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PREVALENCE AND ANTIBIOTIC RESISTANCE PROFILING OF GRAM POSITIVE BACTERIA RECOVERED FROM KITCHEN VEGETABLE WASTES

Mahzaib Mubarak[1], Rabia Shahdin[2], Ayesha Siddiqa[*3]

*[1,2,*3]Institute of Microbiology and Molecular Genetics, University of Punjab, Lahore*

[1]mahzaibmubarak111@gmail.com, [2]rabia.res.mmg@pu.edu.pk, [*3]ayesha.mmg@pu.edu.pk

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Corresponding Author: *
Ayesha Siddiqa

Abstract

Background

The emergence of antimicrobial resistance (AMR) in environmental microbial populations is posing a growing threat to public health. Besides, the particular locations such as hospital environments, local niches can also serve as the source of initiating risk of antimicrobial resistance among gram positive bacteria against commonly used antibiotics hence, developing resistance in pathogens of humans such as Staphylococcus aureus, Escherichia coli etc.

Objective

This research determined to study the prevalence and resistance patterns of gram positive bacteria isolated from kitchen vegetable wastes, in order to evaluate their potential role as environmental reservoirs of antibiotic resistance.

Methods

A total of thirty microbial strains were isolated and their morphological and biochemical characterization was performed. The antibiotic susceptibility profiling was performed via antibiotic disc diffusion assay on Muller Hinton agar. Then, zones of inhibition were measured based on CLSI guidelines and index of multidrug resistance of isolates was calculated.

Results

All isolates were gram positive, twenty-eight strains were catalase positive and few were oxidase positive. Antimicrobial susceptibility testing via disc diffusion method displayed high prevalence of resistance to cefotaxime, obtaining no zone of inhibition, indicated widespread β-lactam resistance. In contrast, a number of isolated strains were also sensitive to chloramphenicol and ciprofloxacin, while gentamicin showed intermediate susceptibility in most strains. A few isolates displaying partial resistance patterns, resulted in low multidrug resistance (MDR) indices, although early signs of reduced susceptibility were prominent. Functional assays illustrated that eleven isolates exhibited phosphate solubilization, while ten isolates demonstrated nitrate reduction activity, indicating metabolic diversity. Five isolates (E2, E3, E4, O1, P3) were positive producers of hydrogen cyanide (HCN).

Conclusion

Overall, the findings reveal that kitchen vegetable waste possess diverse antimicrobial resistant gram positive bacteria, particularly to β-lactams. This *therefore, underscores the importance of domestic waste environments in maintaining antimicrobial resistance, and thus calls for environmental monitoring and sustainable waste management strategies to combat antimicrobial resistance dissemination.*

1. INTRODUCTION

Antimicrobial resistance (AMR) is one of the most significant worldwide public health issue and it is estimated that over 10 million of worlds' population is at risk of death due to AMR till 2050 (Tang, Millar, & Moore, 2023). Besides this, it is the leading cause of approximately 1.27 million deaths annually impacting on the management and treatment of infectious diseases (Al-Tawfiq, Ebrahim, & Memish, 2024). Antimicrobial resistance thus makes the antibiotics ineffective, thus leading to the failure of the treatment of infections that were once curable with such drugs. This thus makes the patients seek prolonged treatment and costly drugs for the infections, and in some cases, the infections may become complicated due to the ineffective treatment of the infections (Bassetti et al., 2020). The ineffectiveness of the drugs thus increases the mortality rate among the immunocompromised, elderly patients, and those in hospitals. Apart from the health effects of antimicrobial resistance, the economic effects of antimicrobial resistance are evident. The economic effects of antimicrobial resistance result from the cost of treating, intensive care and tests to investigate the cause of infections. These economic effects hence make antimicrobial resistance a socioeconomic problem of concern worldwide (Pantea, Croitoru, Burduniuc, Balan, & Anton, 2023).

The importance of the environment as a significant reservoir of antibiotic resistance has received more attention in recent years. Environmental media such as soil, water systems, wastewater, and organic waste have been identified as major reservoirs for antibiotic-resistant bacteria and antibiotic resistance genes (Anand et al., 2021). Antibiotics, as well as metals and other contaminants in these media, have been seen to exert a selective pressure that enhances the maintenance of antibiotic resistance (Vats, Kaur, & Rishi, 2022). For example, it has been demonstrated that there is the presence of a number of resistance determinants in pure ecosystems. This is an indication that the dissemination of AMR is widespread. Water systems and wastewater have been seen to be antibiotic-resistant reservoirs (Meradji et al., 2025).

