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Isolation and identification of pathogenic microorganisms from dump soil and sewage water in Tenali, India: Implications for public health and waste management

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Abstract

Environmental pollution caused by improper waste disposal significantly threatens public health. This study aimed to isolate and identify pathogenic microorganisms from sewage sludge and wastewater collected from the dump sites in Tenali, Vijayawada, and Guntur in Andhra Pradesh, India. A total of five samples, including four soil samples from different stages of decomposition and one sewage water sample, were analyzed for microbial contamination. The pathogen screening involved serial dilution, nutrient agar culturing, and biochemical characterization. We isolated six major pathogenic species: *Escherichia coli, Staphylococcus* species, *Bacillus* species, *Actinomycetes* species, *Trichoderma* species, and *Aspergillus niger*. The microbial load varied with the soil decomposition time, with higher pathogen counts in freshly dumped soil that declined over time, possibly due to phytoremediation and seasonal rainfall effects. The study highlights the presence of diverse pathogenic microorganisms in waste-contaminated environments, emphasizing the need for proper waste management and further investigation into the impact of leachate on microbial diversity.

Keywords: Pathogen Screening; Microbial Contamination; Dump Soil; Sewage Water; Waste Management; Environmental Microbiology.

1. Introduction

Rapid urbanization and industrialization have led to significant challenges in waste management, resulting in environmental contamination and public health concerns. Improper disposal of municipal waste contributes to the proliferation of pathogenic microorganisms, posing risks to human and animal health (Chawla *et al.,* 2023). Likewise, municipal sewage and solid waste harbour diverse microbial communities, including bacteria and fungi, some of which are also pathogenic. These pathogens can spread through soil, water and air, leading to outbreaks of infectious diseases in nearby populations (World Health Organization, 2020).

Several studies have highlighted the increasing microbial load in urban waste due to factors such as inadequate sewage treatment, improper disposal of biomedical waste and industrial effluents (Sobsey *and* Hill, 2008). In addition to the solid and sewage waste, landfill leachate, the liquid that percolates through decomposing waste, acts as a significant carrier of microbial pathogens and toxic compounds. It often contains high loads of organic matter, heavy metals and pathogenic microorganisms, which can infiltrate soil and groundwater systems, posing a direct threat to environmental and public health (Kjeldsen *et al.,* 2002). Studies have identified *E. coli*, *Salmonella* spp., *Clostridium* spp., and various fungi in leachate from untreated or poorly managed landfill sites (Slack *et al.,* 2005).

Microbial leachate contamination has been linked to gastrointestinal diseases, respiratory infections, and skin disorders in populations residing near waste disposal sites (Mor *et al.,* 2006). Moreover, the antibiotic resistance traits of some leachate-associated microbes further complicate treatment strategies and represent a growing public health concern

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(Kalka, 2012). The presence of biofilm-forming bacteria in leachate environments also enhances microbial survival, persistence, and resistance to disinfection methods, thus increasing their capacity to spread and infect (Tewari *et al.,* 2018).

Given the public health risks posed by sewage-borne pathogens, this study aims to isolate and identify microbial contaminants from dump soil and sewage water of different cities located in Andhra Pradesh. Furthermore, this research explores the seasonal variation in microbial load and the potential role of phytoremediation in reducing pathogen activity in waste-contaminated environments. Understanding the microbial composition and resistance patterns in waste systems is crucial for developing effective waste management strategies and mitigating health risks.

2. Materials and methodology

2.1. Sample collection

2.1.1. Study area

We collected 5 samples (dump soil-4 *and* sewage water-1) were collected one liter from in and around the municipal corporation of Tenali (Altitude: 16.236719 *and* Longitude: 80.647476), Vijayawada and Guntur in Andhra Pradesh, India, 522202. Samples were kept in sterile containers for further work.

2.1.2. Sampling technique

For data collection, samples were picked from four different zones with selected municipal grounds (four sides, like EAST, WEST, NORTH and SOUTH).

2.1.3. Sample size

A total in this work 20 samples were collected using a prevalence rate of 55%, an error of 3% and a standard normal of 1.10 by applying this formula (N=Z2 PQ/e2).

2.1.4. Sample collection

We collected twenty samples (four soil types and one sewage water-1), each one liter, from in and around the municipal corporation of Tenali, Vijayawada and Guntur, Andhra Pradesh, India, 522202. The samples were kept in sterile containers for further work.

2.2. Screening of pathogen

Soil samples were weighed to 1g, mixed into 1ml of distilled water, centrifuged and homogenized at 2,000 rpm at 25°C for 4 minutes for separation and serial diluted (10^{-1}to10^{-3}) concentration. These samples were mixed in freshly prepared nutrient agar media (NAM) at low temperatures and plates were stored in an incubation chamber (37°C) for 24-48 hours for proper growth. In the case of sewage water, 1 *ml* of sample was serially diluted to different concentrations and inoculated into the NAM media and for control, we kept an empty plate. We identified different kinds of pathogens after post-incubation. The following standard characterization tests were performed in triplicate: colony identification, Gram staining, motility tests and methyl red analysis of the specific pathogens on NAM-agar plates. In this work, pure pathogen cultures were identified based on their morphological and physiological characteristics with different standard methods.

