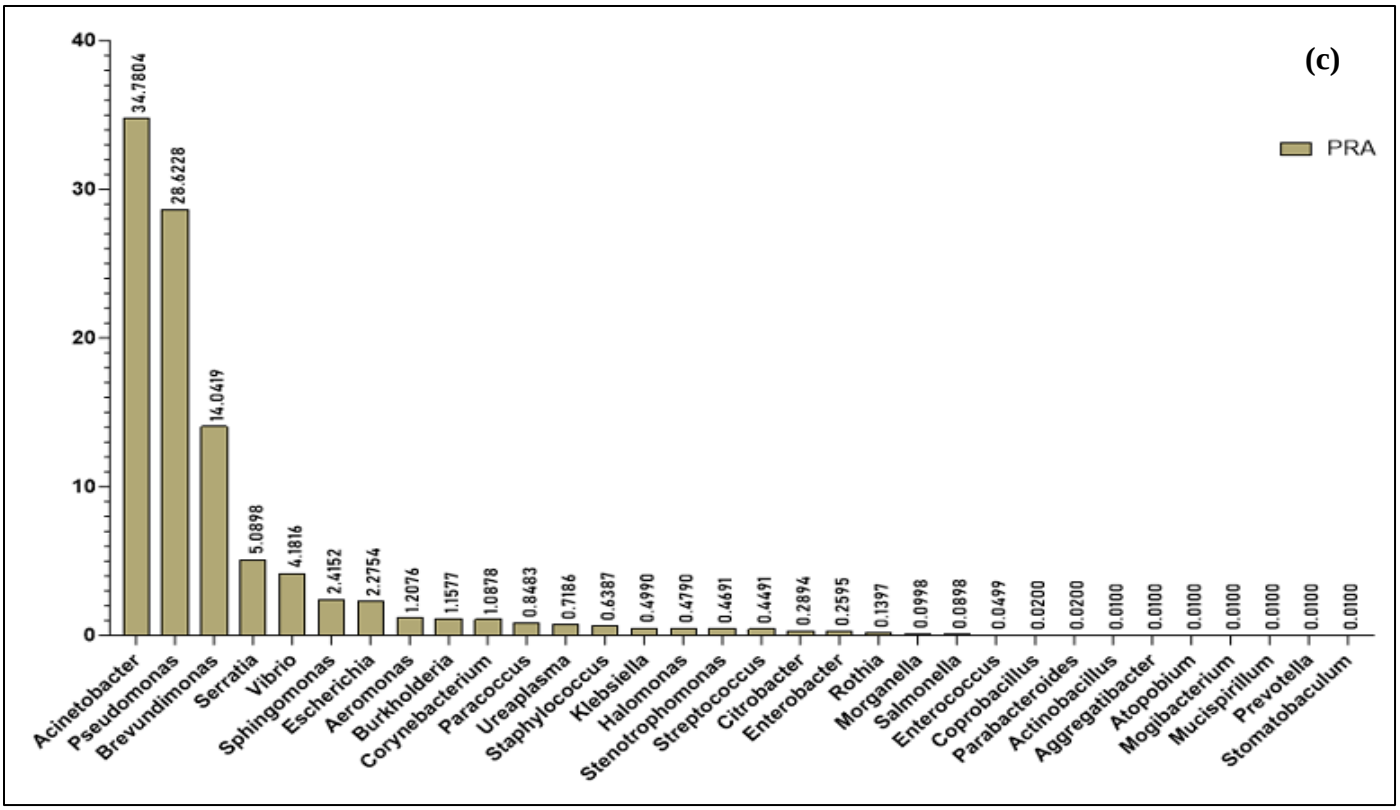
