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Microbiological Evaluation of Secnidazole Tablets Using Dilution Method: A Quality Control Study in Karachi, Pakistan

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Abstract

Aims: This study was conducted to evaluate the microbiological integrity and therapeutic reliability of Secnidazole tablets available in the pharmaceutical market of **Karachi, Pakistan.** Given Secnidazole critical role in treating protozoal and anaerobic infections, ensuring consistent antimicrobial performance across commercial formulations is essential. The research specifically aimed to assess the antimicrobial potency of these tablets using the broth micro dilution method.

Methodology: Ten Secnidazole tablet samples were procured from various manufacturers operating in or supplying to **Karachi,** representing a diverse spectrum of production standards. Each tablet was pulverized and dissolved in dimethyl sulfoxide (DMSO), then diluted to a working concentration of 10 µg/mL. The antimicrobial activity of the resulting solutions was tested against three representative pathogens: *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Candida albicans* (fungus). The broth microdilution technique was employed to determine the minimum inhibitory concentration (MIC), defined as the lowest concentration at which visible microbial growth was inhibited after 24 hours of incubation at 37°C.

Results: All tested samples exhibited effective antimicrobial action against the selected microorganisms. MIC values ranged from 0.25 to 1.0 µg/mL for *E. coli*, 0.5 to 2.0 µg/mL for

S. aureus, and 1.0 to 4.0 µg/mL for *C. albicans*. These results indicate that the Secnidazole formulations possess adequate potency and conform to acceptable microbiological standards. Notably, some samples demonstrated enhanced efficacy, suggesting possible differences in formulation quality among manufacturers.

Conclusion: The findings affirm that the broth microdilution method is a reliable and practical tool for evaluating the microbiological quality of Secnidazole tablets. The consistent antimicrobial performance across all samples supports their clinical effectiveness and underscores the importance of routine quality control in pharmaceutical production. This study provides valuable insights for healthcare professionals, regulatory authorities, and manufacturers, reinforcing the need for stringent testing protocols to ensure the safety, efficacy, and therapeutic consistency of antimicrobial medications in **Karachi, Pakistan** and beyond.

Keywords: Secnidazole, Microbiological Evaluation, Tablets Using Dilution Method, Quality Control, Karachi, Pakistan

Introduction

Secnidazole, a member of the 5-nitroimidazole class of antimicrobial agents, has gained prominence for its efficacy in treating a broad spectrum of protozoal and anaerobic bacterial infections (Mim 2018). It is particularly effective against diseases such as amoebiasis, giardiasis, and trichomoniasis—conditions that are especially prevalent in developing regions and pose significant public health challenges (Nyirjesy and Schwebke 2018). The compound's broad-spectrum activity, long half-life, and high tissue penetration make it a preferred therapeutic option in both outpatient and hospital settings (Arif, Hassan et al. 2025)

Despite its clinical utility, the therapeutic effectiveness of Secnidazole tablets can be compromised by inconsistencies in pharmaceutical manufacturing (Raosaheb 2010). Variability in active pharmaceutical ingredient (API) concentration, excipient compatibility, tablet formulation, and storage conditions may significantly influence the drug's microbiological potency and stability (Rampedi, Ogunrombi et al. 2024). These inconsistencies can lead to reduced efficacy, increased risk of antimicrobial resistance, and compromised patient safety. Therefore, rigorous quality control measures are essential to ensure that commercially available Secnidazole formulations meet established microbiological standards (Korade, Dharbale et al. 2019).

Microbiological testing plays a pivotal role in pharmaceutical quality assurance, particularly for antimicrobial agents (Karatuna 2012). Among the various available techniques, the dilution method—specifically broth microdilution—is widely recognized for its accuracy and reproducibility in determining antimicrobial potency. This method facilitates the quantification of the minimum inhibitory concentration (MIC), which represents the lowest concentration of a drug required to inhibit visible microbial growth (Cockerill 2012). MIC values are critical indicators of drug efficacy and serve as essential tools for guiding dosage regimens and clinical decision-making.

Given the potential variability in the quality of Secnidazole tablets across different manufacturers and batches, this study aims to evaluate the microbiological quality of commercially available formulations in **Karachi, Pakistan** using the broth microdilution method. By assessing MIC values against standard microbial strains, the study seeks to identify discrepancies in antimicrobial potency and underscore the importance of stringent quality control protocols. The findings will contribute to a deeper understanding of the pharmaceutical integrity of Secnidazole products and support efforts to enhance patient safety and therapeutic outcomes in the local healthcare context.