2.3. Separation of pathogens from municipal soil and sewage water

1 g of soil samples was diluted into 10 ml of sterile Millipore water as dilution 10^{-1}. Using a sterile spatula to prepare a homogenous paste from this dilution, similarly, serial dilutions were prepared up to 10^{-3} and these dilutions were evenly poured on a nutrient agar medium. Microorganisms isolated on nutrient agar medium were streaked on selective media such as Mullar and LB agar media for identification of *E.coli,* Mannitol salt agar (MSA) for *S. aureus* and Xylose Lysine Deoxycholate (XLD) for *Salmonella*. The petri plates were kept at 37 °C for up to 72 hours. Colonies observed on these selective media were used for further analysis.

2.4. Aerobic colony count

All the municipal soil and sewage water samples were collected from different municipal locations. Street food samples from street vendors and stalls were segregated and labeled accordingly. About 1 g of samples was weighed and minced with the help of Sterile surgical scissors in a laminar airflow chamber (LAFC) for maintaining aseptic conditions.

Samples were transferred into a 250 *ml* conical flask containing 50 *ml* of peptone water. The mixture was shaken vigorously with the help of a vortex mixer for 5 minutes. 1 *ml* of an aliquot from each sample and peptone water solution were taken and 1-fold serial dilutions were made between 10^{-1} to 10^{-3} and plated 0.1 ml dilutions on LB medium for enumeration of total aerobic count. Plates are then incubated at 37° C for 24 hours. Later, bacterial colonies were counted with the help of a colony counter machine (VTS colony counter, India).

2.5. Voges - Proskauer test

All bacterial cultures were exposed to the Voges-Proskauer test for confirmation to determine the capability of microorganisms to produce acidic end products such as ethanol and acetyl methyl cainol from the organic acid. The isolated bacterial cultures were inoculated into MR-VP broth media. All the plates were incubated at 37 °C for 48 hours. After the incubation period, 12 drops of freshly prepared 5 % α-naphthol solution and 2 drops of 40% potassium hydroxide (KOH) were added to all the inoculated and control tubes. Development of pink or light red color may be intense at the surface, which indicates a positive test, while no change in color indicates a negative test in cultures.

2.6. Indole test

This was performed to find out whether the organism was able to oxidize tryptophan into indole, pyruvic acid and ammonia. Isolated bacteria from different soil and sewage water varieties were inoculated into tryptone broth. Whereas, an empty plate was taken for control and incubated at 37° C for up to 48 hours. After incubation, the Kovac reagent was added to the inoculated and control plates. The development of red color at the top layer in the form of a ring indicates a positive test, while its absence indicates a negative reaction for indole production from the cultures.

2.7. Methyl Red test

The methyl red test was conducted to determine whether the microbes perform mixed acid fermentation when glucose was used as a carbon source. If bacteria ferment glucose, the pH decreases, which can be identified by the pH indicator methyl red. The isolated bacterial species were inoculated into MR-VP broth. The inoculated and control plates were incubated at 37 °C for 48 hours. After incubation, methyl red was added to the inoculated and control plates. Indication of red colour is the fermentation of sugar and its absence indicates significantly low or that fermentation did not happen in the cultures.

2.8. Citrate utilization

A citrate test was performed to determine the ability of microorganisms to utilize citrate as a carbon source. The utilization of citrate in microorganisms depends upon the presence of an enzyme citrate permease that facilitates the transport of citrate into bacterial cells. The isolated microorganisms were inoculated in Simmons citrate agar plates and incubated at 37 °C for 48 hours. After incubation, plates were analyzed for color change in the plate from green to blue, indicating a positive test for citrate utilization. Retaining the same color indicates a negative result for citrate utilization in bacterial cultures.

2.9. Oxidase test

The oxidase test was used for the determination of the ability of bacteria to produce certain cytochrome c oxidases in bacterial species. The enzyme oxidase can oxidize N, N, N, N- N-tetramethyl-phenylenediamine (TMPD) dihydrochloride. For this test, a loop full of culture was rubbed on the filter paper soaked in 1% 4 (N)-TP. The color change in filter paper to purple within a few seconds indicates the positive oxidase results in bacterial species.

2.10. Catalase test

The catalase test was used to determine the ability of microorganisms to produce the catalase enzyme, which breaks down hydrogen peroxide to oxygen along with water. Here, we inoculated a large loop of bacterial cells with hydrogen peroxide. After a few seconds, gas bubbles indicated positive results for the samples.

2.11. Carbohydrate fermentation test

The carbohydrate fermentation test is used to determine whether bacterial species can metabolize carbohydrates and is used for identifying microorganisms. The procedure of this test action of organisms on a carbohydrate substrate results in acidification of the medium, which is detected by pH indicator dye. To identify the production of acid from fermentation, a Durham's tube is placed in each tube to capture gas produced by metabolism. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. Phenol red carbohydrate broth

or basal media was prepared. Phenol red acts as a pH indicator and an inverted Durham tube was used to demonstrate gas production in bacterial cultures.

3. Results

3.1. Estimation of Pathogenic Bacteria in Different Sewage Samples

The isolation of pathogenic bacteria and fungi from soil and sewage water samples was carried out using conventional culture-based methods. This approach included a sequence of steps starting with non-selective enrichment, followed by selective or differential plating techniques. The isolated microorganisms were then identified based on morphological, biochemical and serological characteristics. A total of 16 soil and 4 sewage water samples were collected from various regions in Andhra Pradesh. This isolation process allowed for the identification of multiple sewage-borne pathogens.