Kitchen and vegetable wastes have been identified as an understudied but important environment for understanding microbial diversity and antibiotic resistance (Soong, Chew, & Gew, 2025). Kitchen wastes are rich in vegetable matter and are likely to be in contact with human-associated microbes. This makes kitchen waste an ideal environment for microbes to thrive in and for them to interact with each other (Yang et al., 2024). Additionally, kitchen waste disposal has been identified as a possible pathway for the entry of resistant microbes into the soil and water habitats. Antimicrobial resistance in microbes in kitchen trash, especially in gram-positive microbes, is understudied (Kusi, Ojewole, Ojewole, & Nwi-Mozu, 2022).

There are many environmental and food-related ecosystems where gram-positive bacteria are found. *Staphylococcus*, *Bacillus*, and *Enterococcus* are some of the gram-positive bacteria that have been recognized for their ability to survive in different environments (Alenzi et al., 2026). The increasing resistance of gram-positive bacteria to antibiotics such as beta-lactams and other broad-spectrum antibiotics has made them an important subject of interest. Several environmental and clinical situations have reported an increase in gram-positive bacteria that are resistant to multiple antibiotics (Rajput, Nahar, & Rahman, 2024). A health concern has been raised in the potential modes of transmission of gram-positive bacteria. The Kirby-Bauer disk diffusion test is an important tool in assessing the resistance of bacteria and identifying emerging trends in the bacterial population (Yin et al., 2023).

In light of this, the current investigation seeks to identify the patterns of antimicrobial resistance in Gram-positive bacteria that were recovered from kitchen vegetable waste. After recovering and characterizing a number of bacterial isolates, their antibiotic resistance profiles were examined. Kitchen vegetable waste is identified as a potential source of antimicrobial-resistant bacteria through this study, which aims to shed light on the resistance trends of environmental isolates. This adds to the body of evidence highlighting the significance of environmental monitoring of AMR, which is essential in understanding the role of kitchen vegetable waste in disseminating resistance and helping to address the global AMR crisis.

2. Materials and methods

2.1. Isolation of bacterial strains

Vegetable peels were collected from kitchen areas to isolate the bacterial strains using aseptic conditions. The temperature, pH, visible necrosis, surface texture, moisture content of peels was recorded at the time of sampling. The sample dilutions were prepared and 50µl of each dilution is plated on nutrient agar plates. The plates were then incubated at 37°C for 24 hours before isolating bacterial strains that formed colonies on the plates. The isolated bacterial strains were then streaked on nutrient agar plates.

2.2. Morphological and biochemical characterization

The bacterial strains were then characterized based on their morphology and various biochemical parameters. The colony morphology of the isolates was observed based on their color, surface, elevation, edges and size. Gram staining was performed to determine whether the strains were gram positive or negative. Spore staining was performed the ability of production of spores by bacterial strains under dormant conditions. Further, catalase test, oxidase test, methyl red (MR) test, Voges-Proskauer (VP) test, starch hydrolysis test, citrate utilization test, nitrate reduction test, phosphate solubilization, indole production test was performed to establish the biochemical profile of bacterial strains.

2.3. Antibiotic Susceptibility Testing

The antibiotic resistance pattern of the isolated bacterial strains was determined by the Kirby Bauer disk diffusion test on Muller Hinton Agar. The lawn of bacterial isolate was set via sterile cotton swabs. Commercially available antibiotic disks such as gentamicin (CN10), chloramphenicol (C30), ciprofloxacin (CIP5), and cefotaxime (CTX30) were placed at equal distances on the surface of agar. The plates were then incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters. The bacteria were classified as sensitive, intermediate, or resistant based on the CLSI guidelines.

2.4. Screening for Active Traits

Some of the selected functional characteristics of isolated bacterial strains related to environmental adaptability were also examined. The production of hydrogen cyanide (HCN) was checked by using sodium picrate-impregnated filter paper. These tests were only conducted to evaluate the competitiveness of the isolates, although the major emphasis was profiling of antimicrobial resistance patterns.

