Antibiotic Sensitivity for Nitrofurantoin from Pharmaceutical Industrial Environmental Samples

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***Abstract*—Antimicrobial resistance (AMR) is a major global health problem that reduces the effectiveness of antibiotics and makes infections harder to treat. It is responsible for over 1.27 million deaths every year worldwide. Nitrofurantoin is a commonly used antibiotic for treating urinary tract infections, but resistance to this drug is increasing due to environmental contamination.**
This study aims to evaluate the antibiotic sensitivity of Nitrofurantoin against bacteria isolated from environmental samples such as water, soil, and sewage. These sources act as reservoirs for resistant microorganisms due to pollution from pharmaceutical industries and improper waste disposal.
The Kirby–Bauer disc diffusion method was used to test the sensitivity of bacterial isolates. The results showed different levels of sensitivity and resistance among the isolates.
The study highlights the importance of proper antibiotic use, effective waste management, and regular monitoring to control the spread of antimicrobial resistance and protect public health.

***Index Terms*—Antimicrobial resistance, Antibiotic sensitivity, Nitrofurantoin, Diffusion method Kirby bauer Environmental samples.**

I. INTRODUCTION

Antimicrobials are medicines that kill or slow the growth of germs (bacteria, viruses, fungus) that cause diseases. Antibiotics are the most commonly prescribed for the antimicrobial.
Antimicrobial resistance (AMR) occurs when some of the types of microorganisms that can develop resistance to antimicrobials include:

Bacteria – develop resistance to antibiotics
Viruses – develop resistance to antiviral medicines
Fungi – develop resistance to antifungal medicines
AMR refers to the broader term antimicrobial resistance. This term includes microorganisms that develop resistance to antimicrobial medicines.
Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health threats of the 21st century. AMR manifests when microorganisms, encompassing bacteria, fungi, parasites, and viruses, undergo evolutionary processes leading to their resistance against antimicrobial medications, such as antibiotics, commonly employed for treating such infections.
The prevalent issue is largely attributed to the repercussions of antibiotic overuse or irresponsible utilization across diverse contexts, predominantly in clinical treatment, agricultural practices, animal healthcare, war crisis, and the food system. Often dubbed the "Silent Pandemic," AMR necessitates immediate and efficacious intervention rather than being relegated to a future scenario.
In the absence of preventive measures, projections indicate that by 2050, AMR could potentially supersede all other causes of mortality worldwide. Globally, estimates indicate that the direct fatalities linked to AMR surpassed 1.2 million in 2019, with a foreseen escalation to approximately 10 million deaths annually by 2050 if inadequate measures are implemented to curb AMR.
The issue of AMR presents a substantial risk to the overall well-being of the global population and is increasingly becoming a matter of great apprehension on a worldwide scale. AMR pertains to the capacity of

microorganisms, encompassing bacteria, viruses, fungi, and parasites, to withstand the therapeutic impact of previously efficacious drugs in combating them.

This phenomenon undermines the efficacy of antibiotics, antivirals, and other pharmaceuticals, resulting in heightened morbidity, mortality, and healthcare expenditures. Addressing AMR has emerged as a pivotal global health imperative, necessitating prompt and synchronized endeavours from governmental bodies, healthcare practitioners, scholars, and the general populace.

AMR occurs when microorganisms like bacteria, viruses, fungi, and parasites resist antimicrobial drugs, making standard treatments ineffective and increasing the risk of infection.

AMR is a natural phenomenon; however, human influences have dramatically accelerated and exacerbated its progression over recent decades.

Microbes are under selective pressure to become resistant and acquire adaptive mutations or genes when antimicrobial agents are misused or overused in healthcare, veterinary, and agricultural settings. This then enables their survival and persistence in environments saturated with antibiotics and antiseptics that would previously have readily destroyed them.

Bacteria and other microbes have a remarkable ability to rapidly adapt, mutate, and share adaptive genetic elements via horizontal gene transfer mechanisms, allowing them to develop diverse resistance mechanisms.

Microorganisms that develop AMR may make human and animal diseases challenging to cure. Resistance prolongs sickness, increases spread risk, lengthens hospital stays, requires more costly therapies, and raises fatality rates. This escalating cycle of resistance development is not only a contemporary concern but has roots deeply embedded in the history of antimicrobial use.