Figure 1 Isolation of different bacterial species from sewage soil and water

3.2. Comparison of soil and sewage water contamination and pathogen load across Tenali, Vijayawada and Guntur

When comparing contamination and pathogen loads in soil and sewage water samples across Tenali, Vijayawada and Guntur, notable differences were observed. Generally, soil samples exhibited higher contamination levels than sewage water, with significant variation in bacterial and fungal load between zones. In Tenali, particularly in South Tenali, soil samples such as SOUTH TENALI-1 and SOUTH TENALI-3 showed consistently high contamination levels. SOUTH TENALI-2 and 4 had moderate contamination, while in North Tenali, NORTH TENALI-1 and 2 were highly contaminated and NORTH TENALI-3 and 4 showed moderate levels. Sewage water samples, specifically SOUTH TENALI-5 and NORTH TENALI-5, had relatively lower contamination, with NORTH TENALI-5 having the least pollution.

Pathogen analysis in Tenali revealed that *E. coli* and *Staphylococcus* spp. were the most dominant in soil, especially in high-contamination zones like SOUTH TENALI-1 and 3. *Bacillus* species and *Actinomycetes* species were present at moderate levels across soil samples. In sewage water, *E. coli* and *Pseudomonas* species were found in moderate concentrations, particularly in SOUTH TENALI-5, but the pathogen diversity was lower compared to soil, suggesting possible dilution or microbial competition in water bodies.

Whereas, in Vijayawada, contamination and pathogen levels were highly variable. Soil samples VIJAYAWADA-1 and 2 showed high contamination, while VIJAYAWADA-3 and 5 had moderate to low levels, reflecting differences in local land use and pollution sources. Sewage water (VIJAYAWADA-6) showed mixed contamination levels. Bacterial diversity was highest in Vijayawada's soil samples, where *E. coli*, *Bacillus* species, and *Staphylococcus* spp. were prevalent in VIJAYAWADA-1 and 2. *Clostridium* spp., an anaerobe associated with soil and wastewater contamination, was also detected in moderate amounts. *Aspergillus* and *Trichoderma* species were the primary fungal contaminants. In sewage water, *P. aeruginosa* and *Klebsiella* species were frequently detected, with VIJAYAWADA-6 showing the highest bacterial load among all sewage samples.

Surprisingly, Guntur had a more balanced contamination profile compared to the other two cities. Soil and sewage samples showed moderate to high contamination levels. GUNTUR-1 was highly contaminated, GUNTUR-2 and 4 showed

moderate contamination and GUNTUR-3 had low contamination in some zones. The sewage sample, GUNTUR-5, exhibited both high and low contamination levels, pointing to localized pollution sources. Pathogen load was moderate across Guntur's soil samples, with the presence of *E. coli, Staphylococcus, Bacillus* and *Actinomycetes* species. Fungal species such as *A. niger* and *Penicillium* were prominent in high-contamination soil samples. In sewage water, *P. aeruginosa* and *E. faecalis* were present but at lower levels than in Vijayawada, possibly due to better microbial self-purification or dilution.

3.3. Total plate count of isolates

The total plate count (TPC) of microbial isolates from soil and sewage samples focused on bacteria such *as E. coli, Staphylococcus, Bacillus, Actinomycetes* and *Trichoderma.* The results revealed that *Actinomycetes* species had the highest colony count at the 10^{-1} dilution (2547), followed by *Bacillus* (1975), *Alcaligenes* (1758), *Brevibacterium* (1874) and *E. coli* (1680). *Shigella* species had the lowest presence, with only 57 colonies at 10^{-3} dilution. *Brevibacterium* showed a spike in colony count at higher dilutions, indicating its widespread presence even at low concentrations. This trend illustrates the dominance of *Actinomycetes, Bacillus* and *Brevibacterium*, while *Shigella* was the least prevalent compared to other pathogens (Figure 2).