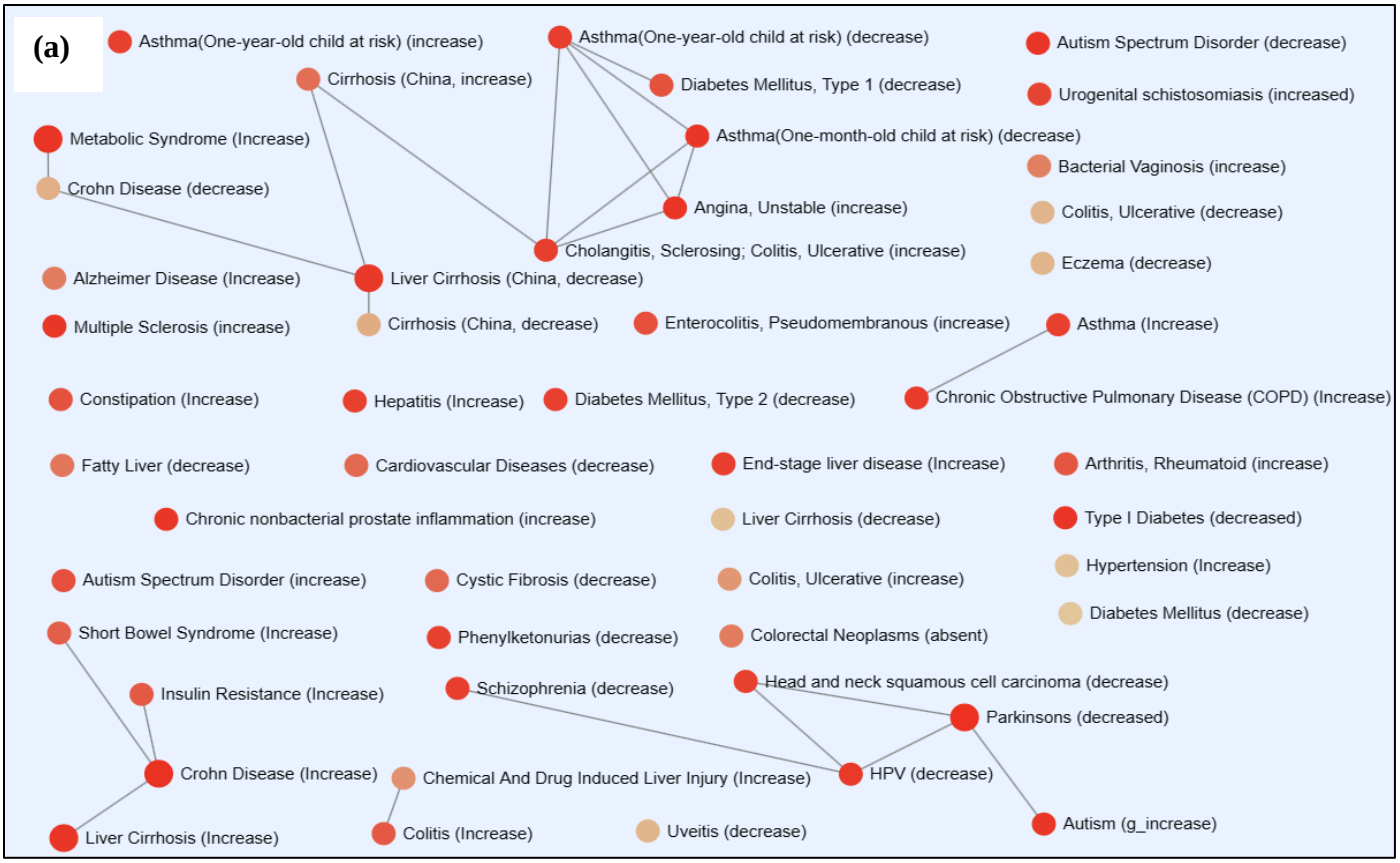


