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ANTIBIOTIC USE IN AGRICULTURE AND ITS ROLE IN THE SPREAD OF ANTIMICROBIAL RESISTANT BACTERIA

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ABSTRACT

Antibiotics were a significant advancement in medicine, yet infections from resistant bacteria are now on the rise, posing a major public health challenge. These bacteria can reach humans through food or contact and can cause severe illnesses that are harder to treat, leading to increased illness and death rates. Additionally, resistant strains can spread into the environment via animal waste, amplifying resistance in the environmental microbiome. The study explored the antimicrobial resistance profile of bacteria in soil, which is a highly diverse environment and a reservoir for resistance genes. In addition, the aim of this study was to isolate soil bacteria from compost and assess their antibiotic resistance profiles using the diffusion test method. In this study, 24 different antibiotics were used to evaluate the resistance profiles of the isolated bacteria. Results showed that 8 out of 20 bacterial isolates exhibited resistance to the tested antibiotics. 60% of the isolates demonstrated resistance to chloramphenicol and nitrofurantoin, whereas only 10% of the isolates exhibited resistance to gentamicin, representing the lowest observed resistance rate. This highlights the need for stronger regulations on antibiotic production, distribution, use, as well as global collaboration to support developing countries in improving antibiotic use and resistance surveillance.

Keywords: antimicrobial resistance, agriculture, environmental sources, soil bacteria, food safety, farm animals

INTRODUCTION

Since their introduction in the 1930s, antibiotics have saved millions of lives; however, the increasing prevalence of antibiotic-resistant bacterial pathogens is a major concern. Initially, research focused on clinical settings as the main sites of antibiotic resistance evolution. In the past two decades, attention has shifted to the environment as a significant source of resistance genes and a key area for the development of antibiotic

resistance (Czekalski et al. 2014). Almost concurrently, antimicrobials have been utilized in agriculture to prevent, control, and treat infections, as well as to enhance growth and feed efficiency (Economou and Gousia 2015). Antimicrobials and their bioactive metabolites can enter different environmental compartments through various pathways. They may infiltrate water bodies through urban wastewater and reach agricultural systems via the application of sewage sludge, manure, sediment, or biosolids that contain antibiotics. Because of their high solubility in water, these ecotoxic substances can quickly disperse into aquatic and soil ecosystems, resulting in accumulation that poses a serious risk to the quality of receiving waters, such as groundwater and soil environments (Serwecińska 2020). Agriculture is widely recognized as a major source of antimicrobial resistance pollution (Stanley et al. 2022). Antibiotics are frequently added to commercial poultry feed across the global industry, as they are generally recognized to promote growth, improve feed conversion rates, and reduce mortality rates by inhibiting pathogenic bacteria. Although their positive impact is widely observed, the precise biological mechanisms behind these effects remain unclear (Lin et al., 2013). Understanding the mechanisms by which antibiotics affect gut microbiota is essential, as this knowledge may help to identify productivity-enhancing microbial traits and support the development of antibiotic-free growth strategies in animal feed, despite the industry's move away from antibiotic use (Crisol-Martínez et al. 2017). Soil is one of the most diverse microbiota systems, rich in bacterial and fungal populations and serves as a primary source for numerous clinical antibiotics (Stanley et al. 2022). The structure and composition of native bacterial communities in soil significantly influence the abundance of antibiotic resistance genes (ARGs) across various soil types (Macedo et al. 2020). Antimicrobial use across human and animal health sectors, including in agriculture and aquaculture, can drive antimicrobial resistance (AMR) development and spread among bacteria. AMR traits can be passed down through generations within a bacterial species via vertical gene transfer, but horizontal gene transfer (HGT) also facilitates the spread of these traits both within species and between unrelated bacterial species. HGT, which occurs through processes such as conjugation, transformation, or transduction, enables the broader dissemination of resistance genes across bacterial communities, as detailed in several reviews (Yazdankhah et al. 2018). Current evidence of human exposure to resistance originating from soil is limited, potentially underestimating the actual risk. Studies have shown that antibiotic resistance genes, along with their associated genetic elements like plasmids and integrons, are present in untouched environments as well as in human and animal populations that have not been exposed to antibiotics. This finding implies that resistance genes can remain viable and proliferate in the environment due to their incorporation into effective gene-transfer systems, even without the use of antibiotics (Fletcher 2015). The high density of microbial populations in soil enhances genetic exchange, which plays a significant role in the emergence of microbial resistance, particularly in the context of antibiotic exposure. Furthermore, antibiotics have been shown to impact various ecological processes, including nitrogen transformation, methanogenesis, and sulfate reduction (Ding and He 2010). Pathogenic bacterial strains that exhibit resistance to multiple antibiotics have been found in various food sources, including fish, poultry, milk, dairy products, and vegetables. Some resistant bacterial strains are capable of causing severe infections in humans and have developed resistance to a majority of available antimicrobial agents. This situation raises significant public health concerns regarding the transmission of resistant pathogens through the food chain, underscoring the need for better monitoring and control measures in food production systems (Serwecińska 2020). Multiple strains of multidrug-resistant (MDR) bacteria have been detected in soil surrounding animal farms and contaminated rivers, indicating their potential role in the spread of antibiotic resistance. These bacteria, which are increasingly recognized for their clinical significance due to their resistance to various antibiotics, exhibit traits characteristic of both environmental and

