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Characterization of Multi Heavy Metal Tolerant Bacterial Isolate from Middle Gangetic Plains, India

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ABSTRACT
The research focuses on antibiotic resistance profiles and bacterial compatibility in multi-heavy metal-tolerant bacterial strains. It reveals significant concerns about antibiotic resistance in these bacteria, including resistance to penicillin, streptomycin, tetracycline, ampicillin, and vancomycin. Notably, 96% of the isolates exhibited resistance to vancomycin. The study emphasizes the need for prudent antibiotic use to combat its resistance. Conversely, tetracycline and penicillin showed lower resistance levels at 85% and 77%, respectively, in line with previous research showing diverse resistance patterns among antibiotics and bacterial strains. This highlights the complexity of antibiotic resistance mechanisms, requiring a comprehensive approach. The research also explores bacterial compatibility, revealing that 23 out of 46 bacterial isolates exhibited complete compatibility, suggesting cooperative interactions. Some specific isolates displayed compatibility at 96%, while few had lower compatibility at 75%, indicating the potential for antagonistic interactions. In summary, this study underscores the complex dynamics of antibiotic resistance and compatibility in multi-heavy metal-tolerant bacteria, with implications for clinical antibiotic use and microbial interactions in natural environments. With advancement in microbial ecosystems research, it is essential to unravel these mechanisms and deliberate strategies for addressing antibiotic resistance as a sustainable approach.

Key words: Heavy metal tolerant bacteria, Biocompatibility, Antibiotic Resistance

INTRODUCTION

The Middle Gangetic Plains, spanning across the states of Uttar Pradesh and Bihar in northern India, represent a vital agricultural region characterized by fertile alluvial soil and a rich history of crop cultivation. However, this region faces an escalating environmental challenge that threatens both its agricultural productivity and ecological integrity: heavy metal contamination. The introduction of heavy metals, such as arsenic (As), cadmium (Cd), zinc (Zn), lead (Pb), chromium (Cr), and cobalt (Co), into the soils of the Middle Gangetic Plains has raised concerns regarding soil quality, food safety, and the sustainability of agriculture (Kumar et al. 2021).

Heavy metals enter the environment through various pathways, including industrial activities, irrigation with contaminated water, and the use of agrochemicals. These metals, even at trace levels, can persist in the soil and accumulate over time, posing a potential risk to crops, ecosystems, and human health. The exceptional combination of agricultural prominence and heavy metal contamination in the Middle Gangetic Plains makes it significant pivotal point for research aimed at understanding the dynamics of heavy metal pollution in agricultural landscapes (Dubey et al. 2018).

This study delves into the complex problem of heavy metal pollution in the Middle Gangetic Plains of Uttar Pradesh and Bihar, giving special attention to the examination of antibiotic resistance and antagonistic interactions within the native microbial populations in these metal-polluted locations. Antibiotic resistance has emerged as a pressing global public health issue, making it imperative to comprehend its occurrence and patterns in regions impacted by heavy metal contamination (Fu et al. 2023).

The coexistence of antibiotic resistance and heavy metal resistance in bacteria is a subject of growing importance and intrigue in the field of microbiology and environmental science (Edet et al. 2023). The dual resistances to antibiotics and heavy metals have been separately documented, but their potential interconnectedness and ecological significance remain underexplored. Recent research suggests that there may be cross-resistance or co-selection phenomena, (Ali et al. 2022) where the evolution of resistance to one stressor (e.g., heavy metals) may inadvertently confer resistance to another (e.g., antibiotics), or vice versa (Claudia and Thomas 2012). Such interplay between antibiotic resistance and heavy metal resistance in bacteria underscores the need for a holistic understanding of bacterial adaptation and its broader ecological consequences. Additionally, the potential competitions among microbes in these environments have substantial ramifications for the functioning of ecosystems and strategies for bioremediation (Krishna and Yamarthi 2019).

This study employs a comprehensive approach that encompasses the isolation and characterization of multi-heavy metal-tolerant bacteria and their assessment for antibiotic susceptibility. Additionally, the research investigates the antagonistic behaviors of these bacterial isolates. By examining these interconnected aspects, this paper aims to contribute valuable insights into the complex relationship between heavy metal contamination, antibiotic resistance, and microbial interactions in the unique context of the Middle Gangetic Plains.

