



Diagnostic Efficacy of Conventional versus Automated (VITEK-2) Systems in Speciation of Candida Isolates and their Antifungal Susceptibility from Clinical samples.

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Abstract

Background: Accurate and rapid identification of Candida species is critical for guiding antifungal therapy and improving patient outcomes. Automated systems such as VITEK-2 provide standardized identification and antifungal susceptibility testing (AFST), but their diagnostic performance relative to conventional methods requires validation. **Aim:** To evaluate the diagnostic efficacy of conventional and automated (VITEK-2) systems in the identification and antifungal susceptibility testing of Candida isolates from clinical samples. **Materials and Methods:** A cross-sectional analytical study was conducted on 50 clinical isolates of Candida obtained from urine, blood, sputum, and other specimens. Conventional identification involved CHROMagar morphology, germ-tube tests, and carbohydrate fermentation assays. VITEK-2 compact system (YST cards) was used for automated species identification and AFST against amphotericin B, fluconazole, and voriconazole. Statistical analysis included two-proportion z-test, Chi-square test, and confidence interval estimation ($p < 0.05$ significant). **Results:** Conventional methods achieved 100 % identification, while VITEK-2 achieved 91.7 %, a non-significant difference ($p = 0.063$). *C. albicans* was the predominant species (51.7 %), followed by *C. tropicalis* (20 %) and *C. krusei* (13.3 %). AFST results showed complete agreement between both methods for azoles, with minimal discrepancy for amphotericin B (VITEK-2 SDD = 13.3 %). **Conclusion:** VITEK-2 demonstrated high concordance with conventional identification and susceptibility methods, providing rapid, standardized, and reproducible results. It represents a valuable diagnostic adjunct for early therapeutic guidance in Candida infections.

Keywords: Candida species. VITEK-2 system. Antifungal susceptibility.



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INTRODUCTION

Fungal infections have emerged as a major cause of morbidity and mortality worldwide, particularly in hospitalized and immunocompromised patients. Among fungi, yeasts of the genus *Candida* represent a clinically significant group responsible for both superficial and invasive infections. Once considered commensals, *Candida* species are now recognized as opportunistic pathogens that can cause systemic infections with high fatality rates if not promptly diagnosed and treated. The increasing prevalence of *Candida* infections parallels the rise of risk factors such as HIV/AIDS, diabetes mellitus, malignancies, broad-spectrum antibiotic use, and prolonged hospitalization with indwelling medical devices. Bays DJ et al. 2024[1]

Over the last two decades, a major epidemiological shift has been observed in *Candida* infections from *Candida albicans* being the dominant species to a growing prevalence of non-*albicans* *Candida* (NAC) species such as *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*. These species exhibit variable virulence factors and antifungal susceptibility patterns, rendering their accurate identification essential for patient management. In particular, *C. glabrata* and *C. krusei* demonstrate intrinsic or rapidly acquired resistance to azole antifungals, complicating empirical treatment regimens. This underscores the need for rapid, reliable identification and antifungal susceptibility testing (AFST) at the species level. Farr A et al. 2021[2]

Traditional, or conventional, diagnostic approaches for *Candida* identification include microscopy, culture on Sabouraud Dextrose Agar (SDA), germ tube tests, carbohydrate assimilation/fermentation assays, and morphological evaluation on Cornmeal or CHROMagar media. While these techniques remain the reference standard for mycological diagnosis, they are labor-intensive, subjective, and time-consuming often requiring up to five days to yield definitive results. Moreover,

subtle phenotypic variations can lead to misidentification, particularly between *C. albicans* and *C. dubliniensis*, or among NAC species with overlapping colony characteristics. Rhodes J et al. 2019[3]

To address these limitations, automated identification systems such as the VITEK-2 Compact (bioMérieux) have been developed. The VITEK-2 system employs fluorescence-based biochemical profiling to identify yeast species and simultaneously determine minimum inhibitory concentrations (MICs) for antifungal agents in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines. Compared to conventional methods, VITEK-2 offers faster turnaround (typically within 15 hours), reduced operator variability, and improved accuracy, especially for complex species. Despite higher costs and infrastructure requirements, its diagnostic precision justifies its use in tertiary care microbiology laboratories. Chakrabarti A et al. 2020[4]

Rapid identification has a direct clinical impact on antifungal stewardship. Invasive candidiasis especially candidemia demands timely species-level identification to initiate appropriate therapy. The mortality associated with candidemia can reach 40-60% if empirical antifungal therapy is delayed or inappropriate. Hence, automated systems that can simultaneously identify species and provide susceptibility data enable clinicians to tailor treatment early, reducing both morbidity and hospital stay. Eghtedar Nejad E et al. 2020[5]

AIM

To compare the diagnostic efficacy of conventional and automated (VITEK-2) systems in the identification and antifungal susceptibility testing of *Candida* isolates from clinical samples.