clinical bacteria. This dual nature raises concerns about their potential to act as pathogens and highlights the importance of monitoring these soil microorganisms in relation to antibiotic resistance (Wang and Dongchang 2015). These findings highlight the intricate interactions between microbial communities and their environment, emphasizing the need for further research to understand the broader ecological implications of antibiotic use in soil ecosystems.

MATERIALS AND METHODS

Sample Collection

The sample was collected from the composting facility in Resen, North Macedonia in march 2024 using sterile bags along with sterile spatula. All the samples were transferred to the Microbiology laboratory and processed within 24 hours (Kostandinovska et al. 2024).

Bacterial Isolation

Microorganisms were isolated from the soil sample using a serial dilution method (1:10) combined with the spread plate technique. A 0.1 ml aliquot was transferred onto Ashby agar, which had been sterilized by autoclaving. The inoculated agar plates were then incubated at 30°C for 5-7 days in an inverted position. To obtain pure cultures, isolated colonies were carefully picked and streaked onto fresh Ashby agar plates. The resulting pure bacterial cultures were maintained in slant tubes and stored at 4°C for later use (Ilesanmi et al. 2020).

Antibiotic Susceptibility Test

The antimicrobial susceptibility profile of the isolates was evaluated using a modified Kirby-Bauer disc diffusion technique on nutrient agar. Each isolate was inoculated onto a distinct nutrient agar plate, to which an antibiotic disc was applied and then incubated at 30°C for a duration of 48 hours. The antibiotic discs were uniformly distributed over the agar medium using a dispenser, while a sterile rod was utilized to gently press each disc onto the agar surface (Dike-Ndudim et al. 2021). This methodology facilitates the assessment of bacterial resistance patterns and the efficacy of various antibiotics.

RESULTS AND DISCUSSION

The bacterial isolates from the soil sample were isolated and sampled on Ashby agar plates. Morphological analysis showed that the isolates were Gram-positive, rod-shaped, sporulating bacteria (Figure 1). Overall, twenty bacterial strains were isolated from the compost sample in this study. During the morphological assessment, all strains were identified as Gram-positive bacilli, primarily organized in chains. They exhibited oval-shaped ends and demonstrated visible spore formation when subjected to Gram staining. This characteristic morphology is consistent with descriptions of certain bacterial groups, highlighting their potential implications in various ecological and clinical contexts.

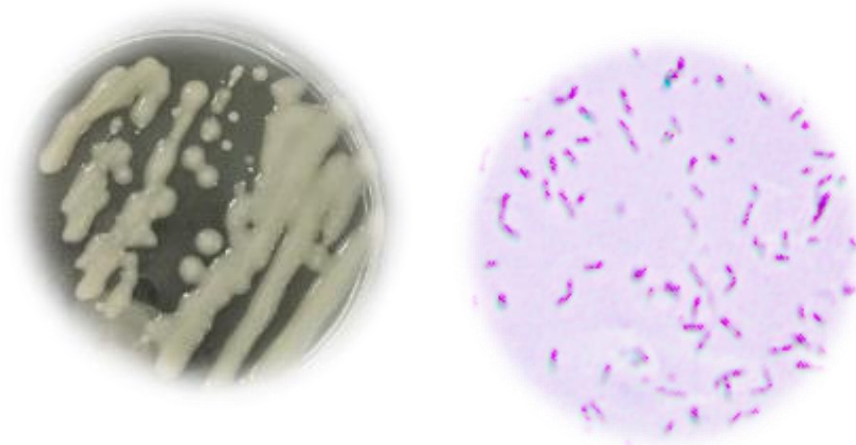


Figure 1. Macroscopic and microscopic characteristics of the isolate B6.