The history of AMR traces back to the discovery of penicillin in 1928 by Alexander Fleming and the subsequent mass production and utilization of antibiotics in the 1940s. However, resistant organisms emerged almost immediately thereafter.

The first cases of penicillin-resistant Staphylococcus aureus were reported in 1942, along with tetracycline resistance by 1953. The widespread agricultural use of antibiotics in the 1950–60s also accelerated resistance. MRSA was reported in 1961, followed by resistance to multiple antibiotic classes. The 1980s saw a global epidemic of MDR tuberculosis. In the 1990s, gram-negative pathogens such as Escherichia coli and Klebsiella pneumoniae developed ESBL resistance.

The rise of MDR diminished the number of available effective antibiotics and resulted in the withdrawal of pharmaceutical companies from antibiotic research. This perfect storm of increasing resistance and lack of new drug development continues to strain healthcare systems today.

We have now entered a dangerous post-antibiotic era where common infections and minor injuries can once again become lethal. If solutions are not urgently implemented, it is expected.

II. ANTI BIOTIC SENSITIVITY

Antibiotic sensitivity (or susceptibility) testing determines which antibiotics effectively kill or inhibit specific bacteria or fungi causing an infection. This laboratory test, often using methods like disk diffusion (Kirby–Bauer), measures the “zone of inhibition”—an area without bacterial growth—to guide targeted, effective treatment, rather than relying on broad-spectrum, empirical therapy.

- PURPOSE:

The primary goal is to identify the best antibiotic to treat an infection, especially when dealing with antibiotic-resistant bacteria.

- METHODOLOGY:

A sample (blood, urine, etc.) is taken, and bacteria are cultured on agar plates, then exposed to antibiotic-impregnated disks to measure the growth inhibition.

- RESULT & INTERPRETATION:

The size of the inhibition zone is measured in millimetres, comparing it to established breakpoints to classify the bacteria as “sensitive” (susceptible), “intermediate,” or “resistant.”

DRUG PROFILE (NITROFURANTOIN)

Nitrofurantoin is a synthetic nitrofuran antibiotic primarily used to treat urinary tract infections. Its chemical and physical properties as of 2026 are detailed below:

CHEMICAL COMPONENTS:
It consists of a five-membered furan ring with a nitro group at the 5-position, connected via a methylene amino bridge to a hydantoin (imidazolidine-2,4-dione) ring.

IUPAC Name:
1-[(E)-(5-nitrofuran-2-yl) methylidene amino] imidazolidine-2,4-dione.

MOLECULAR WEIGHT:
Approximately 238.16 g/mol (reported values range from 238.157 to 238.2 g/mol).

MELTING POINT:
270–272 °C (decomposition occurs at this temperature). Some sources list a slightly lower range of roughly 268 °C.

BOILING POINT:
Not applicable, as the substance decomposes upon melting. A rough theoretical estimate of 380.75 °C has been cited in some databases.

SOLUBILITY:
Water: Very slightly soluble (approximately 190 mg/L at 37 °C). Solubility is pH-dependent; it is a weak acid with a pKa of 7.2.

ORGANIC SOLVENTS:
Soluble in dimethylformamide (DMF) (80 g/L), DMSO (25 mg/mL), and ethanol (500 mg/L or ~15 mg/mL).
Recent 2024–2025 journals indicate that nitrofurantoin remains a first-line therapy for uncomplicated urinary tract infections (UTIs) due to low resistance rates, even as it faces scrutiny for potential pulmonary risks during long-term use. Studies show global resistance of uropathogenic E. coli to nitrofurantoin is relatively low (around 6.9–7.6% between 2015–2024).

Key Findings from 2024–2025 Journals
SAFETY & EFFICACY:
A 2025 study highlighted its safety, efficacy, and cost-effectiveness for treating UTIs in pregnancy Risks:
A 2025 database review highlighted the need for monitoring regarding its risk of pulmonary complications, particularly in long-term, recurrent UTI cases.

COMPARATIVE EFFICACY:
Research in 2024 showed that while effective, it may have slightly different symptom relief profiles compared to fosfomycin.

DEVELOPMENTS:
Investigations into improving its delivery mechanisms (e.g., self-emulsifying systems) are ongoing to address drug formulation challenges.