MATERIAL AND METHODS

For sampling and isolation of microbes, the rhizospheric soil from the agriculture fields and the underground water samples from hand pumps were collected in the month of December from 17 different arsenic-contaminated locations. The samples were stored at 4°C for further analysis. The samples were subjected to increasing concentration of heavy metal (up to 1.5mM) for three enrichment cycles, following which the isolates were screened for heavy metal resistance for 72 hours at 28 ± 20°C. The presence of growth was regarded as a positive indicator of tolerance to that specific heavy metal. These isolates were further used for antibiotics resistivity and compatibility analysis.

Antibiotic resistance of multi-heavy metal tolerant bacteria was evaluated using agar well diffusion method for the antibiotics viz. Penicillin (10 µg/ml), Streptomycin (25 µg/ml), Tetracycline (30 µg/ml), Ampicillin (25 µg/ml), and Vancomycin (30 µg/ml). Plates were incubated at 30^0C for 24 hrs. The isolates that gave a zone of inhibition were denoted as antibiotic sensitive, while the isolates that did not have any zone of inhibition were recorded as antibiotic resistance. All the experiments were performed in triplicates (Nath et al. 2019).

The biocompatibility test for the isolates was done by cross-streaking method (Balouiri et al. 2016). Using a sterile inoculating loop one bacterial isolate was streaked straight across the center of nutrient agar plate (Streak A). The other isolates were tested by streaking them perpendicularly across the streak A. The plates were incubated at 30^0C for 24 hrs. and plates were observed for the presence of growth or no growth across the line of streak intersect indicating the presence of biocompatibility among the isolates. All the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Antibiotic resistance

After three rounds of heavy metal enrichment of all the samples, 46 heavy metal tolerant microbes were isolated. Majority of the isolates were resistant to all the five antibiotics *viz*. Penicillin, Streptomycin, Tetracycline, Ampicillin and Vancomycin. Isolates (96%) showed highest resistance to Vancomycin, while only 49% of the isolates were resistant to Tetracycline and 77% of isolates to penicillin. Resistance to Streptomycin and Tetracycline by the isolates were 85 and 89%, respectively (Figs. 1, 2).

In the context of our research findings, it is evident that the antibiotic resistance profiles of the isolates are of substantial concern. The observed resistance to a range of antibiotics indicates a worrisome trend of antibiotic resistance among these multi-heavy metal-tolerant isolates. This aligns with a growing body of research that has documented the prevalence of antibiotic resistance in bacterial populations across various environments (Vats et al. 2022).

The highest number of isolates exhibiting resistance to Vancomycin is particularly alarming as Vancomycin is often considered a “last resort” antibiotic for the treatment of serious bacterial infections, making the emergence of resistance to this drug a matter of grave concern (Boneca and Chiosis 2003). On the contrary, isolates demonstrated comparatively lesser levels of resistance tetracycline and penicillin. This diversity in resistance patterns is consistent with prior studies, emphasizing the substantial variations in resistance among different antibiotics and bacterial strains (Sao 2017). Our findings emphasize the importance of cautious