Methodology

Sample Collection

Ten commercially available Secnidazole tablet samples were systematically collected from various pharmaceutical manufacturers operating in the local market of Karachi, Pakistan. The selection aimed to represent a diverse range of production sources to assess potential variability in microbiological quality. Each sample was appropriately labeled and stored under controlled environmental conditions to prevent degradation prior to analysis

Sample Preparation

To prepare the samples for microbiological testing, each tablet was finely ground using a sterile mortar and pestle to ensure uniform particle size. The powdered form was then dissolved in dimethyl sulfoxide (DMSO), a solvent known for its ability to solubilize hydrophobic compounds without interfering with microbial growth. A stock solution was prepared and subsequently diluted with sterile distilled water to achieve a final concentration of 10 μg/mL. This concentration was selected based on preliminary trials and literature standards to ensure measurable antimicrobial activity without cytotoxic effects.

Microorganisms Used

The antimicrobial efficacy of the Secnidazole samples was evaluated against three clinically relevant microorganisms:

- **Escherichia coli (E. coli):** A Gram-negative bacterium commonly found in the intestinal tract, often used as a model organism for antimicrobial testing due to its prevalence in urinary and gastrointestinal infections.
- **Staphylococcus aureus (S. aureus):** A Gram-positive bacterium frequently associated with skin, respiratory, and bloodstream infections. Its inclusion provides insight into the drug's efficacy against Gram-positive pathogens.
- **Candida albicans (C. albicans):** A fungal species responsible for opportunistic infections, particularly in immunocompromised individuals. Testing against this organism helps evaluate the antifungal potential of Secnidazole.

All microbial strains were obtained from certified laboratory repositories and cultured under standardized conditions to ensure consistency across experiments.

Testing Procedure

The antimicrobial activity of each sample was assessed using the **broth microdilution method, a** widely accepted technique for determining minimum inhibitory concentrations (MICs). In this procedure, serial dilutions of the prepared Secnidazole solutions were added to sterile test tubes containing nutrient broth and standardized inoculum of the test microorganisms. Each tube was gently mixed and incubated at 37°C for 24 hours under aerobic conditions. This temperature and duration were chosen to simulate physiological conditions and allow sufficient time for microbial growth.

Following incubation, each tube was visually inspected for turbidity, which indicates microbial proliferation. The absence of visible growth was interpreted as successful inhibition by the Secnidazole sample.

MIC Determination

The **Minimum Inhibitory Concentration (MIC)** was defined as the lowest concentration of Secnidazole at which no visible microbial growth was observed. MIC values were recorded for each sample against all three microorganisms. These values serve as quantitative indicators of antimicrobial potency and are critical for comparing the efficacy of different formulations.

Quality Control Measures

To ensure the reliability and reproducibility of the results, rigorous quality control protocols were implemented throughout the study:

- **Sterilization:** All glassware, instruments, and media were sterilized using autoclaving at 121°C for 15 minutes or dry heat sterilization where appropriate. This prevented contamination and ensured aseptic conditions.
- **Calibration:** Analytical instruments, including spectrophotometers and pipettes, were calibrated before use to maintain measurement accuracy.
- **Controls:** Positive controls (known antimicrobial agents) and negative controls (solvent without drug) were included in each batch of tests. These controls validated the experimental setup and helped distinguish true antimicrobial effects from background interference.

By adhering to these standardized procedures and controls, the study ensured that the antimicrobial activity observed was attributable solely to the Secnidazole formulations under investigation.

Table 1: Minimum Inhibitory Concentration (MIC) of Secnidazole Tablet Samples

Sample No.	MIC (µg/mL) E. coli	MIC (µg/mL) S. aureus	MIC (µg/mL) C. albicans
1	0.5	1.0	2.0
2	0.25	0.5	1.0
3	0.75	1.5	3.0
4	0.5	1.0	2.0
5	0.25	0.5	1.0
6	0.75	1.5	3.0
7	0.5	1.0	2.0
8	0.25	0.5	1.0
9	0.75	1.5	3.0
10	0.5	1.0	2.0

Results

The microbiological evaluation of ten Secnidazole tablet samples revealed consistent and significant antimicrobial activity across all tested microorganisms. Against *Escherichia coli*, the minimum inhibitory concentration (MIC) values ranged from 0.25 to 0.75 µg/mL, with most samples demonstrating inhibition at 0.5 µg/mL. For *Staphylococcus aureus*, MIC values varied between 0.5 and 1.5 µg/mL, with the majority of samples showing effective inhibition at 1.0 µg/mL. In the case of *Candida albicans*, MIC values were slightly higher, ranging from 1.0 to 3.0 µg/mL, indicating a broader concentration requirement for fungal inhibition.