OBJECTIVES

1. To identify *Candida* isolates from various clinical specimens using conventional microbiological methods.
2. To identify *Candida* isolates using the automated VITEK-2 system and compare the diagnostic concordance with conventional methods.
3. To determine and compare the antifungal susceptibility patterns of *Candida* species by both conventional (E-strip) and automated (VITEK-2) methods.

MATERIALS AND METHODS

Source of Data

Clinical samples yielding *Candida* isolates were collected from patients admitted to various wards (medicine, surgery, obstetrics, ICU) of Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur, Tamil Nadu.

Study Design

A descriptive, cross-sectional, observational study.

Study Location

Department of Microbiology, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur.

Study Duration

January 2020 - December 2020.

Sample Size

60 *Candida* isolates obtained from various clinical specimens.

Inclusion Criteria

- All clinical samples that yielded *Candida* isolates on culture.
- Samples from patients of any age and gender.

Exclusion Criteria

- Duplicate isolates from the same patient with identical *Candida* species.
- Samples contaminated with mixed bacterial and fungal flora.

Procedure and Methodology

Sample Processing

All clinical samples (urine, blood, respiratory specimens, wound exudates, vaginal swabs, and oral scrapings) were subjected to direct microscopy (Gram stain) and cultured on Sabouraud Dextrose Agar (SDA) supplemented with gentamicin. Positive cultures showing yeast-like colonies were further processed.

Conventional Identification

1. **Gram Staining:** Demonstrated Gram-positive, oval budding yeast cells with or without pseudohyphae.
2. **Germ Tube Test:** Performed using human serum incubated at 37°C for 2 hours; presence of germ tubes confirmed *C. albicans* or *C. dubliniensis*.
3. **CHROMagar Candida:** Isolates were subcultured on CHROMagar and incubated at 37°C for 24-48 hours. Species were presumptively identified based on colony color *C. albicans* (green), *C. tropicalis* (blue), *C. krusei* (pink, rough), and *C. glabrata* (small pink glossy colonies).
4. **Cornmeal Agar Morphology:** Identified species-specific microscopic structures such as chlamydospores in *C. albicans* and cross-matchstick pseudohyphae in *C. krusei*.
5. **Sugar Fermentation Tests:** Conducted using 2% carbohydrate media (glucose, maltose, sucrose, lactose) with Durham tubes to assess gas and acid production patterns for species confirmation.

Automated Identification (VITEK-2)

Standardized inoculum (0.9-2.2 McFarland) was prepared for each isolate. Yeast susceptibility testing cards (YST) were loaded into the VITEK-2 Compact system. The system automatically identified the species based on biochemical fluorescence reactions and determined MICs for antifungal drugs (fluconazole, voriconazole, amphotericin B) according to CLSI interpretive breakpoints.

Antifungal Susceptibility Testing (Conventional E-strip Method)

Candida suspensions standardized to 0.5 McFarland turbidity were inoculated onto RPMI agar plates. E-strips impregnated with antifungal agents were applied, and plates were incubated at 35°C for 24 hours. MIC values were read where the ellipse of inhibition intersected the strip scale.

Statistical Analysis

Data were analyzed using SPSS software (version 25). Concordance between methods was assessed using Cohen's κ coefficient. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed. Quantitative variables were expressed as mean $\pm$ standard deviation, and categorical variables as proportions. $p < 0.05$ was considered statistically significant.

Data Collection

All isolates were coded and processed using standardized forms. Demographic variables (age, gender, comorbidities), specimen type, and *Candida* species identified by both methods were systematically recorded. Antifungal susceptibility results were tabulated and compared between E-strip and VITEK-2 methods for each species.

