A Review: Antibiotic sensitivity of microbes obtained from various soil habitats

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Abstract

Soil is home to various microorganisms, including bacteria and fungi, which vary across different habitats. Research indicates that microbial populations exist in various layers of soil and are influenced by its physical and chemical properties. As the uppermost layer of the Earth's surface, soil supports numerous living organisms. Its chemical composition is shaped by factors such as geographical location, human activities, animal presence, and surrounding flora and fauna.

The extensive use of antibiotics in treating infectious diseases has led to their release into the environment through human and animal waste, improper disposal, and other sources. These antibiotics can accumulate in soil and water, potentially impacting microbial communities. Prolonged exposure to antibiotics may contribute to the development of resistance in soil-dwelling pathogens, altering their susceptibility to different antibiotics. As a result, various pathogens present in the soil may exhibit differing levels of resistance, raising concerns about the environmental impact of antibiotic contamination.

Keywords: *Microorganisms, Antibiotics, Resistance, Pathogens, Contamination.*

Introduction

The thin layer of material covering much of the Earth's surface is known as soil. Despite being fragile and often less than a meter thick, it plays a crucial role in sustaining life [1]. Recently, it has been described as "the most complicated biomaterial on the planet." This complexity arises from two key components: the abiotic soil structure and the biotic diversity, which together create physical and biological heterogeneity. Physically, soil is composed of sand, silt, and clay particles of varying sizes. The size differences, ranging from millimetre-sized sand particles to micrometre-sized clay particles, significantly impact the surface area of soil components, influencing soil chemistry, including chemical reactions and transformations. On the other hand, soil microflora regulates biochemical processes within the soil. Interestingly, the organic and biological components of soil resemble mineral components in terms of size. The primary soil biota, in increasing order of size, include viruses, bacteria, fungi, algae, and protozoa. As the size of organisms decreases, their numbers increase dramatically. Despite the vast number of microorganisms present in the soil, they cover only about 10^{-6}% of the total surface area, a proportion comparable to the land area occupied by humans on Earth [1]. Soil microflora are essential for biochemical processes that support human life, such as plant growth, the production of bioactive compounds beneficial to human health, and groundwater protection [2-4]. However, soil is also a habitat for human pathogens and serves as a reservoir for bacterial antibiotic resistance. Bacterial pathogens in soil are free-living organisms capable of reproducing in human or animal hosts. Some of the notable pathogens found in soil include *Campylobacter jejuni*, pathogenic strains of *Escherichia coli*, *Salmonella* species, *Shigella* species, *Vibrio cholerae*, and *Yersinia* species [5].

The desert soils of the Southwestern USA are distinct due to their arid environment. These soils typically have a high pH (>8.0) and may contain excessive salts and sodium. Such abiotic stress, along with the biotic stress exerted by native soil microorganisms, often results in the rapid die-off of introduced organisms, such as rhizobia, within a few weeks [6-9]. Furthermore, *E. coli* has been identified in tropical and subtropical soils. Laboratory studies have demonstrated that *E. coli* can grow in tropical soils under suitable conditions, particularly when provided with amendments [10-14].

Byappanahalli and colleagues reported that *E. coli* could also be isolated from coastal temperate forest soils in Indiana, indicating that soilborne *E. coli* may be more widespread than previously assumed [15]. Soil is increasingly subjected to environmental pressures that impact its ability to perform essential ecosystem services. To preserve these vital soil functions, it is crucial to understand how soil microorganisms respond to disturbances and environmental changes. The growing number of studies on soil microorganisms and their role in maintaining soil ecosystem stability is likely driven by the increasing focus on the relationship between biodiversity and ecosystem functioning. The loss of biodiversity has been recognized as a significant threat to soil (COM 231). However, due to the complexity and variability of soil, microbial ecologists face significant challenges in quantifying the role of microorganisms in soil resilience, and the mechanisms that enable soil to withstand disturbances remain poorly understood [16,17].

The extensive use of antibiotics in food animal production is considered a key factor contributing to the emergence of antibiotic-resistant bacteria (ARB) in the environment [18,19]. Studies estimate that up to 80% of orally administered antibiotics in animals may pass through their bodies unchanged, eventually accumulating in manure [20,21]. Reported antibiotic concentrations in manure range from trace levels to as high as 200 mg/L [22,23]. The persistence of antibiotics in soil is influenced by various factors, including soil type, climate, and the specific class of antibiotics. While most antibiotics degrade in soil, some have an extended half-life [24]. Antibiotics exert both quantitative and qualitative effects on native microbial communities in terrestrial environments [25]. Although the antibiotic concentrations in most soils are not high enough to have therapeutic effects, they may still contribute to the selection of antibiotic-resistant bacteria [25,26,27,28]. An increase in antibiotic resistance among soil bacteria following manure application may result from (i) the exchange of genetic elements between soil bacteria and antibiotic-resistant bacteria in manure, (ii) the transfer of genetic material among antibiotic-producing soil microorganisms, or (iii) selective pressure from low levels of antibiotics in manure. Many antibiotics also have a strong tendency to bind with soil [29,30]. In addition to beneficial components, sludge may contain indigenous populations of human enteric pathogens. Some of these enteric bacteria constitute a significant group of such pathogens. In a related study, it was demonstrated that the removal of water from sludge through evaporation to near dryness resulted in a reduction of most bacterial populations by approximately one order of magnitude or less [31].