Figure.3. Column chart representation showing percent abundance value of Prevalent Pathogenic bacterial genera present in (a) PC; (b) MC and (c) OC samples under study



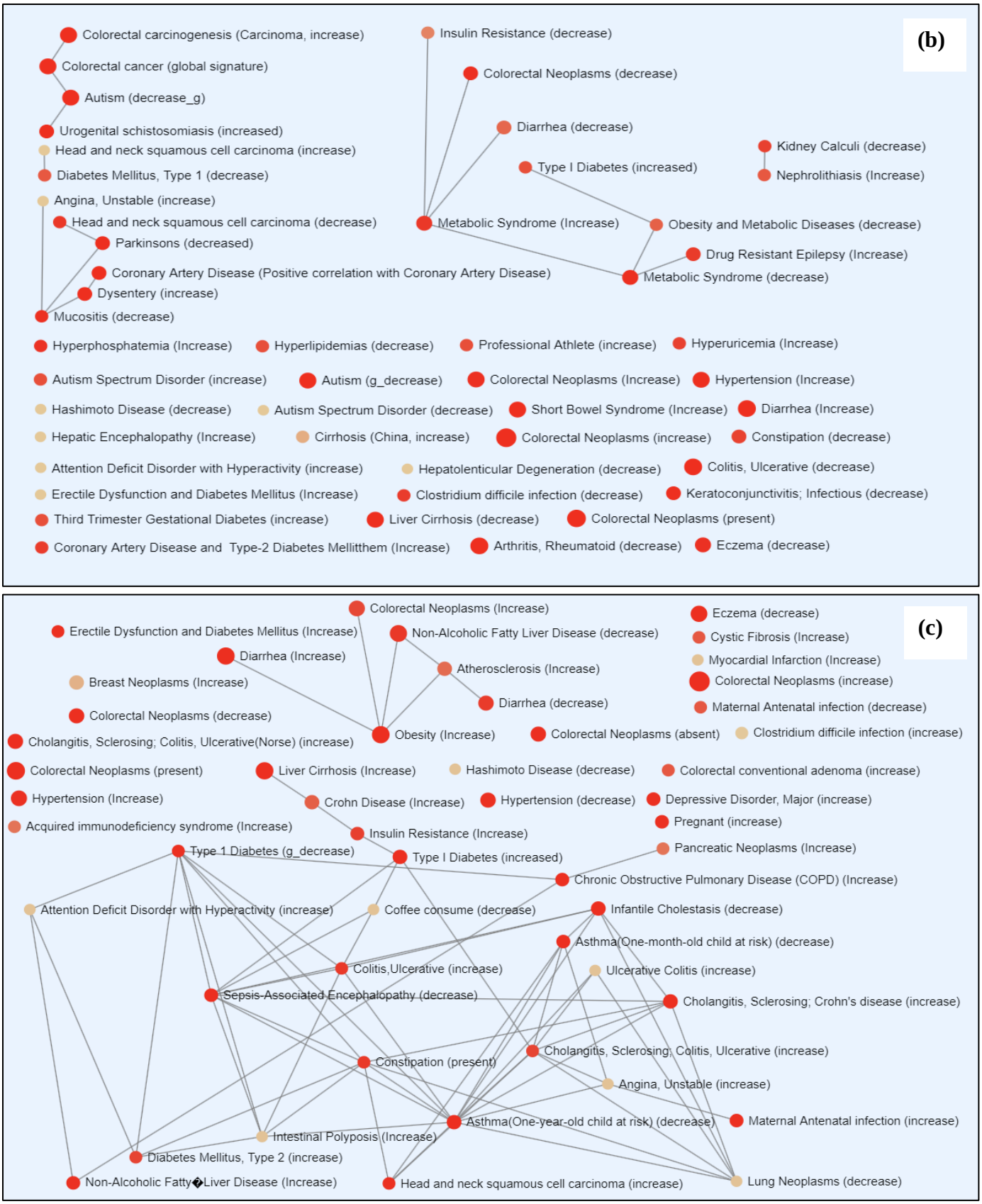


Figure.4. Disease Network predicting the pathogenic load of the pathogenic bacterial members across the samples under study (a) PC; (b) MC and (c)OC

Comparative analysis

Comparative analysis of wastewater bacterial profiles revealed 150 bacterial genera to be common across the three samples under study (Figure.5). A column chart had been prepared to illustrate the relative abundances of the top 20 Pathogenic bacterial genera across different samples under study (Figure.6).

Notably, *Pseudomonas* demonstrates relatively consistent abundance in both Pre-COVID (PC) and Post-COVID (OC) samples, with about half the titre had been observed in Mid-COVID (MC) wastewater samples. Several genera such as *Polaribacter, Prevotella, Ilumatobacter, Cloacibacterium, Gaiella, Rothia, and Arthrobacter* exhibited significantly higher abundance in wastewater collected before the COVID-19 period i.e in PC sample. However, these genera had minimal presence in both MC and OCsamples. On the other hand,*Methylotenera, Sphingomonas, Rhizobium* exhibited a marked increase in abundances during mid-COVID period with a significant reduction in post-COVID phase (Figure.6). A sharp rise in the abundances of *Rheinheimera* and *Acholeplasma* is noticeable in the OC sample (Figure.6).

