Journal of Advanced Zoology
ISSN: 0253-7214
Volume ***40*** *Issue* ***1*** *Year* ***2019*** *Page* ***159-165***

Microbial Communities and Antibiotic Resistance Genes in Agricultural Landscapes: An Indian Study

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Abstract

Antibiotic resistance genes (ARGs) have become an environmental and public health crisis in all parts of the world due to their emergence and spread in agricultural ecosystems. The soilwater interface in India, where intensive agricultural farming and the extensive use of antibiotics in livestock farming is prevalent, is a significant sink and a pathway of transmitting resistant microorganisms. The paper presents a research on the microbial community organization and ARGs profiles of agricultural soils and irrigation waters of the chosen farms, Maharashtra and Punjab. A varied microbial community comprising of Proteobacteria, Firmicutes and Actinobacteria, and large amounts of ARGs linked with both human and veterinary antibiotics, were identified using a combination of field sampling, physicochemical analysis, 16S rRNA gene sequencing and quantitative PCR of selected ARGs (tet, sul, bla, qnr). The comparative analysis showed that there was increased ARG abundance in the regions having increased manure and wastewater irrigation level. The findings indicate the significant interconnection between agricultural activities, microbial ecology, and distributing of resistance. Results highlight the importance of the policy intervention and sustainable practices in reducing the ARG proliferation in the Indian agricultural systems.

Keywords: agriculture soil, microbial community, antibiotic resistance genes, environment microbiology, India.

Introduction

The resistance to antibiotics has become one of the biggest issues facing world health in the 21 st century. Although the issue of clinical misuse of antibiotics is well-recognized, the environmental aspect of resistance is becoming more and more acknowledged (Berendonk et al., 2015). Given the widespread application of antibiotics in the agricultural sector to treat crops and livestock and in aquaculture, agricultural landscapes serve as primary sources and sources of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Zhu et al., 2013). In India, the high rate of agriculture development and the use of untreated manure and wastewater to irrigate the lands provide the soil and water systems with the favorable conditions of the ARGs proliferation (Diwan et al., 2017).

The relationships between micro communities and ARGs in such environments are complicated, including horizontal gene transfer, antibiotic residue selective pressure and environmental conditions that include soil pH, organic carbon, and moisture (Martínez, 2008). This knowledge of the structure of these microbial communities and the spread of ARGs is paramount in the development of specific interventions to slow down the dissemination to the environment and subsequent human exposure.

The paper is a report of a field and a laboratory based research work in Indian agricultural fields aimed at characterizing the microbial communities and ARGs in terms of their diversity, abundance, and their relationship with agricultural activities.

Background of the Study

India is both the largest human health and one of the leading veterinary antibiotic consumers in the world (Van Boeckel et al., 2015). The antibiotics are not metabolized and released in large amounts in soil and water systems in their form of manure applied to the soil and wastewater used to irrigate the soil (Kumar et al., 2020). The agricultural soils therefore become ideal microbial habitats where microbes adapt and exchange genes especially via plasmids and transposons (Heuer & Smalla, 2012).

In more intensive agricultural places such as Punjab and Maharashtra where intensive farming overlaps with livestock farming, repetitive introduction of antibiotic residues into the environment leads to the selection of the resistant strains within the indigenous microbial communities in the soil. A number of researches have revealed that Indian agricultural soils contain genes that provide resistance to tetracyclines, sulfonamides, and 8-lactams (Sharma et al., 2020; Sahoo et al., 2012). These ARGs may be transmitted to human pathogens under different environment pathways, such as runoff, food chain and direct human contact.

Justification

Although the topic of Indian agricultural landscapes has been examined systematically all over the globe, there is little study of such landscapes in relation to other regions. Majority of the obtained data are piecemeal, local or laboratory microcosm-based. It would be necessary to:

Know the presence of microbial taxa and resistance genes in the Indian soils and irrigation waters, their baseline. Identification of hotspots and farming methods that facilitate the spread of resistance. Provide policy-related evidence to create interventions on local and national levels. This research fills this knowledge gap through field sampling and molecular analysis to describe microbiome of soil and water in the principal agricultural areas in India and ARG profiles.

Objectives of the Study

- To investigate the microbial community structure of agricultural soil and irrigation waters of sampled Indian areas.
- To determine the concentration of the chosen antibiotic resistance genes (tet, sul, bla, qnr) by means of qPCR.
- To determine the correlation among the farming practices, physicochemical properties, and ARG distribution.
- To offer evidence-based suggestions on the reduction of the spread of ARGs in the agricultural setting.

Literature Review

Research in the world has defined agricultural landscapes as a source of antibiotic resistance (Zhu et al., 2013; Berendonk et al., 2015). Zhu et al. (2013) also illustrated in China that the abundance of ARG in manure-fertilized soils was many times higher than in pristine soils. The European studies were also characterized by similar trends in which frequent applications of manures led to the rise of ARGs including tet(M) and sul1 (Heuer and Smalla, 2012).