The analysis of inhibitory zones derived from the application of antibiotic discs revealed that three isolates (B7, B10, and B18) out of a total of twenty exhibited no sensitivity to any of the antibiotics tested, as evidenced by the lack of observable inhibition zones. In contrast, all remaining isolates demonstrated distinct inhibition zones, indicating effective growth suppression by various antibiotics. Among the isolates that displayed the least sensitivity, the isolates B3, B4, B8, and B9 were notable, as they only formed inhibition zones in response to certain antibiotic types. Conversely, the isolate B20 emerged as the most sensitive, exhibiting inhibition zones for each antibiotic tested (Figure 2). This highlights the variability in antibiotic susceptibility among the isolated strains, emphasizing the importance of antimicrobial susceptibility testing in understanding bacterial resistance patterns.



Figure 2. Inhibition zones of isolate 20 against the antibiotics Apidaecin, Augmentin, Tetracycline, Rifampicin, and Erythromycin

Up to 53% of the isolates showed inhibition zones exceeding 15 mm, while only 25% exhibited zones within the 0–5 mm range. Antibiotic susceptibility testing revealed that 60% of all isolates were resistant to Chloramphenicol and Nitrofurantoin, while only 10% showed resistance to Gentamicin, the lowest resistance observed across all antibiotics tested. Antibiotic resistance associated with soil microorganisms has become a global concern, with growing evidence linking antimicrobial drug usage to the emergence of resistant strains. The resistance profiles observed in the bacterial isolates from this study raise significant public health concerns, as these bacteria may be transferred to the food chain or enter clinical environments. In another study, *Bacillus* species exhibited complete resistance to Amoxicillin and Ceftazidime, with a notable 60% resistance to Ciprofloxacin and Erythromycin, while showing full sensitivity to Levofloxacin and high sensitivity to Streptomycin (80%) and Cefalexin (80%) (Chibuike et al. 2024). The use of antibiotics in livestock farming and the

presence of antibiotic-resistant bacteria and resistance-carrying plasmids in manure have raised concerns that the application of such manure on agricultural fields could spread resistance to crops intended for human or animal consumption (Gaze et al. 2013). The susceptibility profile of *Bacillus* species in another research showed 100% sensitivity to Cloxacillin, Ofloxacin, and Cefuroxime, alongside complete resistance to Ceftazidime and Augmentin, and a low resistance rate to Erythromycin (9.1%) (Harry et al. 2023). This pattern may reflect high public awareness regarding antibiotic usage. The findings of this study, corroborated by other studies, indicate that antibiotic resistance within the microbial community may vary by geographic location and could be influenced by exposure to human and animal medicinal waste, including antibiotic residues. This suggests that environmental factors, such as regional antibiotic use, may play a role in shaping resistance patterns (Ajuzieogu et al. 2024).

CONCLUSION

In conclusion, this study successfully isolated 20 bacterial strains belonging to the genus *Bacillus* from compost sample. Notably, 60% of these isolates demonstrated resistance to Chloramphenicol and Nitrofurantoin, while a smaller proportion (10%) exhibited resistance to Gentamicin, indicating a substantial variation in their antibiotic susceptibility profiles. Additionally, the research found that 52% of the isolates formed inhibition zones exceeding 15 mm, while 25% displayed zones within the 0-5 mm range, and 22% showed inhibition zones between 5-15 mm. These results highlight the significance of understanding the ecological roles and antibiotic resistance patterns of *Bacillus* species in agricultural contexts, as this knowledge may guide the development of sustainable practices aimed at mitigating antibiotic resistance in the environment. Future investigations should further examine the implications of these findings and evaluate the potential application of these isolates in agricultural practices.

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