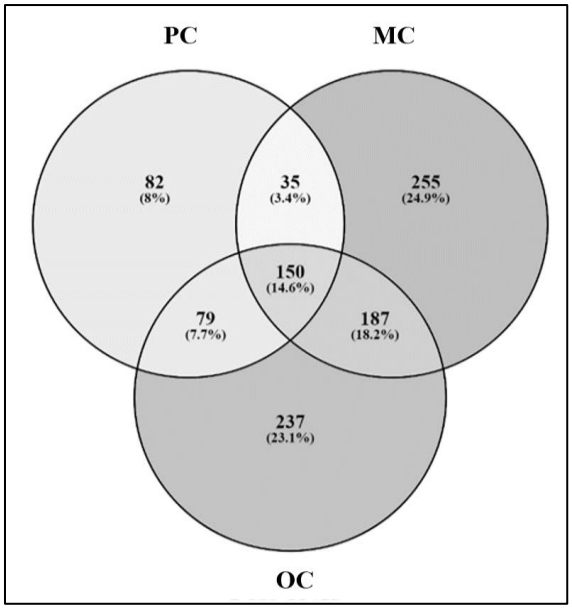


Figure. 5. Venn diagram of unique and common bacterial genera across the three sample under study

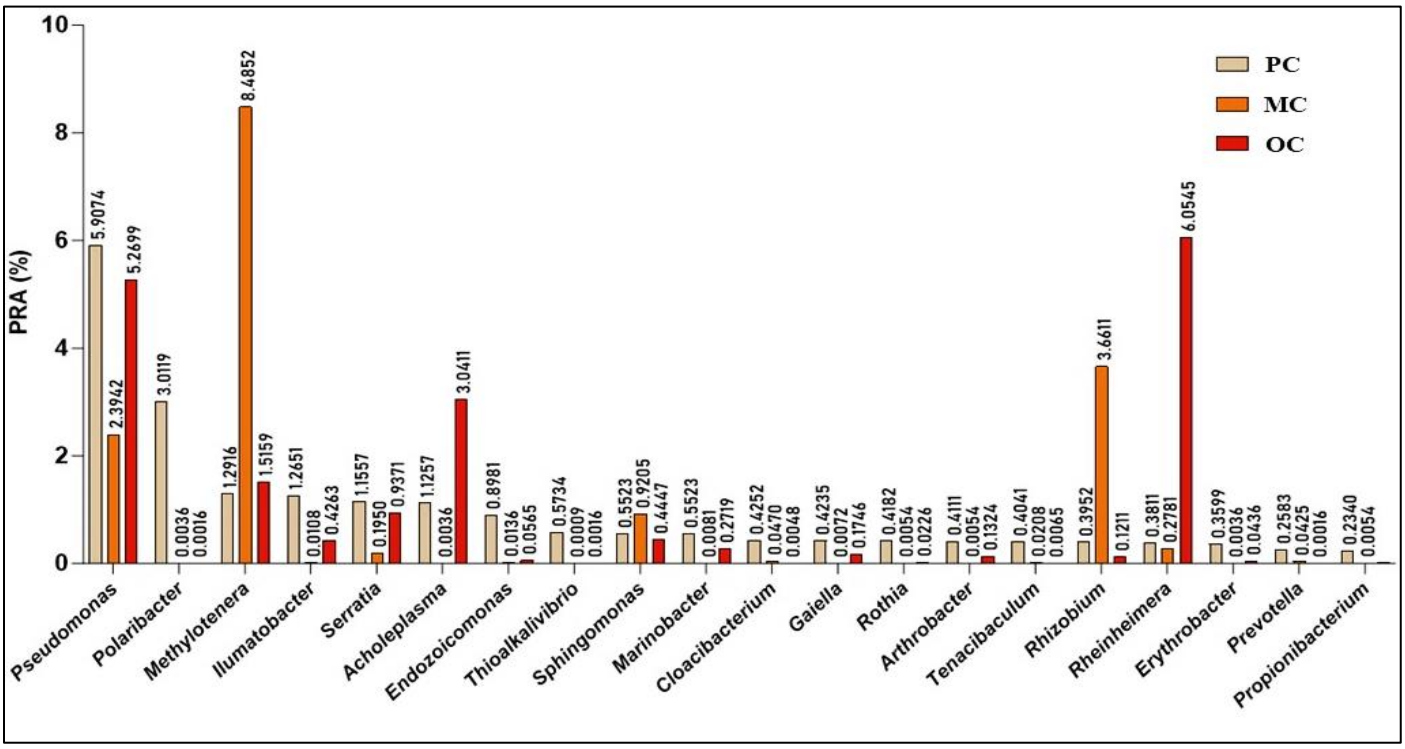


Figure. 6. Comparative abundances of top 20 common genera across the samples under study

The heatmap of core functions exhibited by the bacterial community across the three wastewater samples under study has been shown in figure 7. MC sample exhibited greater number of enriched pathways as compared to the wastewater samples collected during pre and post pandemic period. Notably, during the middle of the COVID era, the MC sample exhibited a distinctive peak in enrichment for various antibiotic resistance pathways such as beta-lactam, vancomycin, and platinum drug resistance (Figure.7).

India's COVID-19 pandemic began in January 2020, with Kerala reporting the first positive case. The number of cases soared to nearly one lakh sixty-four thousand within a

month, reaching 38.2 million by June 2022, marking a shift in the overall trend(Figure.8). The Indian Government implemented a strict nationwide lockdown on March 25, 2020, halting non-essential activities like transportation, businesses, and religious gatherings, extending the lockdown multiple times initially starting with 21 days [24]. Indian government launched the "Shramik Special" initiative at six weeks into lockdown, facilitating the safe return of migrants to their home states. Starting in May 2020, 4621 special trains were operated, rescuing 6 million passengers. Out of 600 million internal migrants, over 100 million workers undertook reverse migration during the first wave of pandemic primarily due to employment difficulties. The Sundarbans, with a significant migrant population employed in Kerala, also experienced a substantial influx of the migrant work force, with 250,000 to 300,000 returning to their families accounting for roughly 51% of the households in this area [3, 25, 26, 27].