In India, evidence is growing. According to Sahoo et al. (2012), there was widespread of ARGs in the soils where wastewater was used as an irrigation in the peri-urban regions. The study conducted by Diwan et al. (2017) identified multidrug-resistant E. coli in rural water bodies around the livestock farms. Sharma et al. (2020) detected various ARGs in paddy soils in Punjab such as blaCTX-M and qnrS. These results are consistent with the trends in the rest of the world but also show the local forces, such as informal antibiotic use and discharge of untreated effluent.

Microbial community and ARG profiles can now be characterized at the same time courtesy of recent developments in high-throughput sequencing (Nesme & Simonet, 2015). A combination of 16S rRNA gene sequencing and qPCR is currently a regular practice in comprehending the ecology of resistance on the landscape level.

6. Materials and Methodology

6.1 Study Sites

The experiment was done in two large agricultural areas of India:

Punjab: This region is characterized by heavy crops of wheat-rice and the use of manure.

Maharashtra: This is characterized by mixed cultivation as well as regular irrigation with wastewater.

Each region was chosen five farms which were the manure-based fields, wastewater-irrigated fields and control (low-input) fields.

6.2 Sample Collection

- Soil samples: Taken at the top 15 cm with the help of sterile augers, merged at five locations in each field.

Water samples: The samples were collected in sterile containers in irrigation channels and tube wells.

Transportation of the samples was done in ice and they were processed within 24 hours.

6.3 Physicochemical Analysis

Standard APHA (2017) protocols determined soil PH, moisture content, organic carbon and level of nutrients (N, P, K). HPLC was used to test water samples regarding their pH, EC, nitrate, and remaining antibiotics.

6.4 Analysis of Microbial Community

MoBio PowerSoil kits were used to extract DNA. The 16S rRNA gene V3 -V4 amplified and sequenced on the Illumina MiSeq platform. Processing of sequences was done with the help of QIIME2; it was done with SILVA database to classify OTUs.

6.5 Quantification of ARGs

Four target genes were tested using quantitative PCR:

In addition, tetracycline resistance is present in bacterial cells (tet(M)).

- sul1 (sulfonamide resistance)
- blaCTX-M (β-lactam resistance)
- qnrS (quinolone resistance)

The copies of the genes were standardized against the abundance of 16S rRNA genes.

6.6 Statistical Analysis

QIIME2 was used to measure alpha and beta diversity measures. ANOVA was used to evaluate the differences between types of fields ($p < 0.05$). Pearson correlation in R was used to produce correlations between ARGs and environmental variables.

Results and Discussion

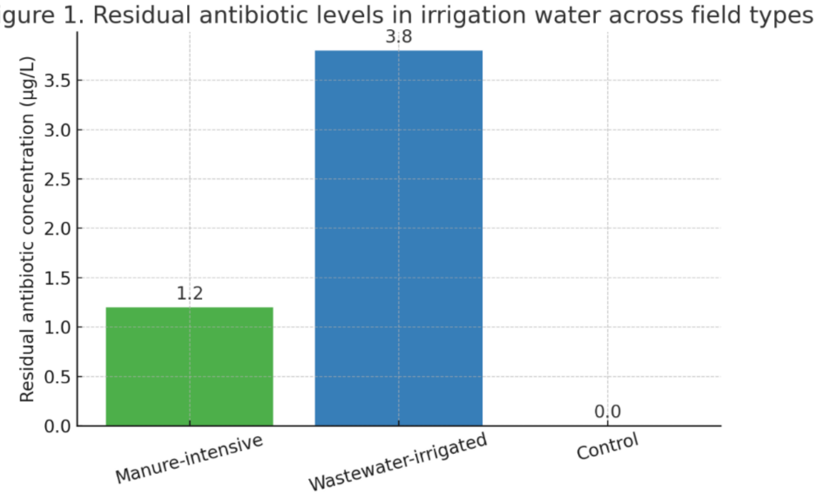
7.1 Physicochemical Characteristics of Soil and water.

There was a significant difference in physicochemical parameters of soil and irrigation waters in different field types (Table 1). Fields that were subjected to manure had more organic carbon and nutrients whereas fields that were irrigated with wastewater had higher EC and nitrate levels than control areas.

Table 1. Physicochemical characteristics of soils and irrigation waters across different field types (mean ± SD).