3. Results

Thirty bacterial strains were successfully isolated from the collected samples. All bacterial isolates were primarily gram positive comprising both rods and cocci morphology. Some of the bacterial isolates displayed visible spore forming ability when stained with malachite green. All isolated strains were positive for catalase test except E4 and GR1. GP1, GP3 and S1 were positive for oxidase activity. T1, CU2, CU3, CA1, CA2, CA3, E1, E2, CB1 and O3 were positive for methyl red test while P3, T1, T3, CU1, CA3, E2, GI1, GI2, GP1, S1, O1, O2 and O3 are positive for Voges Proskauer test. P1, P2, P3, CU2, CA2, CA3, E2, GI1, GI2, GP1, GP2, CB1 and CB3 were positive for starch hydrolyzation. P1, P3, T1, T3, CA1, GI1, GI2, S1, S2 and GR1 were positive for indole test. P1, P3, CU1, CU3, CA2, CA3, GP1, GP2, CB1, S1 and GR1 were positive for phosphate solubilization. P1, P2, T3, CU1, E3, CB3, O1, O2 and GR1 were the strains negative for nitrate

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reduction indicating a good potential of majority of strains to reduce nitrate (table 1).

The antimicrobial susceptibility profiles of the isolated strains determined a variety of resistance patterns across the tested antibiotics. All the bacterial isolates were resistant to cefatoxamine except CU2, E2, GI1 and GI3 having intermediate zones of inhibitions demonstrating reduced susceptibility to such types of ß-lactam antibiotics. Most of the bacterial strains were sensitive to ciprofloxacin except for T1, CU1, GI1, GI3, GP2 and S1 which displayed intermediate zones of approximately ranging from 17mm to 20mm. While, only S3 exhibited intermediate zone of inhibition of 19mm against chloramphenicol. All strains had intermediate zones of inhibition against gentamicin indicating partial tolerance to it. In general, bacterial isolates such as E1, E2, GR1, and GR2 showed relatively higher sensitivity to a range of antibiotics, while others showed reduced sensitivity to one or more antibiotics, representing variability in antibiotic resistance among the isolates (figure 1,2,3). By this pattern of resistance, the presence of diverse antimicrobial resistance traits could be highlighted among the bacterial isolates obtained from kitchen waste (table 2). Functional assays revealed that P3, E2, E3 and E4 produced hydrogen cyanide (HCN) showing their environment adaptability (table 1).

3.1. Multidrug Resistance (MDR) Analysis

The antimicrobial resistance profile of the isolated strains was established in order to determine the multidrug resistance patterns using the multi drug resistance analysis. The MDR index of the isolates would be calculated on the basis of the recorded resistance to the antibiotics used in the study such as gentamicin, chloramphenicol, ciprofloxacin, and cefotaxime. The results of the analysis determined that the isolates were resistant only to cefotaxime, remaining sensitive to chloramphenicol and ciprofloxacin. However, some of the isolates were also observed to have intermediate susceptibility to gentamicin, indicating partial resistance. Therefore, the calculated MDR index of the majority of the isolates was relatively low, as the isolates were mostly observed to be resistant only to a single antibiotic (figure 4).

4. Discussion

The current study suggests that kitchen waste including vegetable peels can serve as a reservoir for antimicrobial resistant bacteria particularly gram positive ones (Alenzi et al., 2026). From previous researches, it is observed that gram positive bacteria are a major source of blood stream and other infections in humans. Among these, Vancomycin resistant Enterococci (VRE) and methicillin resistant *Staphylococcus aureus* are of major concern these days (Jubeh, Breijyeh, & Karaman, 2020) . The biochemical profiling of thirty isolated bacterial strains indicated that organic waste ecosystems contain bacteria which can hydrolyze starch, solubilize phosphate and reduce nitrate. Majority of strains are catalase positive and oxidase negative. While it is also observed that various foodborne pathogenic bacteria such as *Bacillus cereus, Escherichia coli, Staphylococcus aureus*, various species of *Salmonella, Vibrio* and *Listeria* are frequently found in nutrient rich kitchen waste materials such as vegetable peels, contributing to the development of antibiotic resistance (Budiati, Suryaningsih, & Bethiana, 2022).