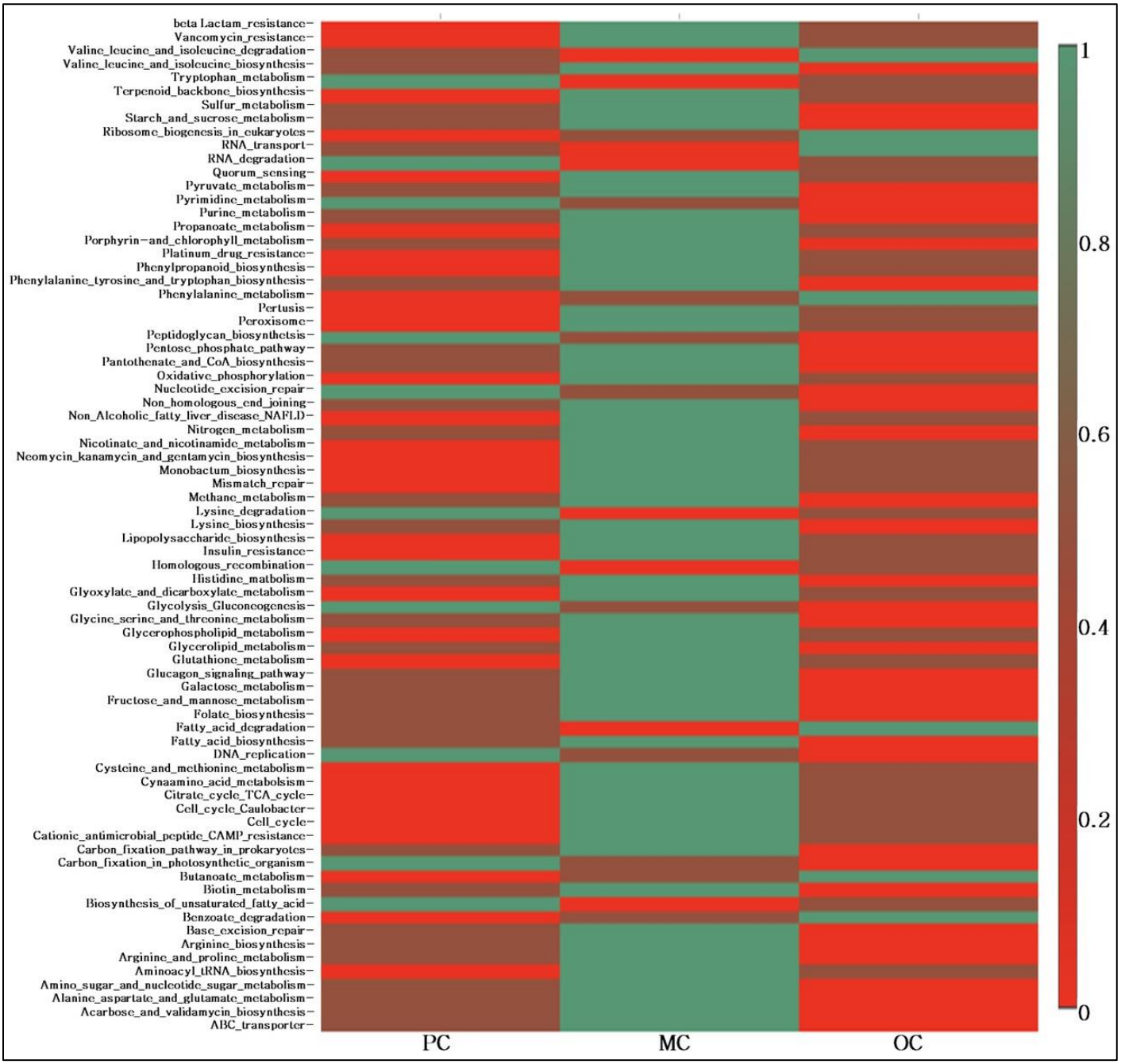


Figure. 7. Functional profiling of bacterial community through heatmap representation ofthe Enriched Metabolic Pathways expressed by each of the community across the samples under study