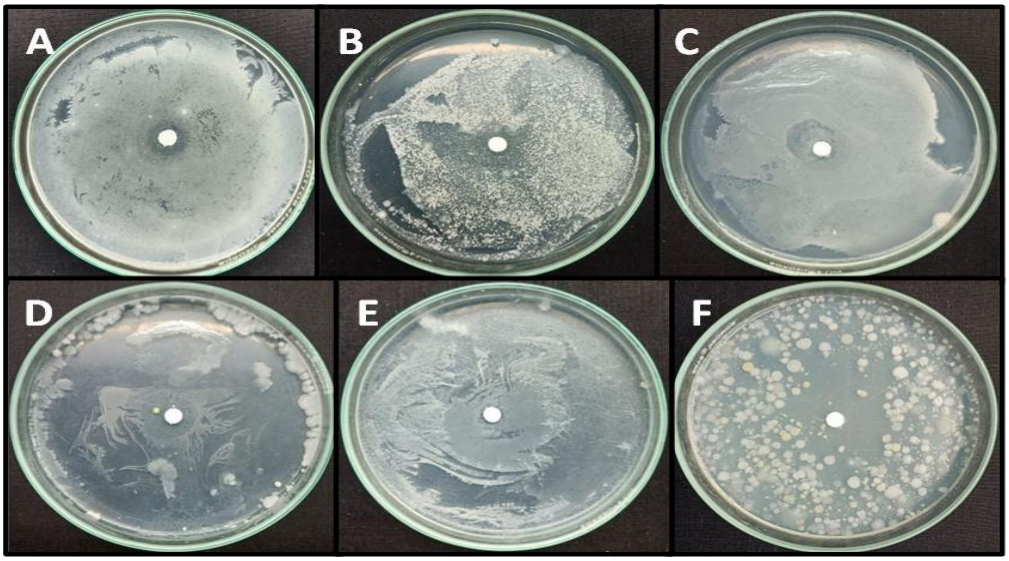

Figure 2 Different municipal soil samples and their pathogen colonies, along with morphological characterization (A-F)

3.4. Analysis of microbial contamination in sewage soil and water

Microbial contamination analysis across regions revealed specific bacterial distribution patterns. In South Tenali, *E. coli* (198 CFU) was found in SOUTH TENALI-4, *Alcaligenes* (155 CFU) in SOUTH TENALI-3, and *Bacillus* (274 CFU) in SOUTH TENALI-5. *Shigella* species were absent in all South Tenali samples, and CFU/ml ranged from 0.13×10^6 to 0.25×10^{11}. North Tenali exhibited a broader microbial diversity. *E. coli* was present in NORTH TENALI-3 and 5, while *Alcaligenes* showed a high count in NORTH TENALI-4 (571 CFU). *Trichoderma* was also common in NORTH TENALI-3 and 5. *Shigella* was detected in NORTH TENALI-1 and 4. CFU/ml values ranged from 1.1×10^3 to 1.5×10^{10}. Vijayawada had the highest microbial abundance overall. *E. coli* was present in VIJAYAWADA-3 and 5, *Alcaligenes* was dominant in VIJAYAWADA-2 (578 CFU) and *Bacillus* appeared in multiple samples. *Trichoderma* was abundant in VIJAYAWADA-2 and 3. *Shigella* was found only in VIJAYAWADA-1. CFU/ml values ranged from 0.26×10^{11} to 1.8×10^{11}, confirming high bacterial loads. Whereas, Guntur showed lower microbial loads compared to other areas. E. coli and Alcaligenes were detected in GUNTUR-2 and 5, *Brevibacterium* in GUNTUR-1, and Bacillus in GUNTUR-4. *Shigella* was present in GUNTUR-1 and 3. CFU/ml ranged from 0.4×10^3 to 0.9×10^7.

North Tenali showed a wide variety of pathogens, including *E. coli*, *Shigella, Bacillus* and *Trichoderma.* South Tenali had moderate contamination with dominance of *E. coli* and *Bacillus*. Guntur had relatively lower bacterial loads but showed significant presence of *Brevibacterium* and *Shigella.* These findings suggest regional differences in microbial contamination, with potential implications for public health. Selective media were instrumental in isolating specific pathogens. McConkey agar was used for detecting *E. coli*, Mannitol Salt Agar (MSA) for *S. aureus*, EMB for gram-negative bacteria and XLD for *Salmonella*. Following inoculation and incubation at 37°C for 72 hours, characteristic colonies were

identified for further analysis. The morphological characterization of isolated bacteria included assessments of colony pigmentation, shape, margin and elevation, along with Gram staining, motility and structural alterations. Most colonies appeared white, round, with entire margins and either flat, raised, or convex elevation, depending on their origin. These morphological traits, along with Gram staining (positive or negative) and motility observations, provided preliminary identification, supporting the biochemical and serological tests for pathogen confirmation (Figure 3).

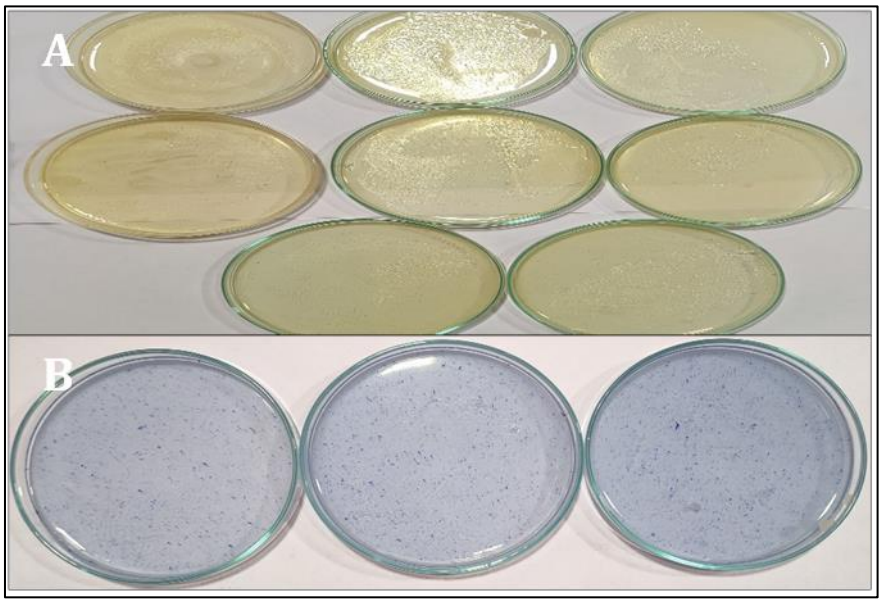

Figure 3 Selective media for bacterial growth: (A) XLD media for Salmonella and Shigella; (B) EMB media for Gram-negative bacteria

3.5. Characterization of bacteria isolated from sewage soil and water samples in dump areas:

Bacteria isolated from sewage-contaminated soil and water samples in dump areas across Tenali, Vijayawada and Guntur were characterized using standard microbiological techniques. This included assessments based on colony morphology, microscopic features and a suite of biochemical tests, namely the Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate, Catalase, Oxidase and Fermentation tests. These analyses were essential for identifying bacterial species, determining their metabolic capabilities and evaluating potential pathogenicity in environmental contexts.