Among the isolated strains, resistance patterns were observed by commonly established antimicrobial susceptibility tests. A prominent trend of resistance to the β-lactam drug cefotaxime was observed over a large range of strains which is consistent with the findings of (Furukawa, Misawa, & Moore, 2018) who also observed resistant patterns of bacteria from domestic food waste. The main reason of resistance to β-lactam antibiotics is mainly due to presence of enzymes called β-lactamases having the ability to inactivate a wide range of antibiotics so, this is the main concern by which issue of antimicrobial resistance can be eliminated (Pandey & Cascella, 2023). Research also supports that antibiotic resistance is also significantly influenced by different environmental niches. This indicates that the hospital niche is no longer the only setting where resistance to clinically relevant antibiotics can occur. Research also supports that antibiotic

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resistance is also significantly influenced by different environmental niches (Spinu & Rzewuska, 2022). However, from our observations the isolated strains somehow displayed sensitivity to ciprofloxacin and chloramphenicol, indicating that these antibiotics are still active against the environmental isolates. But, the resistance to these two classes against gram negative *E.coli* and other gram positive bacteria such as *Listeria monocytogens* have been frequently reposted previously (Das et al., 2023) (Kawacka, Pietrzak, Schmidt, & Olejnik-Schmidt, 2023).Only a few isolates were observed to have partial or multiple resistances to more than one tested antibiotics and none of the isolates met the criteria of multidrug resistance i.e., the resistance must be to three or more antibiotic classes to declare the strain as MDR (Terreni, Taccani, & Pregnolato, 2021). Hence, actual multidrug resistance pattern was not there, the existence of a consistent level of resistance to particular drugs can suggest a growing trend of antimicrobial resistance in bacteria derived from kitchen waste material and it can serve as a selective environment for the survival of many resistant bacteria, adding to the environmental locations of AMR (Hazards et al., 2021). It was also observed that a few number of isolates displayed intermediate gentamicin susceptibility serving as the first sign of resistance. These findings still hold significance as this limited resistance can lead to the growth of large resistant infections. The heterogeneous character of antimicrobial resistance evolution will be evident by the presence of sensitive, intermediate, and resistant phenotypes within the same environmental niche (Baquero et al., 2021). In another study it was estimated that 25% of the *Staphylococcus aureus* isolates from household waste water in same community were resistant to majority of tested antibiotics as compare to isolates collected from hospital waste water displaying 100% resistance pattern. It suggests that these bacteria isolated from kitchen waste may have less resistance activity than hospitals and such established antimicrobial resistance niches but can have the potential to initiate and establish the antibacterial resistance pool very effectively (Fatema, 2023). The additional observation that these isolated strains can produce HCN have the potential to survive themselves in the competitive niche which the kitchen waste environment is serving (Lipková et al., 2021).

Overall, the findings support that antimicrobial resistant gram positive bacteria in kitchen vegetable waste may represent a significant environmental niche other than established AMR locations to be studied and mitigated. Such conditions have the active potential to contribute to the persistence and dissemination of resistance phenotypes, as observed by the high levels of cefotaxime resistance and the emergence of decreased susceptibility to other antibiotics. The current study presents the dire need of a coordinated strategy to monitor the antimicrobial resistance in the environment and the contribution of household trash to the spread of antibiotic resistance. The development of methods that can aid in diminishing the emergence of antibiotic resistance majorly depends on the environmental approach to antimicrobial resistance research.

5. Conclusion

This study suggests that kitchen waste containing vegetables is not just an organic waste but an important environmental niche inhabited by gram positive bacteria possessing prominent antimicrobial resistance characteristics. The high rate of resistance to cefotaxime, along with emerging reduced susceptibility to other antibiotics, highlights the possible role of such environments in the development of antibiotic resistance. Even though multidrug resistance was not established among the isolates, the presence of intermediate resistance patterns leads to an evolutionary trend towards more complex resistance profiles. Overall, this study points to the need to realize the role of household waste in the environmental aspect of antimicrobial resistance. Combining environmental surveillance with public health approaches may be necessary to control the quiet spread of antibiotic resistance and protect existing antibiotic efficacy.

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Table 1: Morphological and biochemical profiling of isolated bacterial strains from kitchen waste.

