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Original Article

Comparison of the gradient diffusion technique for fluconazole and voriconazole with the VITEK 2 yeast susceptibility system for clinical breakpoints of candida tropicalis isolated from clinical samples.

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Abstract

Background
Modern health care advances in immunosuppressive therapy, particularly related to bone marrow transplantation, and increasing utilization of implantable devices have contributed to the rise of invasive *Candida* infections in recent decades.
Objectives: An automated method based on the broth microdilution MIC technique was used to evaluate the susceptibility of fluconazole and voriconazole with that of the Gradient diffusion technique using brain heart infusion (BHI) agar. Additionally, to identify a low-cost, quick, accurate, and simple way to test for antifungal sensitivity.

Materials and Methods
Over a year, an observational study was conducted from November 2023 to October 2024. The study was carried out at the Department of Microbiology at the Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. The study included 120 participants in total.

Results
Out of 120 isolates of Candida species, a total of 40 isolates were found to be of Candida tropicalis. Most of the isolates were confirmed in blood samples 24 (60%), followed by deep aspirated pus 06 (15%), pleural fluid 05 (12.5%), CSF 03 (7.5%), and peritoneal fluid 02 (5%). Candida tropicalis isolates were sensitive to fluconazole in 37 (92.5%) of the species as well as to voriconazole in 39 (97.5%) of the species through Vitek 2 tests.

Conclusion
A standardized automated test for antifungal susceptibility, the AST-YS08Vitek 2 card system (bioMérieux), was shown to be dependable and produced findings that were comparable to the E test; as a result, it may be used in place of the E test.

Recommendations
Nevertheless, additional research is required to assess the VITEK 2 method's capacity to detect resistant isolates. Retesting of any resistant isolates generating inconsistent findings will be necessary to resolve this problem.

Keywords: *Antifungal sensitivity testing, Fluconazole, Broth micro dilution, Minimum inhibitory concentrations, Voriconazole, VITEK 2*
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Introduction

Over the past few decades, there has been a growing worry about human disease caused by Candida species. Diseases can range from mucocutaneous conditions that are not life-threatening to invasive diseases [1, 2]. As of right now, Candida species rank third in the US for nosocomial bloodstream infections and are the most frequent cause of invasive fungal infections in hospitalized patients [3].

Modern health care advances in immunosuppressive therapy, particularly related to bone marrow transplantation, and increasing utilization of implantable devices have contributed to the rise of invasive *Candida* infections in recent decades [4]. Higher rates of morbidity and mortality among at-risk patients have been brought on by the rising frequency of candidiasis, which has also raised health care expenses [1, 5]. Given the increase in disease prevalence and severity, there has never been a greater need for rapid and accurate methods to screen for antifungal susceptibilities.

Because fluconazole has established breakpoints (susceptible [S], susceptible-dose dependent [S-DD], and resistant [R]), the results of in vitro susceptibility tests can be used to inform antifungal treatment[6]. Voriconazole breakpoints have not yet been determined. In terms of pharmacokinetics, fluconazole is different from voriconazole in that the amount of fluconazole in the blood depends on the dosage; that is, a larger dose of fluconazole results in a greater concentration in the blood (hence the S-DD designation) [7].

Voriconazole's recommended dosage consists of two intravenous loading doses of 6 mg/kg spaced 12 hours apart, followed by a maintenance dose of 4 mg/kg (or 3 mg/kg if tolerance is an issue) every 12 hours[8]. In 18 hours, as opposed to 48–72 hours for the other approaches, the VITEK 2 system (bioMérieux, Inc.) enables both species identification and antifungal susceptibility testing. For amphotericin B (with a range of 11 to 27.8 hours), fluconazole (with a range of 9 to 24.2 hours), and voriconazole (with a range of 8.1 to 25.1 hours), the average time-to-result for the VITEK 2 system is 15 hours [9]. A quick, easy, and recently commercialized method of testing for antibiotic susceptibility is the E-test. When the antifungal agent has been incubated for 24 to 48 hours, the oval-shaped inhibition zone of candidal growth shows the minimal inhibitory concentrations (MICs) [10].

An automated method based on the broth microdilution MIC technique was used to evaluate the susceptibility of fluconazole and voriconazole with that of the Gradient diffusion technique using brain heart infusion (BHI) agar.

Methodology

Study design

Over a year, an observational study was conducted from November 2023 to October 2024. The study was carried out at the Department of Microbiology at the Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India.

Study population

The study included 120 participants in total. Patients of both sexes and all age groups had to meet the inclusion requirements to be enrolled in the study. Additionally, samples from patients who are outside were not included. Sputum, bronchoalveolar lavage (BAL) fluid, urine, stool, and pus from the superficial region are examples of non-sterile samples that are excluded from the study. Additionally, excluded are patients who began using antifungals before sampling.

Study procedure

Other bodily fluids, such as cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid, deep aspirated pus, etc., were cultured on Brain Heart Infusion agar, Sabouraud's dextrose agar, and blood agar. Blood samples were treated for blood culture. Antifungal sensitivity testing (AFST) was performed on all isolates of Candida tropicalis by VITEK 2 (Biomereux India Pvt Ltd) utilizing the AST-YS08 fungal susceptibility card and E test by Himedia Ezy MIC Strip. The two most significant antifungals for Candida tropicalis invasive infections, fluconazole and voriconazole, were assessed in this study by calculating their minimum inhibitory concentration (MIC) using both test techniques. Clinical breakpoints, as outlined in the most recent CLSI guidelines, were used to compare the results.