III. NITROFURANTOIN

Nitrofurantoin is an antibiotic medication that is used for the treatment of uncomplicated lower urinary tract infections. It is effective against most gram-positive and gram-negative organisms. The FDA approved nitrofurantoin in 1953 to treat lower urinary tract infections. Nitrofurantoin is a synthetic antimicrobial created from furan and an added nitro group and a side chain containing hydantoin. Nitrofurantoin was widely used to treat lower urinary tract infections until the 1970s, when trimethoprim-sulfamethoxazole and newer beta-lactam antibiotics became available. Several major guidelines have recently declared nitrofurantoin the first-line therapy for treating uncomplicated lower urinary tract infections. Increasing resistance to newer antibiotics coinciding with an increasing prevalence of extended-spectrum beta-lactamase (ESBL) producing bacteria has led to a resurgence in the prescriptions of nitrofurantoin.
Nitrofurantoin's primary use has remained in treating and prophylaxis of urinary tract infections. Nitrofurantoin is advantageous as it concentrates in the lower urinary tract while maintaining a low serum concentration and does not significantly affect bowel flora. The predominant cause of urinary tract infections is periurethral colonization of bacteria from a faecal reservoir, which then ascends the urinary tract. Researchers think that nitrofurantoin's continued effectiveness and minimal resistance patterns are partly attributable to its minimal effect on bowel flora. Nitrofurantoin is effective against many gram-positive and gram-negative organisms. Nitrofurantoin is bactericidal against most common urinary tract pathogens, including Escherichia coli, Enterococci, Klebsiella, Staphylococcus saprophyticus, and

Enterobacter. Its spectrum of susceptibility also includes Shigella, Salmonella, Citrobacter, Neisseria, Bacteroides, group B streptococcus, Staphylococcus aureus, and Staphylococcus epidermidis. Studies have shown the effectiveness of nitrofurantoin does not differ between ESBL-producing E. coli and non-ESBL-producing E. coli strains.

Resistance to nitrofurantoin remains relatively rare despite several decades of widespread use. Numerous studies demonstrated that nitrofurantoin is an effective prophylactic agent in long-term prophylaxis and compares well to other antibiotics. A population-based survey of in vitro antimicrobial resistance of urinary E. coli isolates among United States outpatients showed a resistance rate of 1.6%. A meta-analysis for clinical cure demonstrated overall equivalence between nitrofurantoin and its comparators when used for uncomplicated urinary tract infections

IV. METHODOLOGY

A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as the poisoned food technique. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytoluminometric methods are recommended, which provide information on the nature of the inhibition effect (bactericidal or bacteriostatic), whether it is time-dependent or concentration-dependent, and the cell damage inflicted on the test microorganism.

Owing to the growing interest in the properties of new antimicrobial products, especially for combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of an extract or a pure compound for its applications in human health, agriculture, and the environment. Therefore, in this review, the techniques for evaluating the in vitro antimicrobial activity were discussed in detail.

ANTIBIOTIC SENSITIVITY TEST

Diffusion Methods (Kirby–Bauer)

Agar Disk-Diffusion Method

Agar disk-diffusion testing, developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeast testing.

Although not all fastidious bacteria can be tested accurately by this method, standardization has been developed to test certain fastidious bacterial pathogens such as Streptococci, Haemophilus influenza, Haemophilus parainfluenza, Neisseria gonorrhoea, and Neisseria meningitis using specific culture media, incubation conditions, and interpretive criteria for inhibition zones.

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper disks (about 6 mm in diameter) containing the test compound at a desired concentration are placed on the agar surface. The Petri dishes are incubated under suitable conditions.

Generally, the antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism. The diameters of inhibition growth zones are then measured. The growth media, temperature, incubation period, and inoculum size are maintained according to CLSI standards.

Antibiotic resistance has emerged as one of the greatest challenges in public health, agriculture, and environmental microbiology. The need for precise and reproducible techniques to evaluate bacterial susceptibility to antibiotics has never been more critical. Among various antibiotic susceptibility testing methods, the disk diffusion method stands out due to its accuracy, simplicity, and cost-effectiveness.

What is the Disk Diffusion Method?

The disk diffusion method, also commonly known as the Kirby–Bauer method, is a standardized microbiological test widely employed to evaluate the sensitivity of bacterial isolates against antibiotics. This assay relies on diffusion of antibiotics from impregnated paper disks into an agar medium inoculated with bacteria.

As the antibiotic diffuses radially from the disk, a gradient of concentration forms. These gradient influences bacterial growth, resulting in clear zones around the disks where bacterial growth is inhibited.