Strains ID	Gram staining	Catalase test	Oxidase test	Methyl red	Voges Proskauer	Starch hydrolysis	Indole test	HCN utilization
P1	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve
P2	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
P3	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
T1	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve
T3	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve
CU1	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
CU2	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
CU3	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
CA1	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve
CA2	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
CA3	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
E1	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
E2	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
E3	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
E4	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
GI1	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve

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GI2	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
GP1	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
GP2	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
GP3	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
CB1	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
CB3	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
S1	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve
S2	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve
S3	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
O1	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve
O2	+ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
O3	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve
GR1	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
GR2	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 2: The heat map visualization is indicating the resistant pattern of cefatoxamine among all tested antibiotics

Isolate	Gentamicin	Chloramphenicol	Ciprofloxacin	Cefatoxamine
P1	I	S	S	R
PR	I	S	S	R
P3	I	S	S	R
T1	I	S	I	R
T3	I	S	S	R
CU1	I	S	I	R
CUR	I	S	S	I
CU3	I	S	S	R
CA1	I	S	S	R
CAR	I	S	S	R
CA3	I	S	S	R
E1	S	S	S	R
ER	S	S	S	I
E3	I	S	S	R
E4	I	S	S	R
GI1	I	S	I	I
GI3	I	S	I	I
GP1	I	S	S	R
GPR	I	S	I	R
GP3	I	S	S	R
CB1	I	S	S	R
CB3	I	S	S	R
S1	I	S	I	R
SR	I	S	S	R
S3	I	I	S	R
O1	I	S	S	R
OR	I	S	S	R
O3	I	S	S	R

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GR1	S	S	S	R
GRR	S	S	S	R

I: intermediate, S: sensitive, R: resistant

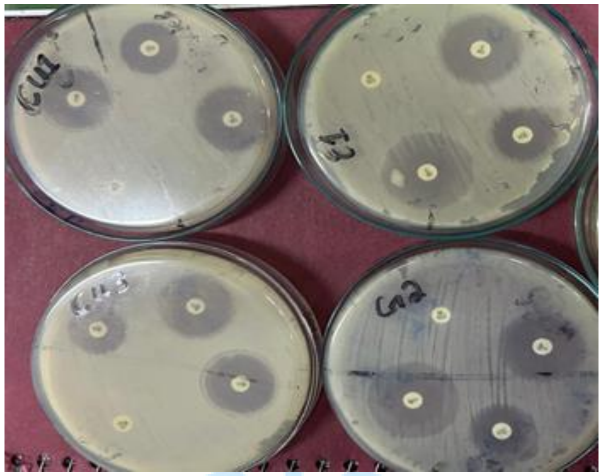

Figure 1: Zone of inhibitions(mm) of various bacterial isolates against tested antibiotics

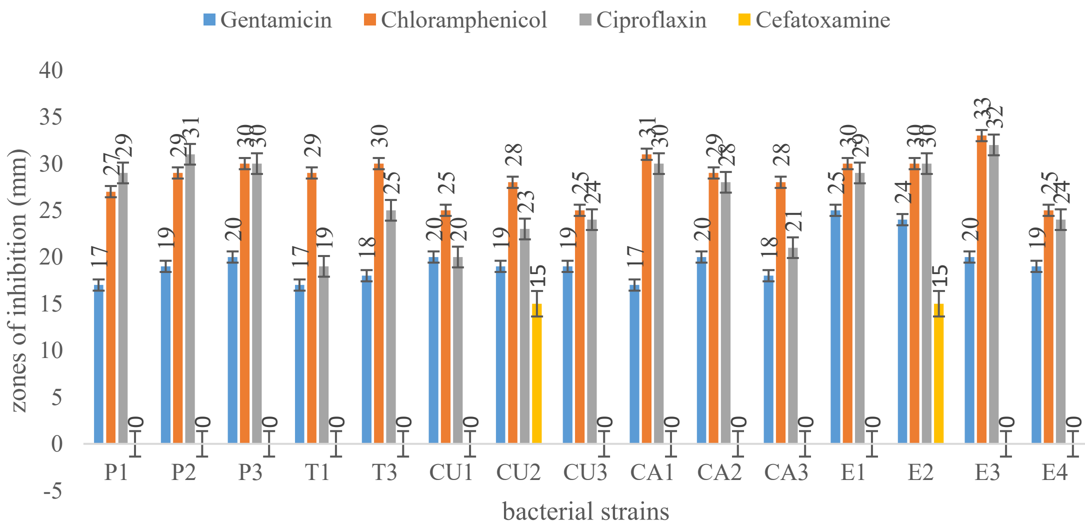

Figure 2: Zone of inhibitions(mm) of various bacterial isolates (P1 to E4) against tested antibiotics