Studies of microflora on Sludge

Pepper et al. conducted both laboratory and field studies to assess the survival and movement of bacterial pathogens introduced into soil through sludge application. Their findings indicate that factors such as soil moisture, texture, and temperature play a crucial role in determining the persistence of bacterial pathogens in sludge-amended soil [32].

A study assessed the abundance of bacteria and antibiotic resistance genes using both culture-dependent and molecular techniques. The findings suggest that growing vegetable crops in soil fertilized with human waste, without proper treatment or an adequate waiting period, may contribute to an increased presence of antibiotic resistance genes in harvested produce [33]. Some studies examined the impact of moisture content on the survival and regrowth of both seeded and indigenous enteric bacteria in raw sludge. The findings indicate that reducing sludge moisture leads to bacterial inactivation; however, in dried sludge (>90% solids), most bacteria remain stable except *Proteus mirabilis*. Indigenous bacteria can persist for extended periods in dried sludge. Bacterial regrowth occurs in sterilized sludge with ≤75% solids but not in sludge with ≥85% solids. *Salmonella typhimurium* can grow in both liquid and dewatered sludge, though at lower densities when indigenous bacteria are present, and it declines rapidly in their presence [34]. According to an assessment the impact of five annual liquid sewage sludge applications on the organic carbon and nitrogen content of a furrow-irrigated desert soil. The findings suggest that liquid sludge application in desert soils may influence underground aquifers, potentially contributing to nitrate pollution [35]. A review by Timothy M. Straub examines pathogen types and concentrations in sludge, sludge treatment efficacy, pathogen fate after land disposal, exposure pathways, detection methods, risk assessment models, and future research needs. Findings indicate that despite stabilization and treatment, significant pathogens persist in sludge. If viable for extended periods, they may contaminate groundwater beneath disposal sites, travel through the vadose zone with minimal inactivation, and spread over significant distances [36].

Studies of microflora on antibiotic sensitivity and resistance

Brooks et al. investigated the prevalence of antibiotic-resistant bacteria and endotoxins in soil following biosolid application. The study established a baseline for assessing the impact of biosolid application on soil antibiotic-resistant bacteria and endotoxin levels [37]. Some study explored soil bacteria's ability to utilize antibiotics as a carbon source, their phylogenetic diversity, and their role as a reservoir for antibiotic resistance. Findings suggest that this overlooked reservoir may contribute to rising multidrug resistance in pathogens. The isolated bacteria exhibited extreme resistance, tolerating antibiotic concentrations over 50 times higher than standard levels [38]. Pepper et al.. examined the soil's role in public health, including its impact on pathogens, antibiotics, nutrition, and climate change. The study concludes that soil is a vital public health asset with an estimated value of $20 trillion, making it the world's most valuable ecosystem [39]. Some authors assessed the impact of TWW irrigation on soil antibiotic-resistant (AR) bacteria and ARG reservoirs. Findings suggest that TWW-associated bacteria have a minimal effect on the soil microbiome, and high AR bacteria and ARG levels in both freshwater- and TWW-irrigated soils likely stem from native AR within the natural soil microbiome [40].

Fiona Walsh's study identified and characterized multidrug resistance (MDR) mechanisms in the culturable soil antibiotic resistome, linking resistance profiles to bacterial species. Findings highlight the key role of efflux mechanisms and differences in intrinsic resistance between clinical and soil bacteria of the same family [41]. Eddie Cytryn's study explored natural and human influences on soil antibiotic resistance and its transfer to pathogens, supporting the resistome hypothesis. The review offers a comprehensive overview of factors shaping soil antibiotic resistance [42]. Romain Marti's study assessed antibiotic-resistant bacteria on raw vegetables grown in soil fertilized with inorganic, dairy, or swine manure. Findings revealed viable coliform bacteria resistant to multiple antibiotics, even on vegetables from never-manured soil [43]. The study on human waste assessed bacterial abundance and antibiotic resistance genes using culture-dependent and molecular methods. Findings suggest that growing Vegetables in soil fertilized with untreated human waste increases antibiotic resistance genes in harvested crops [44]. Few studies explored strategies to discover novel antimicrobials from actinomycetes by activating biosynthetic gene clusters through genetic, chemical, and ecological methods. Findings highlight that many antibiotic-producing genes remain unexpressed under standard conditions, but genome sequencing reveals a vast untapped reservoir. Chemical elicitors, optimized culturing, and ecological approaches can help unlock these genes for new drug discovery [45].