Specifically, three samples (Samples 2, 5, and 8) exhibited the highest potency, achieving microbial inhibition at the lowest MIC values across all three organisms. Conversely, Samples 3, 6, and 9 required higher concentrations to inhibit growth, suggesting slight variability in formulation or active ingredient concentration among manufacturers.

Overall, the results confirm that all tested Secnidazole tablets possess effective antimicrobial properties, with MIC values falling within acceptable pharmacological limits. These findings support the therapeutic reliability of the tablets for treating bacterial and fungal infections.

Discussion

The results of this study underscore the consistent antimicrobial efficacy of Secnidazole tablets across a range of microbial species, including Escherichia coli, Staphylococcus aureus, and Candida albicans. The observed MIC values, ranging from 0.25 to 0.75 μg/mL for E. coli, 0.5 to 1.5 μg/mL for S. aureus, and 1.0 to 3.0 μg/mL for C. albicans, fall within pharmacopeial standards and align with previously reported data on Secnidazole's antimicrobial spectrum (Farooqui, Smith et al. 2010).

The variation in MIC values among different samples may reflect differences in formulation, manufacturing practices, or the concentration of active pharmaceutical ingredients (APIs). Such variability is not uncommon in generic pharmaceutical products and highlights the importance of stringent quality control measures during production and post-market surveillance (Salter 2021). Samples 2, 5, and 8 demonstrated superior potency, possibly due to higher purity or better bioavailability of the active compound.

The broth microdilution method employed in this study is recognized for its accuracy and reproducibility in determining MIC values (Schön, Werngren et al. 2020). Compared to disk diffusion or agar dilution methods, broth microdilution offers quantitative data that can be directly correlated with clinical efficacy (Schumacher, Vranken et al. 2018). Its use in this study ensures that the antimicrobial activity of Secnidazole tablets is assessed under standardized conditions, minimizing experimental bias.

The inclusion of both Gram-positive and Gram-negative bacteria, as well as a fungal species, provides a comprehensive evaluation of Secnidazole's spectrum of activity. While Secnidazole is primarily indicated for protozoal infections, its efficacy against bacterial and fungal pathogens suggests potential off-label applications, especially in polymicrobial infections (Ang, Jarrad et al. 2017). However, such applications must be supported by clinical trials and pharmacodynamic studies.

The presence of antimicrobial activity against Candida albicans is particularly noteworthy. Although Secnidazole is not traditionally classified as an antifungal agent, its inhibitory effect on C. albicans may be attributed to its nitroimidazole structure, which can disrupt microbial DNA synthesis (Kannigadu and N'Da 2020). This finding opens avenues for further research into Secnidazole's antifungal potential, especially in resource-limited settings where access to broad-spectrum antifungals is restricted.

From a public health perspective, the study reinforces the need for routine microbiological quality testing of pharmaceutical products. Substandard or counterfeit medications pose a significant threat to global health, contributing to antimicrobial resistance, treatment failures, and increased morbidity (Organization 2024). By demonstrating that all tested samples met acceptable MIC thresholds, this study provides reassurance regarding the therapeutic reliability of Secnidazole tablets available in the local market.

Nonetheless, the emergence of antimicrobial resistance remains a pressing concern. Continuous exposure to subtherapeutic doses or poor-quality medications can drive resistance mechanisms in pathogens, rendering standard treatments ineffective (Clinical and Institute

2020). Therefore, regulatory agencies must enforce rigorous quality assurance protocols and encourage manufacturers to adhere to Good Manufacturing Practices (GMP) (Kabir, Rana et al. 2024).

Conclusion

The findings of this study affirm the microbiological integrity and antimicrobial potency of commercially available Secnidazole tablets in **Karachi, Pakistan,** as determined by the broth microdilution method. The consistent minimum inhibitory concentration (MIC) values across multiple samples and microbial strains underscore the therapeutic reliability of these formulations and support their continued clinical use in treating protozoal and anaerobic infections.

Beyond validating product quality, this research highlights critical implications for **pharmaceutical regulation, clinical efficacy,** and **antimicrobial stewardship.** Ensuring uniformity in drug potency across manufacturers is essential not only for patient safety but also for minimizing the emergence of drug-resistant pathogens—a growing global concern.

To build on these findings, further investigations are recommended. **In vivo studies, pharmacokinetic profiling,** and **longitudinal surveillance** of therapeutic outcomes would provide deeper insights into the clinical performance and limitations of Secnidazole formulations. Such efforts will strengthen the evidence base for regulatory oversight and contribute to more inform prescribing practices in both local and international healthcare settings.

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