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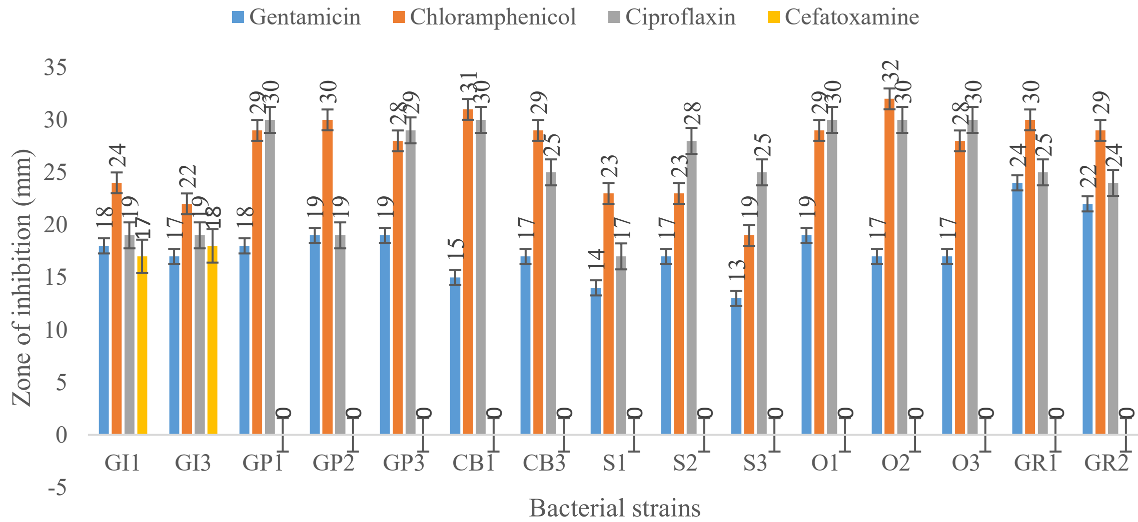

Figure 3: Zone of inhibitions(mm) of various bacterial isolates (GI1 to GR2) against tested antibiotics

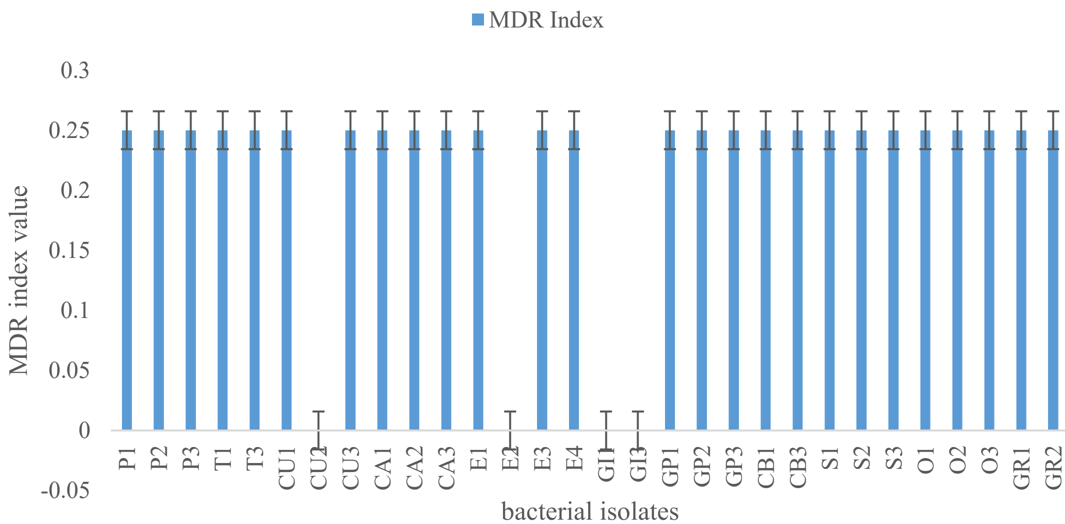

Figure 4: MDR index calculated for all thirty isolated strains