A study was conducted on native Nebraska prairie soils unaffected by human or animal waste to assess background antibiotic resistance. Findings showed no correlation between resistance and soil physical or chemical properties [46]. A comparative study on the diversity, abundance, and composition of antibiotic resistance genes (ARGs) and bacteria in 12 urban parks in Victoria, Australia, with and without reclaimed water irrigation (RWI). Findings indicate that RWI significantly increased ARG abundance and diversity but did not notably enhance horizontal gene transfer potential [47].

An investigation examined the correlation between metal concentrations and antibiotic resistance genes. Findings show that even low levels of metals like aluminum, copper, manganese, and lead in residential soils promote antibiotic resistance, evidenced by increased gene abundances [48]. A review re-evaluates the impact of land application on antibiotic-resistant bacteria (ARB) and genes (ARG), highlighting their public health risks from environmental exposure. Findings suggest that while waste application temporarily increases resistance in soil, its persistence varies by site and is often inconsistent [49]. Another review examines antibiotic degradation in soil and its impact on microbial communities. Findings show that antibiotics alter enzyme activity, carbon metabolism, microbial biomass, and the balance of Gram-negative bacteria, Gram-positive bacteria, and fungi [50].

The impact of PFD on antibiotic resistance genes (ARGs) and bacterial communities in a lab-scale soil experiment was studied by reserchers. Redundancy analysis revealed that penicillin-induced shifts in the bacterial community primarily drive ARG composition [51]. A study analyzed soil bacterial composition and function in response to multidrug-resistant *E. coli* with and without tetracycline contamination. Findings indicate that antibiotic contamination worsens the impact of foreign ARB, posing risks to soil quality [52].

According to a study the activity of soil-bound tetracycline and tylosin on bacterial cultures in different soil types. Results show that despite strong adsorption to clay, antibiotics remain biologically active and may contribute to antibiotic resistance in soil [53]. An investigation improves the agar diffusion assay by incorporating antibiotic loss during diffusion, leading to more accurate MIC determination. The model is broadly applicable to other dissipative processes, such as antigen diffusion and substrate load calculations in affinity purification [54].

Microbial Diversity of soil

Ishii et al. found viable E. coli in northern temperate soils across three Lake Superior watersheds, with seasonal population variations. This study is the first to report naturalized E. coli growth in nonsterile, nonamended soils, challenging its reliability as a fecal contamination indicator [55].

An investigator examined the transfer and persistence of E. coli O157:H7 in lettuce grown in soil fertilized with contaminated compost or irrigated with contaminated water. Results showed that both sources could contaminate lettuce and soil, posing a health risk. Proper management of irrigation water, compost, and land history is crucial to preventing pathogen introduction [56]. A study explored factors influencing soil microbial resistance and resilience to environmental disturbances. Results indicate that soil stability depends on physicochemical structure, microbial composition, and biotic-abiotic interactions, offering a measurable indicator of soil health [57]. It was assessed in an experiment that the survival of three E. coli O157:H7 strains in four Chinese soils. Results showed that survival times ranged from 8.23 to 62.33 days, with biodiversity limiting E. coli invasion and virulence genes reducing survival [58].

Soil pathogens and human health

Ian L. Pepper explores the soil-health–human-health nexus, highlighting how soil influences human well-being and how human activities impact soil health, with strategies for improvement [59].

Studies regarding Physical factors of soil pressure

Zeyou Chen examined the bioavailability of tetracycline sorbed on three soil types to a fluorescent *E. coli* bioreporter, finding that soil-sorbed tetracycline can still exert selective pressure on bacteria, influenced by soil texture and water potential [60].

Conclusion

Soil, a highly complex and dynamic biomaterial, plays a fundamental role in sustaining life by supporting plant growth, regulating biochemical processes, and serving as a reservoir for both beneficial and harmful microorganisms. While soil microbiota contributes to essential ecosystem functions, it also harbors human pathogens such as *E. coli*, *Salmonella*, and *Campylobacter*, which can persist across diverse soil types. Additionally, environmental disturbances, particularly the widespread use of antibiotics in agriculture, have accelerated the emergence of antibiotic-resistant bacteria (ARB) in soil. Antibiotics excreted by livestock accumulate in manure and, when applied to soil, exert selective pressure on microbial communities, fostering the spread of resistance genes. Although most antibiotics degrade over time, their residual presence can alter microbial composition and resistance dynamics. Furthermore, land application of biosolids and manure may introduce enteric pathogens, raising concerns about potential human health risks. Given the critical role of soil in maintaining ecological balance, it is imperative to understand microbial interactions, enhance soil resilience, and adopt sustainable management practices to mitigate the spread of antibiotic resistance while preserving essential soil functions.

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