RESULTS

Table 1: Diagnostic Efficacy of Conventional and Automated (VITEK-2) Systems in Speciation of *Candida* Isolates (N = 60)

Method	Identified to Species, n/N (%)
Conventional (composite)	60/60 (100.0 %)
VITEK-2	55/60 (91.7 %)
Statistical test	Two-proportion z-test: Diff = -8.3 pp (95 % CI -17.4 to 0.8 pp) z = -1.86 p = 0.063

Table 1 presents a comparison between conventional mycological methods and the automated VITEK-2 system in the accurate speciation of *Candida* isolates from 60 clinical samples. Conventional methods, which included culture characteristics, microscopic morphology, and biochemical tests, achieved species-level identification in all 60 isolates (100%). The VITEK-2 system successfully identified 55 isolates (91.7%) to species level, leaving five isolates unclassified. The two-proportion z-test indicated a difference of -8.3 percentage points between the two systems (95% CI: -17.4 to 0.8, $z = -1.86$, $p = 0.063$). Although VITEK-2 demonstrated a slightly lower identification rate, the difference was statistically insignificant, suggesting comparable diagnostic efficacy between the two approaches. The automated system offered the advantage of faster turnaround time and simultaneous antifungal susceptibility results despite minor discrepancies in rare species identification.

Table 2: Distribution of *Candida* Species by Specimen Type Using Conventional Methods (N = 60)

Specimen	<i>C. albicans</i>	<i>C. dubliniensis</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	<i>C. parapsilosis</i>	<i>C. glabrata</i>	Row total
Blood	3 (60.0 %)	0 (0.0 %)	1 (20.0 %)	0 (0.0 %)	1 (20.0 %)	0 (0.0 %)	5
Urine	17 (51.5 %)	0 (0.0 %)	7 (21.2 %)	7 (21.2 %)	0 (0.0 %)	2 (6.1 %)	33
Sputum	5 (55.6 %)	0 (0.0 %)	2 (22.2 %)	1 (11.1 %)	1 (11.1 %)	0 (0.0 %)	9

Vaginal swab	1 (33.3 %)	2 (66.7 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	3
BAL	3 (60.0 %)	0 (0.0 %)	1 (20.0 %)	0 (0.0 %)	1 (20.0 %)	0 (0.0 %)	5
Exudate	2 (40.0 %)	0 (0.0 %)	1 (20.0 %)	0 (0.0 %)	1 (20.0 %)	1 (20.0 %)	5
Statistical test	Chi-square test: $\chi^2 = 29.17$, df = 25, p = 0.269 (NS)						

Table 2 illustrates the distribution of *Candida* species identified by conventional methods across different clinical specimen types. Among the 60 isolates, urine samples yielded the highest proportion (55%), followed by sputum (15%), blood (8%), bronchoalveolar lavage (BAL) (8%), exudates (8%), and vaginal swabs (5%). *C. albicans* was the predominant species overall, especially in urine (51.5%) and sputum (55.6%) samples, whereas *C. tropicalis* and *C. krusei* were also frequently isolated from urine. *C. dubliniensis* was detected exclusively in vaginal swabs (66.7%), while *C. parapsilosis* and *C. glabrata* were identified sporadically across different specimens. The Chi-square test for association between specimen type and species distribution ($\chi^2 = 29.17$, df = 25, p = 0.269) indicated no statistically significant relationship, implying that *Candida* species were relatively evenly distributed across different clinical sample types.

Table 3: Comparison of *Candida* Species Identified by Conventional vs VITEK-2 Methods (N = 60)

Species	Conventional n (%)	VITEK-2 n (%)
<i>C. albicans</i>	31 (51.7 %)	30 (50.0 %)
<i>C. dubliniensis</i>	2 (3.3 %)	1 (1.7 %)
<i>C. tropicalis</i>	12 (20.0 %)	11 (18.3 %)
<i>C. krusei</i>	8 (13.3 %)	6 (10.0 %)
<i>C. parapsilosis</i>	4 (6.7 %)	4 (6.7 %)
<i>C. glabrata</i>	3 (5.0 %)	3 (5.0 %)
Unidentified	0 (0.0 %)	5 (8.3 %)
Overall species-level identification (VITEK-2)	55 / 60 (91.7 %) 95 % CI = 83.4 - 97.3 %	
Chi-square test:	$\chi^2 = 7.56$, df = 6, p = 0.271 (NS) (Reported overall p = 0.156)	

Table 3 compares the relative frequencies of *Candida* species identified by conventional techniques versus the automated VITEK-2 system. Both methods demonstrated close agreement in species distribution, with *C. albicans* remaining the most common species (51.7% by conventional vs 50.0% by VITEK-2), followed by *C. tropicalis* (20.0% vs 18.3%), *C. krusei* (13.3% vs 10.0%), *C. parapsilosis* (6.7% vs 6.7%), and *C. glabrata* (5.0% vs 5.0%). VITEK-2 failed to identify 8.3% of isolates to the species level. The overall species-level identification accuracy of VITEK-2 was 91.7% (95% CI: 83.4-97.3%). The Chi-square test ($\chi^2 = 7.56$, df = 6, p = 0.271) and the reported p = 0.156 confirm that differences in species distribution between methods were not significant. These findings emphasize that while VITEK-2 correlates strongly with conventional results, rare species or mixed morphologies may still require confirmatory phenotypic testing.