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Statistical analysis

Data were initially entered in Microsoft Excel. The data has been presented as n (%).

Ethical approval

The study has been approved by the Institutional Ethics Committee, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, dated 05 October 2023 (Letter No-1211/IEC/IGIMS/2023)

Results

Out of 120 isolates of Candida species, a total of 40 isolates were found to be of Candida tropicalis. Most of the isolates were confirmed in blood samples 24 (60%), followed by deep aspirated pus 06 (15%), pleural fluid 05 (12.5%), CSF 03 (7.5%), and peritoneal fluid 02 (5%).

Table 1. Presence of candida tropicalis in various samples

Samples	Candida Tropicalis Species (n=40)
CSF	03 (7.5%)
Pleural Fluid	05 (12.5%)
Peritoneal Fluid	02 (5%)
Deep Aspirated Pus	06 (15%)
Blood Samples	24 (60%)

Data was presented as n (%)

Candida tropicalis isolates were sensitive to fluconazole in 37 (92.5%) of the species as well as to voriconazole in 39 (97.5%) of the species through Vitek 2 tests. Table 2 shows antifungal susceptibility tests in Candida tropicalis Species.

Table 2. Antifungal susceptibility tests in candida tropicalis species

Tests	Fluconazole		Voriconazole	
	Sensitivity	Resistant	Sensitivity	Resistant
Vitek 2	37 (92.5%)	03 (7.5%)	39 (97.5%)	01 (2.5%)
E-test	38 (95%)	02 (5%)	40 (100%)	00 (0%)

Data was presented as n (%)

Discussion

The present study found that most of the isolates were confirmed in blood samples 24 (60%), followed by deep aspirated pus 06 (15%), pleural fluid 05 (12.5%), CSF 03 (7.5%), and peritoneal fluid 02 (5%). Candida tropicalis isolates were sensitive to fluconazole in 37 (92.5%) of the species as well as to voriconazole in 39 (97.5%) of the species through Vitek 2 tests.

These data show that the Vitek 2 yeast susceptibility method for evaluating fluconazole and voriconazole against Candida tropicalis is not negatively impacted by the new (lower) CBPs and ECVs. In light of the dearth of isolates with known resistance mechanisms, it should be mentioned that a 2009 study by Posteraro et al. included 48 isolates of Candida glabrata and 11 isolates of Candida albicans that were resistant to fluconazole both phenotypically and genotypically (due to mutations in ERG11 and/or efflux pumps). The investigation found that all but two (57/59, 96.6%) of the resistant isolates were accurately classified using the Vitek 2 approach [11].

According to this investigation, the VITEK 2 system and the E-test produced remarkably similar results. Although both Vitek 2 and E-test methods show consistent trends, with E-test showing slightly higher sensitivity percentages. However, the VITEK system's MIC values were available 14–18 hours later, with an average of 16 hours, compared to 48 hours for the E test. This reduced the amount of time needed to optimize antifungal treatment choices. The VITEK2 system may be taken into consideration as an alternative to the E test for antifungal susceptibility testing

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because of its faster turnaround time, even though the results from both approaches were similar.
The Vitek 2 yeast susceptibility method appears to provide rapid, accurate, and reliable findings when testing fluconazole and voriconazole against Candida spp. The Vitek 2 approach, which uses the novel CBPs, consistently detects fluconazole resistance in Candida species and shows strong quantitative and qualitative agreement with the reference BMD technique when testing either fluconazole or voriconazole. One significant step in improving antifungal treatment for candidiasis should be the clinical laboratory's utilization of the Vitek 2 system for antifungal susceptibility testing [12, 13, 14].

Conclusion

A standardized automated test for antifungal susceptibility, the AST-YS08 Vitek 2 card system (bioMérieux), was shown to be dependable and produced findings that were comparable to the E-test; as a result, it may be used in place of the E-test. The E-test may be more accurate, especially for detecting low-level resistance. Hospitals can validate empirical treatment regimens with the help of the surveillance of antifungal susceptibility profiles. All things considered, the VITEK 2 system offers a quick and completely automated way to calculate MICs against Candida species, doing away with the subjectivity of manual MIC determination techniques.

Limitations

The study's sample size was one of its primary drawbacks; a larger sample would have produced better findings. Another disadvantage of the study was that it was only carried out at one centre.

Recommendations

Nevertheless, additional research is required to assess the VITEK 2 method's capacity to detect resistant isolates. Retesting of any resistant isolates generating inconsistent findings will be necessary to resolve this problem.

List of abbreviations

SDD- Susceptible-dose dependent
IGIMS- Indira Gandhi Institute of Medical Sciences
CSF- Cerebrospinal fluid
AFST- Antifungal sensitivity testing
CLSI- Clinical and Laboratory Standards Institute
BMD- Broth micro dilution
MICs- Minimum inhibitory concentrations
AST- Antimicrobial susceptibility test

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Author contributions

All authors contributed equally to the study design, data collection, analysis, and manuscript preparation.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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