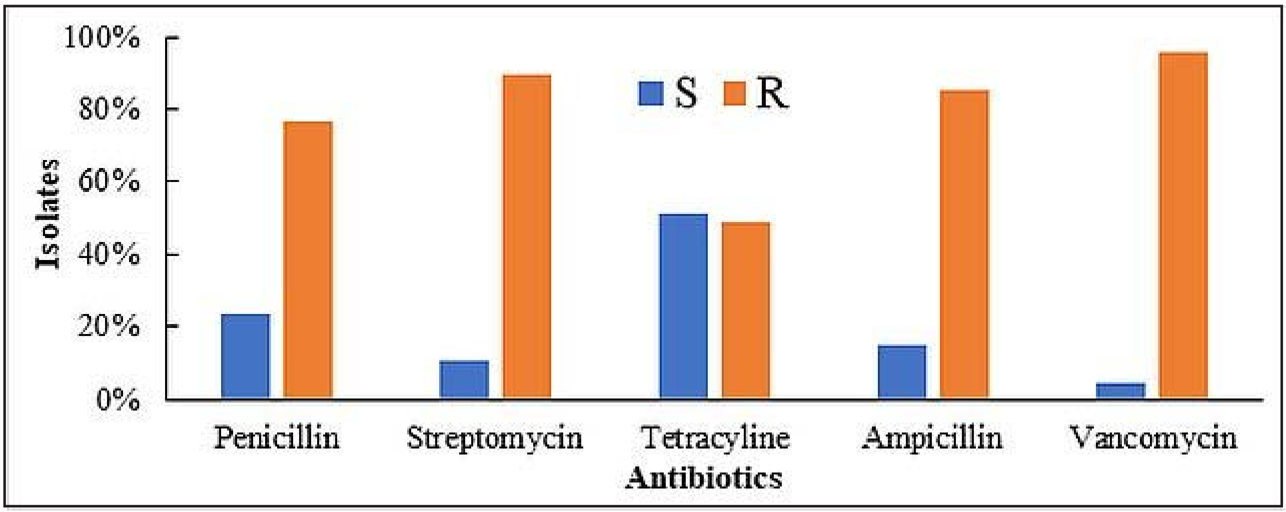
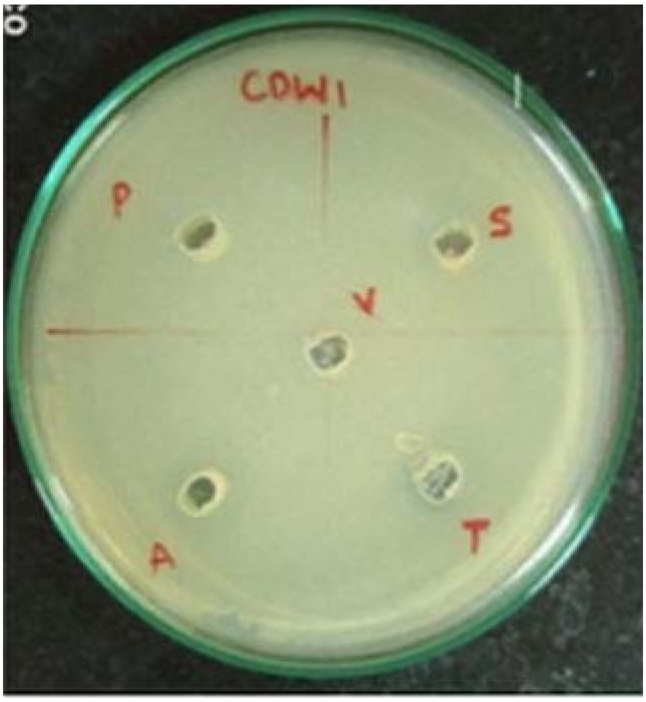


Figure 1. Heavy metal tolerant bacterial isolates exhibiting resistance to antibiotic (S and R denotes Sensitive and Resistance)



Isolate CDW1 showing resistance to all the Antibiotics

Isolate CDW15 showing antibiotic resistance to Streptomycin

Figure 2. Isolates exhibiting antibiotic resistance

antibiotic applications in clinical settings to minimize the selection pressure for resistance.

Biocompatibility among isolates

Twenty three out of the 46 isolates showed total compatibility with all the other isolates (Figs. 3, 4). The isolates CDW1, CDW6, CDW10 showed 96% biocompatibility with other isolates, while isolate CDW21 showed lowest compatibility. The assessment of bacterial compatibility offers insights into the potential interactions and coexistence of these isolates, which has implications for their ecological roles. Total compatibility of bacterial isolates with each other suggesting the possibility of cooperative relationships or niche overlap. This is consistent with other studies (Hibbing et al. 2010).