3.6. Indole test analysis

The Indole test helped assess the isolates' ability to degrade tryptophan to indole, indicating the presence of the enzyme tryptophanase. This metabolic trait was particularly prevalent in samples from Tenali. In South Tenali-1 and -2, as well as North Tenali-3 and -4, Indole-positive results pointed to bacteria such as *E. coli* and *B. cereus*, both linked to fecal contamination and soil-borne infections. Conversely, Indole-negative results in South Tenali-3 and -4 and North Tenali-5 indicated the presence of non-enteric, potentially less harmful environmental bacteria. However, *K. pneumoniae*, identified in North Tenali sewage water, raised concerns due to its association with respiratory and urinary tract infections (Figure 4).

In Vijayawada, Indole-positive results were observed in samples 2, 3 and 4, suggesting the presence of *Proteus* and *Enterobacter* spp., known to cause urinary tract and wound infections. Indole-negative results in Vijayawada-1 and -5 indicated possible dominance of *P. aeruginosa*, an environmental bacterium with high antibiotic resistance. In Guntur, positive Indole results in Guntur-2, -3, and -4 suggested the presence of *Salmonella* and *Vibrio* species, whereas Guntur-1 and -5 likely contained environmental *actinomycetes*. Overall, while Tenali samples indicated high fecal contamination, Vijayawada revealed both enteric and opportunistic pathogens and Guntur showed waterborne pathogen presence. Indole-negative sewage water samples still posed potential risks due to biofilm-forming bacteria like *P. aeruginosa*.

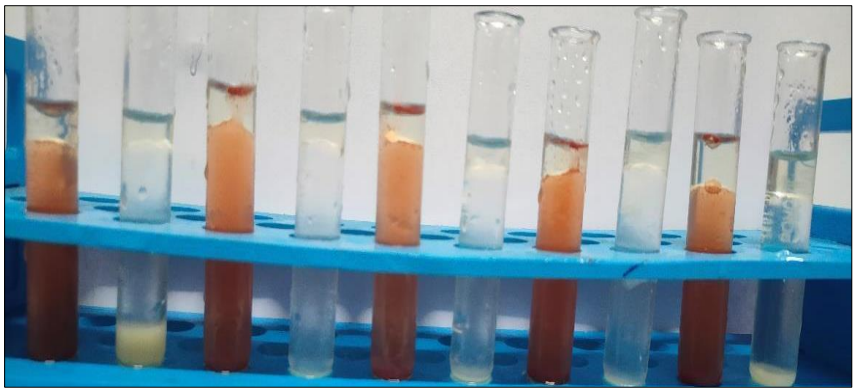

Figure 4 Negative and positive results of several pathogens in the indole test

3.7. MR-VP Test Analysis

MR-positive and VP-negative results in South Tenali-3, -4, North Tenali-2, and Guntur-2 and -3 suggested the presence of acid-producing enteric bacteria such as *E. coli*, *Salmonella* and *Shigella*. Conversely, MR-negative and VP-positive outcomes in South Tenali-1, Vijayawada-1, and Guntur-4 indicated *Klebsiella*, *Enterobacter* and *Bacillus* spp., known for environmental resilience and biofilm formation. North Tenali-5 also showed VP positivity, reflecting contamination by opportunistic bacteria (Figure 5).

Several samples were positive for both MR and VP, indicating metabolically versatile bacteria like *Enterobacter* and *Bacillus cereus*, capable of surviving in diverse conditions. This was evident in samples from South Tenali-2 and -5, North Tenali-1 and -3, Vijayawada-2 and -3, and Guntur-2 and -3. Some isolates from Vijayawada-4 and Guntur-5 were negative for both tests, hinting at non-fermentative bacteria such as *Pseudomonas*, *Acinetobacter*, or *Actinomycetes*, often resistant to antibiotics and thriving in hospital or sewage environments. MR-VP results suggested Tenali had high fecal coliform prevalence, Vijayawada had a mix of enteric and opportunistic pathogens, and Guntur was dominated by waterborne pathogens, emphasizing the need for effective sanitation.