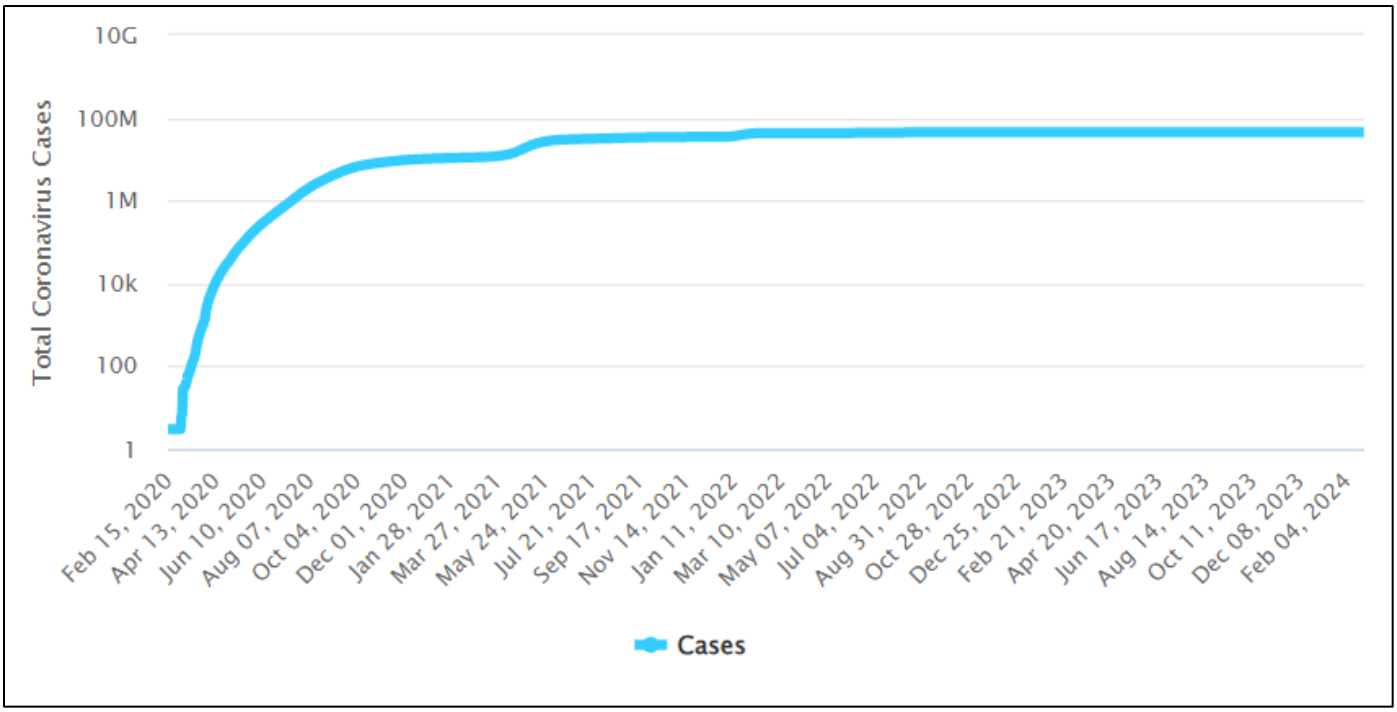


Figure. 8. Statistics of Coronavirus positive cases in India (Source: https://www.worldometers.info/coronavirus/country/india/)

The phenomenon significantly impacted various fields, including healthcare and agriculture sectors and hence made a noticeable shift in wastewater bacterial dynamics. Due to the nature of the pandemic there is sparse information available from official platforms and thus data points in terms of reverse migration and stabilization are mostly restricted to the print and electronic media reports, which are often unreliable. For understanding the possible association of the increase in human population load with the changes in bacterial abundances we performed a simple correlation and results indicate that there is positive correlation among the two variables under study; which was further validated using a simple linear regression analyses. (Supplementary Figure. 2 and 3) Emergence of the pandemic in late 2019 triggered a global emphasis on hygiene practices like handwashing, mask-wearing, and surface disinfection which potentially impacted transmission dynamics of multiple pathogens, including the nosocomial ones like *Pseudomonas, Acinetobacter, Staphylococcus* etc. A retrospective analysis conducted by Smith et al. [28] observed a significant decline in the number of hospital-acquired infections attributed to *Pseudomonas* following the implementation of stringent infection control measures in healthcare facilities. Similarly, a multicenter study byTham et al. [29] reported a reduction in the incidence of methicillin-resistant *Staphylococcus* infections among hospitalized patients during the peak of the pandemic, coinciding with heightened infection control protocols [28, 29]. In line with other observations, our research revealed a significant reduction in *Pseudomonas*titre, by about a half with a percent abundance value of 2.39 during the Mid-COVID period, followed by a subsequent elevation in the Post-COVID phase. The observed decline in abundance could be attributed to several factors associated to population-wide hygiene interventions. Enhanced hand hygiene practices, including the use of alcohol-based hand sanitizers and proper handwashing techniques, could have reduced the likelihood of contamination and subsequent transmission of pathogens via healthcare workers and environmental surfaces.

In contrast, another emerging opportunistic bacterial genus *Sphingomonas* linked to septicaemia, saw a surge in abundance during the Mid-COVID sampling period aligning with the findings by Rohilla et al. [30] where *Sphingomonaspaucimobilis* exhibited significant abundance in urine and blood samples from hospital outpatients in Uttarakhand, India in 2020. Adding to the concern, 61% of *Sphingomonas* isolates showed resistance to colistin and other antibiotics, indicating multidrug-resistant strains [30-33]. COVID infections disrupted environmental bacteriome dynamics, leading to antibiotic overuse and ecological niches favouring*Sphingomonas* growth. Increased patient load in hospitals may have exacerbated contamination levels, fostering *Sphingomonas* growth.