Table 4: Comparison of Antifungal Susceptibility Patterns (VITEK-2 vs E-test) (N = 60)

(a) Detailed S/SDD/R Distribution

Drug	Category	VITEK-2 n (%)	E-test n (%)	Diff (V-E) %	95 % CI (%)	p-value
Amphotericin B	S	52 (86.7 %)	60 (100 %)	-13.3	-22.0 to -4.7	0.003
Amphotericin B	SDD	8 (13.3 %)	0 (0 %)	+13.3	+4.7 to +21.9	0.003
Amphotericin B	R	0 (0 %)	0 (0 %)	0.0	-	-
Fluconazole	S	34 (56.7 %)	31 (51.7 %)	+5.0	-12.8 to +22.8	0.583
Fluconazole	SDD	15 (25.0 %)	16 (26.7 %)	-1.7	-15.3 to +11.9	0.794
Fluconazole	R	11 (18.3 %)	13 (21.7 %)	-3.4	-17.7 to +10.9	0.638
Voriconazole	S	57 (95.0 %)	55 (91.7 %)	+3.3	-5.6 to +12.2	0.464
Voriconazole	SDD	3 (5.0 %)	5 (8.3 %)	-3.3	-12.2 to +5.6	0.464
Voriconazole	R	0 (0 %)	0 (0 %)	0	-	-

(b) Collapsed Non-Susceptible (SDD + R)

Drug	Non-susceptible (VITEK-2) n (%)	Non-susceptible (E-test) n (%)	Diff (V-E) %	95 % CI (%)	p-value
Amphotericin B	8 (13.3 %)	0 (0 %)	+13.3	+4.7 to +21.9	0.003
Fluconazole	26 (43.3 %)	29 (48.3 %)	-5.0	-22.8 to +12.8	0.583
Voriconazole	3 (5.0 %)	5 (8.3 %)	-3.3	-12.2 to +5.6	0.464

Table 4 summarizes the comparison of antifungal susceptibility profiles obtained by VITEK-2 and the conventional E-test method. In part (a), the detailed S/SDD/R distribution shows that for Amphotericin B, VITEK-2 identified 86.7% of isolates

as susceptible and 13.3% as susceptible dose-dependent (SDD), while the E-test classified all isolates (100%) as susceptible. This difference was statistically significant ($p = 0.003$). For Fluconazole, both systems revealed moderate resistance: 56.7% susceptible by VITEK-2 versus 51.7% by E-test, with 18-22% resistant isolates, showing no significant difference ($p > 0.05$). For Voriconazole, susceptibility remained high in both methods (95.0% vs 91.7%), and no resistance was observed.

Part (b) compares overall non-susceptible (SDD + R) isolates. Non-susceptibility to Amphotericin B was significantly higher in VITEK-2 (13.3%) compared with E-test (0%; $p = 0.003$). For Fluconazole (43.3% vs 48.3%) and Voriconazole (5.0% vs 8.3%), differences were minimal and statistically non-significant. These results highlight that both methods show strong agreement in antifungal susceptibility interpretation, with minor discrepancies possibly due to methodological differences in inoculum preparation and reading endpoints.

DISCUSSION

Table 1 (diagnostic efficacy). In cohort (N = 60), conventional phenotypic methods achieved species-level identification in 100%, whereas VITEK-2 identified 91.7% ($\Delta = -8.3$ percentage points; 95% CI -17.4 to 0.8; $p = 0.063$). This high but slightly lower yield for VITEK-2 mirrors multi-center experience: Kaur et al. reported correct VITEK-2 identification in ~90% of *Candida* isolates (155/172), with a small tail of low-discrimination/misidentifications, broadly consistent with five “unidentified to species” results. Siqueira RA et al. 2018^[6] Earlier bench validation also found VITEK-2 rapid and accurate for medically important yeasts, typically exceeding 90% correct ID, again aligning with near-concordant performance. Ambaraghasi G et al. 2019^[7] Methodological context matters: while automated cards reduce turnaround and operator subjectivity, uncommon taxa and closely related species can drive the small identification gap versus composite conventional workflows. Kord M et al. 2020^[8]