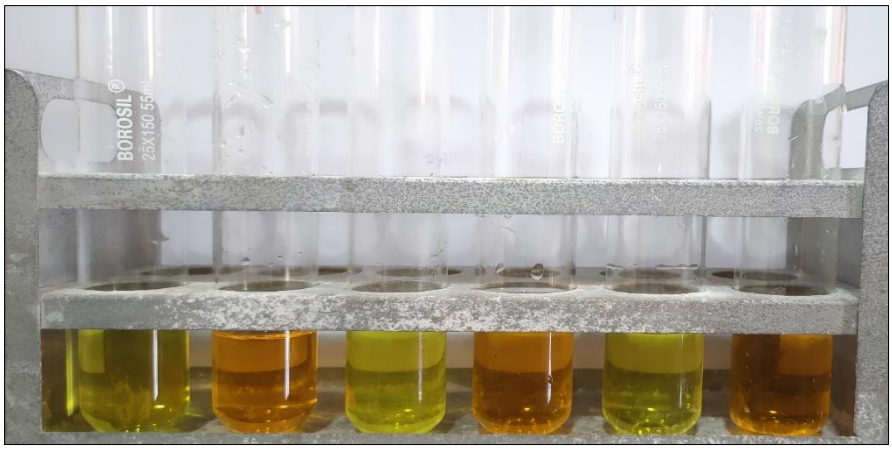

Figure 5 Negative and positive results of several pathogens in the MR-VP test

3.8. Citrate Utilization Test

Citrate utilization, indicative of an organism's ability to metabolize citrate under aerobic conditions, varied across regions. In South Tenali, samples 2, 4 and 5 tested positive, pointing to *Enterobacter*, *Klebsiella* and *Bacillus* spp., while samples 1 and 3 had mixed or negative outcomes, suggesting bacteria like *E. coli* that favour glucose fermentation. In North Tenali, citrate positivity in samples 2 and 3 indicated *K. pneumoniae* or *P. aeruginosa*, whereas North Tenali-1 and -4 showed fermentative bacteria like *Shigella* (Figure 6).

In Vijayawada, only Vijayawada-1 tested citrate-positive, suggesting *Bacillus* or *Enterobacter* spp., while the rest were dominated by non-citrate utilizers such as *actinomycetes*. Guntur 2 and 4 showed strong citrate utilization, likely due to aerobic bacteria such as *Pseudomonas* and *Enterobacter*, while Guntur-3 showed minimal utilization. Sewage samples

from North Tenali-5, Vijayawada-5 and Guntur-5 exhibited mixed to positive results, suggesting the presence of biofilm-forming, opportunistic bacteria capable of thriving in nutrient-deprived environments.

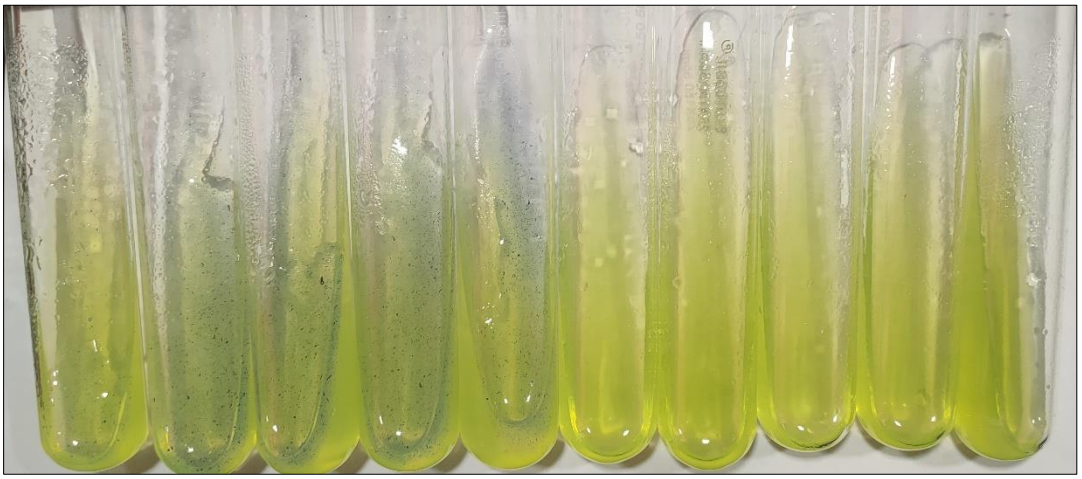

Figure 6 Negative and positive results of several pathogens in the citrate utilization test

3.9. Oxidase Test Analysis

Oxidase testing revealed the distribution of aerobic respiratory bacteria. Out of all isolates, 18 tested oxidase-positive, identifying organisms like *Pseudomonas*, *Vibrio*, and *Neisseria*. North Tenali-2, South Tenali-2 and sewage samples from Vijayawada-5 and Guntur-5 had the highest oxidase positivity. In contrast, 27 isolates were oxidase-negative, pointing to a dominance of facultative anaerobes like *E. coli*, *Klebsiella* and *Salmonella*, especially in North Tenali-1, -3, -4 and Vijayawada-2 and -4 (Figure 7).

Whereas, South Tenali-1 had a mixed profile, South Tenali-2 had a higher oxidative load, while South Tenali-3 was entirely negative. North Tenali-2 had complete oxidase positivity, whereas North Tenali-5 had a mixed microbial community. Vijayawada samples showed partial to full oxidase negativity, with Vijayawada-5 being an exception. In Guntur, mixed results were recorded across locations, with oxidase positivity in sewage (Guntur-5) again suggesting *P. aeruginosa* as a dominant species.