These zones, termed zones of inhibition, provide critical information on bacterial susceptibility.

Disk Diffusion Test Protocol:
Executing the disk diffusion test with high precision is vital for obtaining reproducible results. Below is a detailed step-by-step protocol:

Step 1: Preparation of Bacterial Inoculum
Select isolated colonies from an overnight culture and suspend them in sterile saline. Adjust the turbidity to match the 0.5 McFarland standard, approximately corresponding to a bacterial density of $1–2 \times 10^8$ CFU/mL.

Step 2: Agar Medium Preparation
Mueller–Hinton Agar (MHA) is recommended for its reliable performance and consistency. Pour MHA plates to a uniform thickness of 4 mm to ensure standard diffusion rates and accurate interpretation.

Step 3: Inoculation of Agar Surface
Immerse a sterile cotton swab into the bacterial suspension. Gently press and rotate the swab against the tube's side to remove excess fluid. Streak the swab evenly across the agar surface, rotating the plate about 60 degrees and repeating streaking for full coverage. Allow plates to dry briefly, typically within 5 minutes.

Step 4: Placement of Antibiotic Disks
Place antibiotic-impregnated disks onto the agar surface using sterile forceps or a disk dispenser, ensuring proper spacing between disks.

Step 5: Bacterial inoculum density
The turbidity matching 0.5 McFarland standard ensures consistent bacterial density. Deviations in inoculum density can lead to inaccurate interpretation due to altered diffusion gradients and zone sizes.

Step 6: Incubation Conditions:
Variations in incubation time and temperature significantly affect diffusion rates and bacterial growth. Laboratories, such as Creative Bio labs, meticulously monitor and standardize incubation parameters to ensure reproducibility and accuracy in antibiotic susceptibility testing.

Understanding Disk Diffusion Test Results:
Interpreting the results of the disk diffusion test involves careful measurement of inhibition zones. Clear, defined zones indicate bacterial susceptibility to the antibiotic, whereas diminished or absent zones suggest resistance.

Zone Diameter Interpretation:
Susceptible (S):
Indicates clinical efficacy of the antibiotic at standard dosages.

Intermediate (I):
Implies a potential therapeutic effect achievable at higher concentrations or localized sites where antibiotics accumulate.

Resistant (R):
Demonstrates bacterial resistance, advising against the clinical use of the antibiotic tested.
At Creative Bio labs, we ensure accurate zone measurement and interpretation using advanced imaging systems and digital calipers, minimizing human error and enhancing reliability.

Application of Disk Diffusion Method in Research:
In microbiology research, the disk diffusion method is indispensable in:
Screening Novel Antibiotics:
Scientists employ disk diffusion assays efficiently to evaluate the efficacy of new antibiotic compounds against various bacterial strains, essential for preliminary antimicrobial screening in drug discovery research.

Monitoring Antibiotic Resistance Trends:
Longitudinal studies using disk diffusion tests reveal emerging resistance patterns in microbial populations, essential for epidemiological surveillance and informing public health strategies.

Comparative Effectiveness of Antibiotics:
Disk diffusion facilitates direct comparisons among various antibiotics, determining relative efficacy and guiding antibiotic stewardship policies and research directions in microbiology and clinical trials.

Advantages of Disk Diffusion Over Other Methods:
Despite the availability of automated susceptibility systems, the disk diffusion test remains popular in microbiological laboratories for several compelling reasons:

Cost-effectiveness:
Requires minimal equipment and consumables, making it suitable even for resource-limited settings.

Flexibility:
Adaptable to test numerous antibiotics simultaneously, facilitating broad-spectrum susceptibility profiling.

Simplicity:
Easy to perform, interpret, and troubleshoot compared to more complex automated assays.

V. RESULT

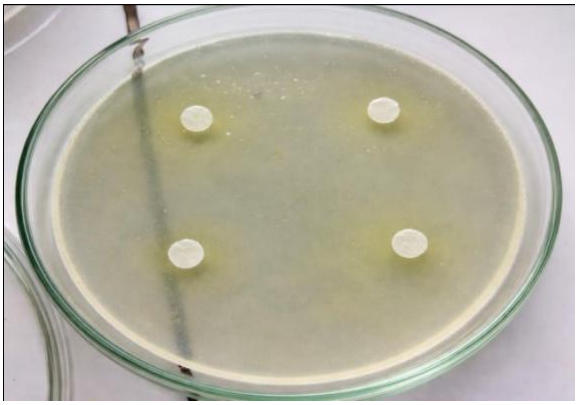

Fig no:1 Sewage sample
Zone of inhibition (Highly resistance)