Parameter	Manure-intensive	Wastewater-irrigated	Control (Low-input)
Soil pH	7.4 ± 0.2	7.6 ± 0.3	7.2 ± 0.1
Organic carbon (%)	1.85 ± 0.12	1.35 ± 0.08	0.95 ± 0.06
Available N (mg/kg)	145 ± 10	120 ± 8	90 ± 7
Moisture (%)	20.4 ± 2.1	18.2 ± 1.9	15.3 ± 1.5
Water EC (µS/cm)	780 ± 35	1020 ± 45	610 ± 30
Nitrate (mg/L)	14.2 ± 1.5	22.5 ± 1.9	9.8 ± 1.1
Residual antibiotics (µg/L)	1.2 ± 0.2	3.8 ± 0.3	ND

Figure 1. below demonstrates the difference in the residual antibiotics of the irrigation water with a significant difference between the wastewater-irrigated and the control fields.



The results in the case study reveal that the level of antibiotic residues in the fields was lower in the past than in the present. The case study shows that the pharmaceutical content of residues in the different fields, including the amount of the antibiotics in the fields, were lower in the past than current.

These variants indicate that the processes of manure and wastewater can dramatically change the chemistry of the soil-water environment by providing excellent habitats to grow microorganisms and even favor the emergence of resistant strains.

The structure of the microbial community will be assessed using pragmatic nonparametric statistics like the Kolmogorov-Smirnoff test to determine how the community size relates to its stability, persistence, and composition changes over time. 7.2 Microbial Community Structure Microbial community structure will be evaluated with the pragmatic nonparametric statistics such as the Kolmogorov-Smirnoff test to identify how the community size is related to its stability, persistence and compositional dynamics over time.

In all of the samples, sequencing provided about 1.2 million high-quality reads grouped into 5,240 OTUs. Proteobacteria presented the largest proportion (35%), Firmicutes (22%), and Actinobacteria (18%) and others (Figure 2).

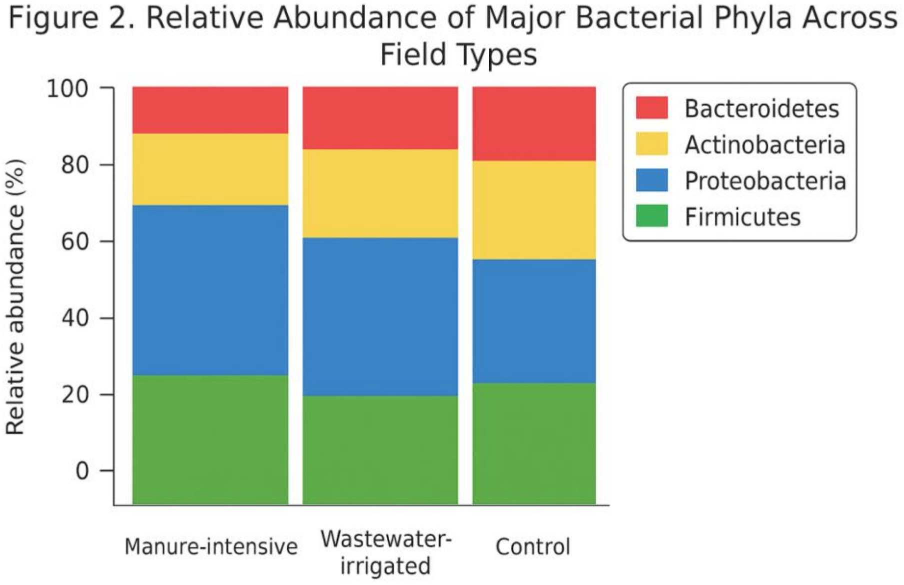


Figure 2. Abundance of major bacterial phyla in soils of various field types relativity

The graph below illustrates a stacked bar chart used to display the phyla along with the type of field in each case:

Fields fertilized with manure and those irrigated with wastewater exhibited greater Firmicutes and Bacteroidetes relative abundance, which are commonly linked with feces contamination and the genome of

resistance (Zhu et al., 2013). The control soils were also highly populated with Actinobacteria and Acidobacteria, which are less affected anthropogenically. Analysis of beta diversity (PCoA) showed that the samples could be easily classified by the type of field, meaning that the management practices have a significant impact on the organization of microbial communities (PERMANOVA, $p < 0.01$). The Abundance of Genes with antibiotic resistance is a characteristic of the bacterial isolate as well.
Quantitative PCR showed that there was a lot more ARG abundance in manure and wastewater fields than in controls (Table 2).

Table 2. Abundance of selected ARGs (log10 gene copies per g soil)

Gene	Manure-intensive	Wastewater-irrigated	Control
tet(M)	6.2 ± 0.3	6.5 ± 0.2	4.8 ± 0.2
sul1	5.9 ± 0.2	6.3 ± 0.3	4.5 ± 0.3
blaCTX-M	5.5 ± 0.3	5.8 ± 0.2	4.0 ± 0.2
qnrS	4.8 ± 0.2	5.1 ± 0.3	3.5 ± 0.2

The soils with the highest ARG levels were those irrigated with wastewater, especially sul1 and tet(M) which are likely to represent the presence of antibiotic residues and fecal contributions. Arguments were still present in control sites, but background environmental resistomes (Martínez, 2008) were 1-2 orders of magnitude lower.