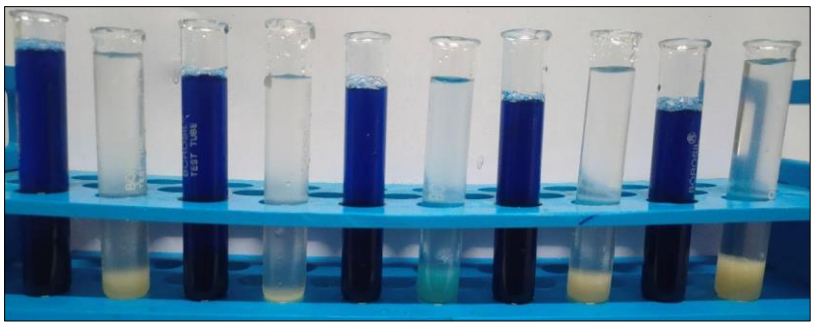

Figure 7 Negative and positive results of several pathogens in the indole test

3.10. Catalase Test Analysis

The catalase test detected bacterial capability to decompose hydrogen peroxide, a trait associated with aerobic and facultative anaerobic organisms like *Staphylococcus*, *Bacillus* and *Pseudomonas*. Whereas, 19 isolates were catalase positive, particularly in North Tenali-2 and -4, Vijayawada-4 and -5 and Guntur-3, suggesting oxidative stress-resistant bacteria. In contrast, all isolates from South Tenali tested catalase-negative, indicating an anaerobic bacterial community composed of genera like *Enterococcus* and *Lactobacillus*.

In North Tenali, catalase positivity was limited to samples 2 and 4, suggesting organisms like *Bacillus* or *Staphylococcus*, while the rest harbored anaerobic types. Vijayawada showed high catalase activity in sewage samples, and Guntur-3 also had catalase-positive isolates, pointing to aerobic organisms capable of enduring oxidative stress (Figure 8).

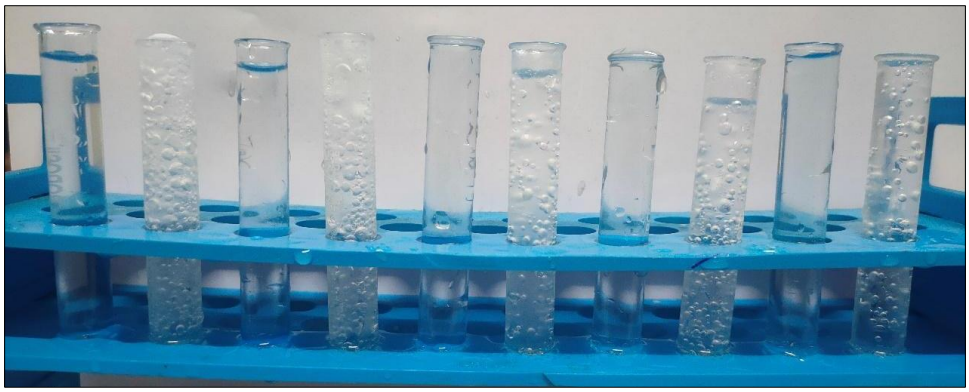

Figure 8 Negative and positive results of several pathogens in the catalase test

4. Discussion

The present study provides a comprehensive analysis of microbial contamination, specifically focusing on pathogenic bacteria and fungi isolated from sewage-contaminated soils and water samples collected from the Tenali, Vijayawada, and Guntur regions of Andhra Pradesh. The results reveal significant regional differences in microbial loads and diversity, largely influenced by anthropogenic activities, population density and waste disposal practices (Figure 1).

A consistent trend across all study sites was the higher microbial contamination in soil samples compared to sewage water. This observation supports the findings of Nwachukwu *et al.* (2010), who highlighted soil's role as a natural reservoir for pathogens, owing to its nutrient-retention capacity and favourable conditions for microbial persistence. The high microbial loads observed in South Tenali and North Tenali, particularly the dominance of *E. coli*, *Staphylococcus* spp., and *Bacillus* spp. align with earlier reports by Igbinosa *et al.* (2011), who found elevated levels of fecal indicators and opportunistic pathogens in urban dump yard soils. In Vijayawada, the detection of diverse bacteria such as *E. coli*, *Clostridium* spp., and *Staphylococcus* spp. in soil indicates severe fecal and organic contamination, potentially attributed to unregulated sewage discharge and industrial effluents. Fawell and Nieuwenhuijsen (2003) similarly noted that untreated municipal wastewater can substantially increase microbial diversity in urban environments. The presence of *P. aeruginosa* and *Klebsiella* spp. in sewage water samples from Vijayawada aligns with the work of Scott *et al.* (2002), who associated these organisms with waterborne infections, particularly in developing regions lacking effective sanitation.

In contrast, Guntur demonstrated moderate contamination, with occasional detection of *Brevibacterium*, *E. coli* and *Actinomycetes*, suggesting relatively improved waste management systems. However, the presence of *Shigella* in some soil samples and *E. faecalis* in sewage water raises public health concerns. These findings echo those of Okoh *et al.* (2007), who found that indicator bacteria levels can vary with environmental factors such as rainfall, pH and microbial interactions.