Before India's lockdown, the Rabi season was underway, with harvesting planned during the lockdown. During the Covid era, various local agricultural practices such as maximizing crop rotation for nitrogen fixation, particularly with leguminous crops like pulses gained attention due to limited accessibilities to chemical and biofertilizers. These practices were noted to have close association with elevation in nitrogen-fixing bacteria titre which may have discharged these symbiotic bacteria into wastewater systems via agricultural runoffs [34]. Consistent with this fact, our data revealed a marked increase in abundances of few of the nitrogen fixers like *Methylotenera*, *Rhizobium* in the wastewater sample collected in the Mid-COVID period. Similar trends were noted in a recent study conducted by Dhar *et al.* [35], where high throughput mapping of rhizospheric soil in the vast areas of the Indian Sundarbans, including Rangabelia, Satjelia, and Kumirmari, revealed the predominance of *Rhizobium* and *Sphingomonas* consistently across all surveyed habitats. These genera were notably associated with the rhizospheres of *Ceriops* plants, renowned for their utilization in both timber and non-timber forest products such as honey, tannins, and medicinal extracts. The pandemic has led to socioeconomic consequences like food insecurity and hence potentially affecting sustainable resource usage in Indian Sundarbans [35, 36, 37]. Increased agricultural practices, including increased fertilizer and pesticide use, can select antibiotic-resistant bacteria, disrupting soil microbiome balance, potentially impacting soil health and plant productivity [38]. Intensified agricultural practices often involve increased fertilizer and pesticide application, which can select for bacterial members, resistant to these chemicals [36]. Studies have shown that antibiotic resistance genes in soil bacteria can increase with higher antibiotic use in agriculture [37]. The rise of certain resistant bacteria might come at the expense of other beneficial groups crucial for nutrient cycling and decomposition. This can disrupt the delicate balance within the soil microbiome, potentially impacting soil health and plant productivity [38].

Prediction of the core sets of enriched metabolic functions in microbial community present across the wastewater samples under our study sites revealed a significant increase in genes associated with several narrow and broad-spectrum antibiotics resistance, possibly linked to the reverse exodus of migrant labourers to Indian Sundarbans. Factors such as limited healthcare access, self-medication, and antibiotic misuse could have contributed to antibiotic resistance. Crowded camps and unsanitary conditions can also facilitate the spread of resistant bacteria as per the documentation by Zhao et al. [39]. Arshad *et al.* [40] emphasized comparable findings in their article, associating the emergence of wastewater bacterial communities in lower-middle-income countries with the unjudicial use of antimicrobial agents.In this particular scenario, sewage surveillance offers a thorough understanding of the microbial dynamics as well as changes in occurrence and abundance of antibiotic-resistant bacteria, linked to the movement of migrant labourers allowing for proactive public health measures to be implemented to protect both migrant populations and the communities they interact with, specifically in a complex ecosystem as the Indian Sundarbans.

CONCLUSION

Data from WBE can guide specific public health actions aimed at reducing the transmission of infectious diseases among migrant labourers and the broader population. These actions may encompass launching vaccination drives, enhancing sanitation facilities, and bolstering health education programs. By quantifying concentration of specific pathogens in sewage, researchers can have an estimate of the number of infected individuals in a community, even if they are not diagnosed. This information can be used to predict future outbreaks and allocate resources effectively. In a nutshell our study suggests that the reverse migration of wage labour during the COVID 19 pandemic posed an adverse effect on the overall microbial dynamics of the region. However, this study underscores the importance of utilizing metagenomics-guided wastewater surveillance as an efficient and cost-effective way to gain valuable insights into the community health and to inform public health policies. Additionally, this study emphasizes the need for careful monitoring and adjustments to sewage system operations to maintain effectiveness amidst demographic changes.

DECLARATION

Authorship contributions

Rupsha Karmakar: Investigation, Formal analysis, Writing Original Draft, Mahashweta Mitra Ghosh: Supervision, Resources, Funding acquisition; Sayak Ganguli: Conceptualization, Investigation, Supervision, Funding acquisition, Writing Review & Editing, All authors approve the final version of the manuscript.

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Ethics approval and consent to participate

Not Applicable as no human/animal samples or respondents were recruited for the study and only environmental samples were analysed.

Competing interests

The authors declare no competinginterest.

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