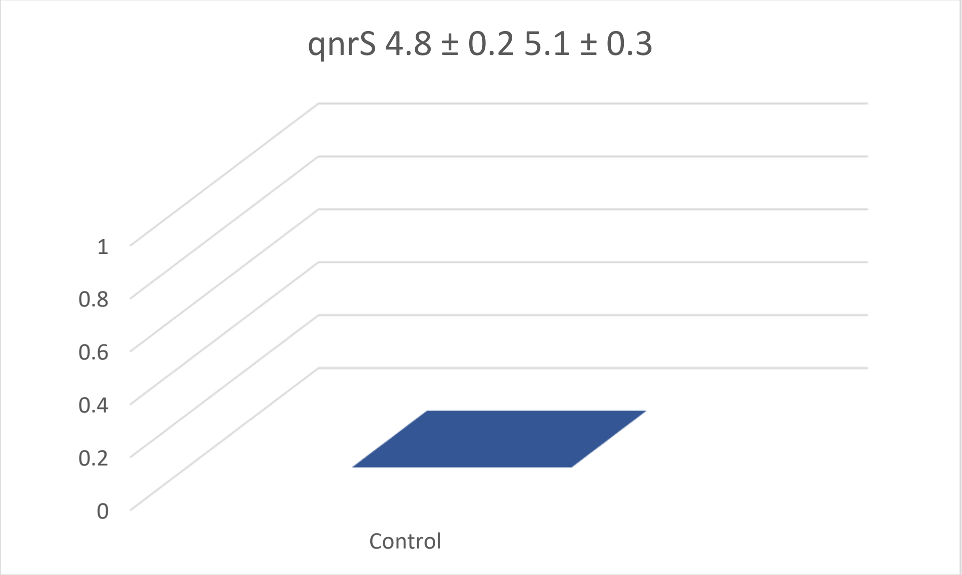


Figure 3: PCoA (beta diversity) plot — *Microbial community clustering by field type.*

7.4 Environmental Factor and ARGs Correlations

Pearson correlation analysis showed the presence of significant positive correlations between ARG abundance and organic carbon ($r = 0.72$, $p < 0.01$), residual antibiotics ($r = 0.81$, $p < 0.01$) and Bacteroidetes abundance ($r = 0.69$, $p < 0.05$). This is in line with the previous evidence on the fact that manure input and antibiotic residues fuel ARG growth (Heuer and Smalla, 2012; Zhu et al., 2013).

7.5 Discussion

These findings are clear evidence that Indian agricultural fields that are receiving manure and waste water inputs are reservoirs of ARGs that support diverse microbial communities that have high resistance gene loads. This is also indicative of some trends being experienced in the rest of the world, with some unique problems in India: informal use of antibiotics, uncontrolled effluent release, and lax regulatory controls (Diwan et al., 2017; Sahoo et al., 2012).
Close dependence between residual antibiotics and ARG abundance implies that the decrease in antibiotic release to the environment would result in the substantial decrease in selective pressure. The further increase in the fecal-associated taxa including Bacteroidetes underlines the role of animal and human waste.

Limitations of the Study

Although this research offers vital baseline information, it is necessary to cite some weaknesses:
- Temporal variation was not represented fully; the samples were only collected in one season of agriculture.
- This was limited to a set of four important genes that were studied by ARGs, which would be more profiled using a metagenomic methodology.
- The potential resistance transfer (e.g. plasmid-mediated mobility) was not directly tested.
- These limitations will be overcome in future work in order to develop a holistic description of agroecosystem ARG dynamics within Indian agroecosystems.

Future Scope

The results leave a number of research and policy initiation opportunities in the future:
- ARGs longitudinal monitoring in seasons and agricultural systems in order to determine temporal processes.
- Metagenomic and metatranscriptomic of functional resistomes and mobile genetic elements.
- Intervention studies that test composting, built wetlands or controlled application of manure in order to reduce the spread of ARG.
- The policy integration, which involves the inclusion of environmental monitoring of ARGs in the national action plans on antimicrobial resistance in India.

Conclusion

The paper has shown that manure-rich and wastewater-covered agricultural landscapes are important sources of antibiotic resistance genes and have a variety of microbial communities due to management practices in India. Environmental factors The abundance of ARG was strongly associated with residual antibiotics and organic inputs, which provided evidence of environmental drivers of the spread of resistance. Enhancing the environmental surveillance, control of waste products and encouraging sustainable farming patterns is important to prevent the spread of ARGs off-farm to other ecosystems and finally to human beings.

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