Total Plate Count (TPC) analysis revealed that *Actinomycetes* and *Bacillus* species were dominant across all sites, particularly at lower dilutions. Their prevalence can be attributed to their spore-forming ability and ecological resilience. This observation aligns with the findings of Zhang *et al.* (2018), who highlighted the adaptability of these genera in polluted soils due to their ability to form biofilms and degrade complex substrates. Selective media and biochemical tests provided further confirmation of key microbial contaminants. Frequent detection of *E. coli* on MacConkey agar in South and North Tenali supports the likelihood of fecal pollution from domestic waste, consistent with the studies of Leclerc *et al.* (2001). Indole-positive reactions in multiple samples from Tenali and Vijayawada indicated the presence of *E. coli* and *Proteus* spp., both considered classic indicators of fecal contamination (Cabral *et al.,* 2010).

MR-VP test results demonstrated that several isolates produced either strong acids (MR+) or neutral end products (VP+), with some positive for both reactions. This metabolic profile suggests the presence of facultative anaerobes such as *Enterobacter* spp. and *Bacillus cereus*, which are known for thriving in complex waste environments. Tallon *et al.* (2005) similarly documented the metabolic versatility of these organisms in sewage and landfill ecosystems. Spatial analysis of microbial diversity indicated site-specific contamination sources. For instance, the elevated CFU counts of *Alcaligenes* in North Tenali-4 and Vijayawada-2 suggest industrial or xenobiotic waste influx, as this genus is commonly linked with bioremediation and degradation of toxic compounds (Kong *et al.,* 2013). Fungal contaminants like

Trichoderma and *Aspergillus* spp. dominated the moist, nutrient-rich soils of Vijayawada, consistent with the tropical soil fungal profile described by Domsch*et al.* (2007).

One important but often overlooked contributor to microbial contamination in urban settings is landfill leachate the liquid formed by rainwater percolating through solid waste, picking up organic and inorganic pollutants. Leachate is not only chemically toxic but also microbiologically active. It often contains high loads of pathogenic bacteria, including *E. coli*, *Salmonella spp.*, *Clostridium spp.*, and even antibiotic-resistant organisms (Kjeldsen *et al.,* 2002; Kalka *et al.,* 2012). These pathogens, when released into surrounding soils and water bodies, significantly amplify health risks to nearby populations.

Several studies have confirmed that leachate from poorly managed landfills can contaminate groundwater and surface water, with microbial loads exceeding WHO standards (Slack *et al.,* 2005; Mor *et al.,* 2006). For instance, Tewari (2018) reported the presence of biofilm-forming and multidrug-resistant bacteria in landfill leachates, which complicates disinfection and contributes to chronic infections in exposed communities.

In the context of this study, dump yard areas in Tenali and Vijayawada, known to receive mixed solid waste without effective leachate containment systems, likely contribute to the observed microbial profiles. The frequent detection of enteric pathogens and facultative anaerobes in soil samples near these zones suggests leachate infiltration into surface soil layers. Furthermore, *A. niger* and *Trichoderma* species, which thrive in moist, high-organic content environments, are often abundant in leachate-affected soils, as also documented by Domsch (2007).

The sporadic detection of *Shigella* across regions points to transient contamination events, possibly linked to seasonal runoff, improper sewage handling, or leachate migration. This pattern mirrors earlier findings by Ramteke (1992) in Indian urban settings, where rainfall and surface water interactions contributed to the rapid spread of enteric pathogens.

Collectively, these results suggest that Vijayawada faces the greatest risk for microbial outbreaks, due to high microbial loads in both soil and water, particularly involving enteric and opportunistic pathogens. Whereas Tenali maintains a proper sanitation system compared to other cities. Tenali, especially the North and South zones, also exhibits a significant health risk, mainly from *E. coli* and *Staphylococcus* contamination, potentially exacerbated by unregulated leachate seepage. Guntur, while comparatively less affected, still shows signs of microbial threats and warrants periodic monitoring, especially during monsoon months when leachate movement increases

5. Conclusion

This study reveals significant microbial contamination in sewage-contaminated soils and water from Tenali, Vijayawada and Guntur, with marked regional variation influenced by waste disposal practices, industrial activities and population density. Soil consistently showed higher microbial loads than water, confirming its role as a reservoir for pathogens. Vijayawada exhibited the highest contamination, particularly with fecal and opportunistic pathogens like *E. coli*, *Clostridium spp.*, *P. aeruginosa*, and *Klebsiella spp.*, likely due to unregulated sewage and industrial discharge. North and South Tenali also displayed heavy microbial loads, dominated by *E. coli*, *Staphylococcus spp.*, and *Bacillus spp.*, indicating substantial fecal pollution. While Guntur showed moderate contamination, the presence of *Shigella* and *E. faecalis* raises health concerns. The widespread detection of resilient microbes such as *Actinomycetes*, *Bacillus spp.*, and fungi like *Trichoderma* and *Aspergillus spp.* highlights the ecological adaptability of these organisms in polluted environments. These findings underscore the urgent need for improved sewage treatment, effective waste management and routine monitoring to prevent the spread of pathogenic microbes and safeguard public health. Going forward, integrating molecular screening for virulence and antibiotic resistance genes may offer deeper insight into the potential threat posed by these environmental pathogens and contribute meaningfully to the global dialogue on antimicrobial resistance (AMR) in natural reservoirs.

Compliance with ethical standards

Disclosure of Conflict of Interest

The authors declare that they have no competing interest

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