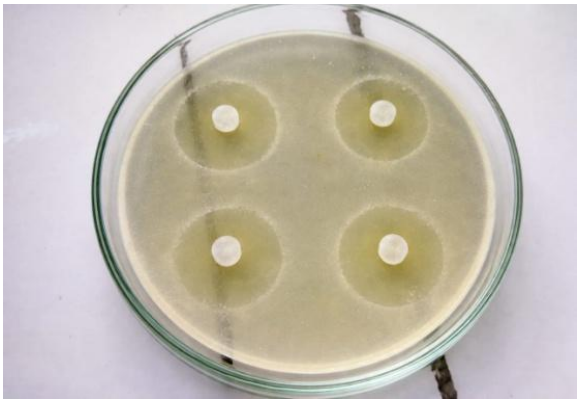

Fig no: 2 Water sample
Zone of inhibition (15mm) (Intermediate)

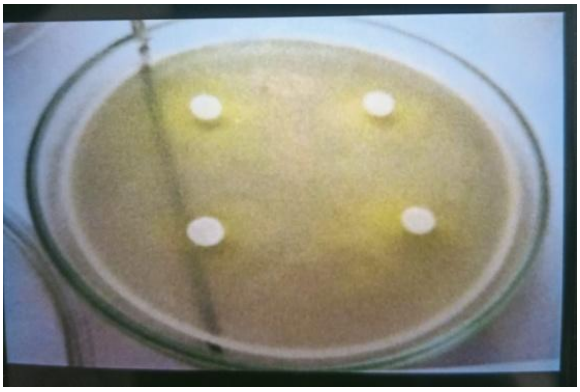

Fig no: 3 Soil sample
Zone of inhibition (< 14mm) (Resistant)

S.NO	SAMPLE	ZONE OF INHIBITION	SENSITIVITY
1.	Sewage	No zone of inhibition	Highly resistant
2.	Water	15mm	Intermediate
3.	Soil	<14mm	Resistant

VI. DISCUSSION

The antimicrobial activity of the sewage, water, and soil samples was evaluated based on the zone of inhibition. The results indicate clear differences in microbial resistance among the samples. The sewage sample showed little to no zone of inhibition, suggesting a high level of resistance. This may be due to the presence of diverse microorganisms that are frequently exposed to contaminants and antibiotics, leading to the development of resistance mechanisms.
The water sample exhibited a moderate zone of inhibition (approximately 15 mm), indicating intermediate sensitivity to the antimicrobial agent. This suggests that the microbial population in water is less resistant compared to sewage, possibly due to lower exposure to pollutants and antibiotics.
The soil sample showed a smaller zone of inhibition (≤14 mm), indicating resistance, although slightly less than that observed in sewage. Soil contains a wide variety of microorganisms, some of which naturally possess resistance traits.
Overall, the findings demonstrate that sewage and soil samples harbour more resistant microorganisms, while water samples show comparatively lower resistance.

These results highlight the environmental variation in antimicrobial resistance patterns.

VII. PREVENTION:

- Use antibiotics only with prescription
- Complete full course; no sharing/leftovers
- Maintain hand hygiene & sanitation
- Take vaccinations
- Follow hospital infection control
- Limit use in animals/agriculture
- Increase public awareness
- Ensure surveillance & monitoring
- Proper waste disposal
- Follow guidelines by World Health Organization

VIII. CONCLUSION:

- Increasing concerns of AMR due to its leading global mortality rates and how its estimated death rates can surpass cancer death rates in the coming years. It is highly necessary for adopting preventive measures in reducing AMR and judicious use of antibiotics in humans, animals, and agriculture.
- Our work was emphasized on identifying the environmental reservoirs from antibiotic-manufacturing pharmaceutical industries contributing to AMR occurrence.
- In our research work soil and sewage samples were found to be resistant and highly resistant for the test antibiotic respectively and the same point were found susceptible for the test antibiotic. From test result found conclusion can be given that antibiotic manufacturing industries could be a contributing factor for development antibiotic resistant gene in the environment.
- Therefore, adapting of proper preventive measure is of high necessity for pharmaceutical industries. We wish that our AMR studies work will contribute to filling a gap in the research world.

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