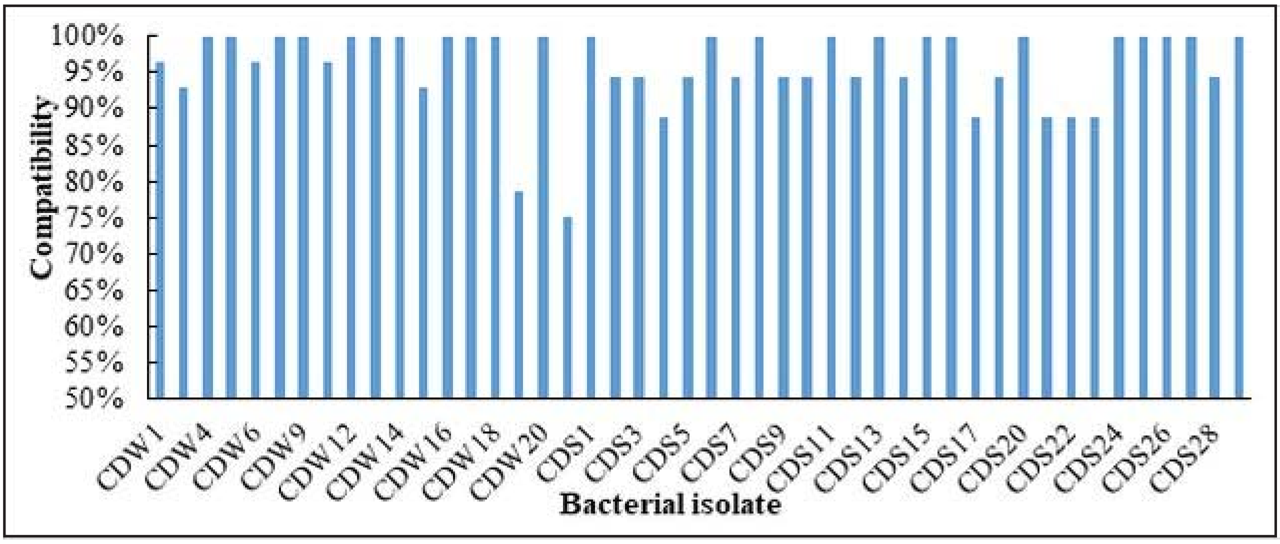
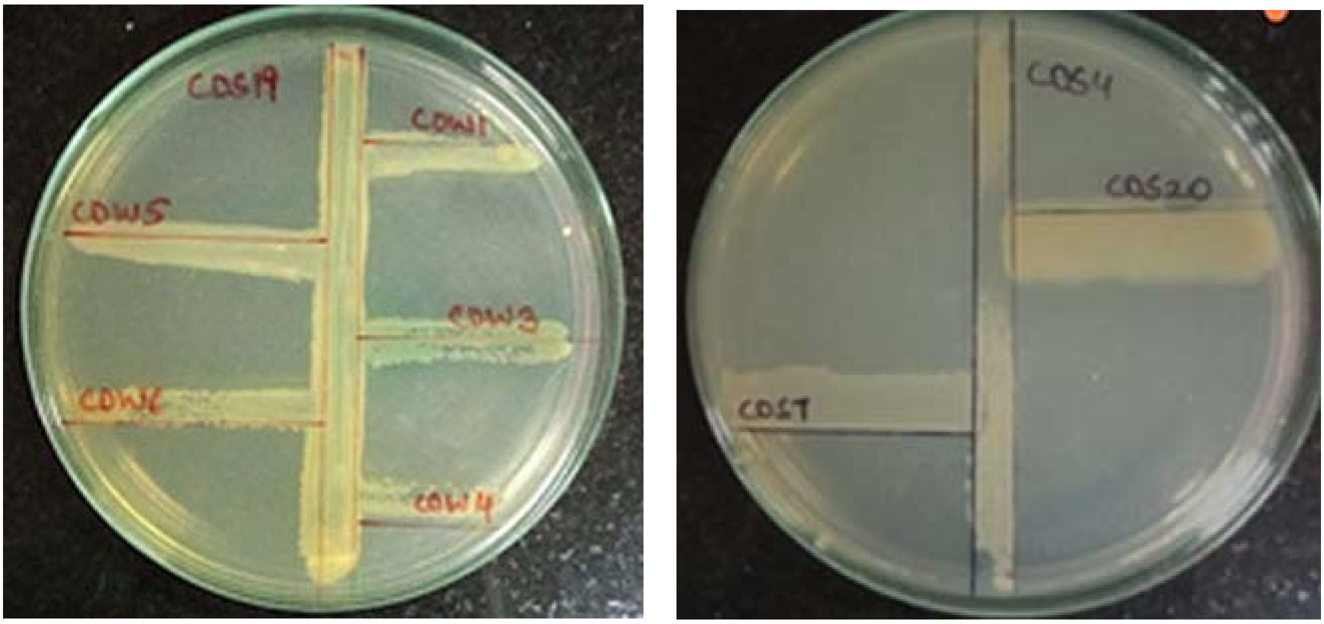


Figure 3. Biocompatibility among the heavy metal tolerant isolates



CDS19 showing compatibility with isolate CDW1, CDW3, CDW4, CDW5, CDW6

CDS4 showing incompatibility with isolate CDS20

Figure 4. Isolates exhibiting compatibility or antagonistic activity

Few isolates, that exhibited more than 90% biocompatibility in this analysis also sheds light on the importance of cooperative interactions among microorganisms in microbial communities (Kumawat et al. 2021). However, only two of the isolates indicate the potential for antagonistic interactions as seen by other studies (Hu et al. 2022).

In conclusion, this research underscores the complexity of antibiotic resistance and compatibility within multi-heavy metal-tolerant bacterial communities. These findings have implications for both clinical antibiotic practice and our understanding of microbial interactions in natural environments. Continued research in this field is essential to unravel the intricate mechanisms governing these phenomena and to practice strategies for mitigating antibiotic resistance and enhancing our comprehension of microbial ecosystems.

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