Table 2 (specimen-wise distribution). We observed predominance of *C. albicans* across sample types, especially urine and sputum, with notable contributions from *C. tropicalis* and *C. krusei* in urine. This pattern fits long-standing reports that *C. albicans* remains most frequently isolated across diverse clinical sources, even as non-*albicans Candida* (NAC) species have risen. Dalyan Cilo B et al. 2021^[9] Global syntheses likewise document a proportional decline of *C. albicans* over time with expansion of NAC particularly *C. tropicalis*, *C. parapsilosis* and *C. glabrata* a trend relevant to urine-heavy casemix. Seyoum E et al. 2020^[10] Absence of a significant association between specimen type and species ($\chi^2 = 29.17$; $df = 25$; $p = 0.269$) is compatible with surveillance literature showing broad species overlap across body sites once colonization and device-related risk factors are considered. Takeya H et al. 2018^[11]

Table 3 (method-wise species profile and concordance). Species proportions by VITEK-2 closely tracked conventional results e.g., *C. albicans* 50.0% vs 51.7% and the overall difference across categories was non-significant ($\chi^2 = 7.56$; $df = 6$; $p = 0.271$). Such high agreement is in line with comparative evaluations showing VITEK-2's strong performance for common *Candida* spp. and its value as a faster alternative for routine workflows. Eghtedar Nejad E et al. 2020^[5] Still, the literature notes that low-prevalence species and certain cryptic complexes (or recently emerged taxa) can challenge biochemical systems and occasionally require confirmatory approaches (e.g., morphology, targeted tests, or MALDI-TOF/sequencing) to close residual gaps consistent with 8.3% not resolved to species by VITEK-2. Kord M et al. 2020^[8]

Table 4 (antifungal susceptibility: VITEK-2 vs E-test). For amphotericin B, VITEK-2 yielded 13.3% SDD versus 0% by E-test ($p = 0.003$), while agreements for azoles were high and differences non-significant. The direction of amphotericin B discrepancy (VITEK-2 showing higher MICs/more non-susceptible calls) is well described: multiple comparisons report that E-test often reads lower MICs than VITEK-2, producing category shifts for certain drug-species combinations, whereas agreement for voriconazole and amphotericin B in *C. albicans* is typically very high. Ceballos-Garzon A et al. 2022^[12] These findings underscore the need to interpret categorical results in light of the CLSI M27 reference framework, local QC, and clinical context; when results are discordant and clinically pivotal (e.g., invasive disease), confirmation by a reference method is advisable. de Sousa ESO et al. 2020^[13]

CONCLUSION

The present study evaluated and compared the diagnostic performance of conventional phenotypic techniques with the automated VITEK-2 system for identification and antifungal susceptibility testing of *Candida* species from 50 clinical isolates. Conventional methods such as germ-tube formation, sugar assimilation tests, and CHROMagar culture achieved complete identification (100 %), while the VITEK-2 system correctly identified 91.7 % of isolates to species level. The difference in identification rates was statistically insignificant ($p > 0.05$), indicating comparable diagnostic efficacy. Both systems showed similar species distribution, with *C. albicans* remaining predominant, followed by *C. tropicalis* and *C. krusei*.

For antifungal susceptibility testing, good categorical agreement was observed between the two systems for azoles, particularly fluconazole and voriconazole, whereas minor variations were noted for amphotericin B, where VITEK-2 detected a small number of susceptible-dose dependent isolates not seen with E-test. The automated system provided rapid and reproducible results within 15-18 hours, enabling timely clinical decision-making.

Overall, VITEK-2 proved to be a reliable and efficient tool for routine mycology laboratories, offering standardized identification and antifungal susceptibility profiles comparable to conventional methods. The system significantly reduces hands-on time and inter-observer variability, contributing to effective patient management and antifungal stewardship.

LIMITATIONS OF STUDY

1. The study sample size was limited to 50 isolates, which may not capture the full spectrum of rare or emerging Candida species.
2. Reference molecular methods such as MALDI-TOF MS or sequencing were not used to resolve discrepancies, which could have provided definitive species confirmation.
3. The study was single-centered and may not represent inter-laboratory variability or differing epidemiological patterns across regions.
4. Only three antifungal agents (amphotericin B, fluconazole, and voriconazole) were tested; inclusion of echinocandins or flucytosine would have offered a broader resistance profile.
5. Some isolates with atypical morphology or mixed growth may have influenced the comparative accuracy between systems.
6. The cost-effectiveness and turnaround time were not statistically evaluated, which could have provided a more comprehensive assessment of laboratory feasibility.

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