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Comparative Evaluation Of Vitek 2 System And Pcr Technique In Identification Of Candida Species

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Abstract:

This research aimed to identify the efficacy of the Vitek 2 system in comparison to the PCR technique in detecting Candida species in the laboratories. Thirty-four swab samples were collected randomly from healthy, non-immunodeficient children; all samples were cultured on the CHROM-Candida agar medium for primary identification and then confirmed the diagnosis by Vitek 2 Compact system and by PCR technique using ITs universal primers.

The data showed that 30 (88.23%) samples grow as positive for Candid spp. on CHROM-Candida agar medium. Fourteen out of 30(46.6%) isolates formed germ tube, the positive result were referred to rapid and presumptive identification technique, which differentiated C. albicans/ C. dubliniensis from other Candida species. All 30 isolates could grow on cornmeal agar ,but 17 (56.6%) of 30 isolates displayed the typical chlamydospores Vitek2 compact system data showed that from these 30 isolates, 24(80%) recognized as C. albicans, 4(13.3%) isolates as C. dubliniensis, and 2(6.6%) isolates as C. tropicalis, shows that C. albicans, C. dubliniensis, and C. tropicalis exhibited sensitivity to all antifungal used in this study. In contrast, the PCR technique showed 30 isolates 24(80%) identified as C. albicans, 4(13.3%) isolates as C. dubliniensis, and 2(6.6%) isolates as C. tropicalis**.** Candida spp. detection by CHROM-Candida agar medium. Finally,Both PCR and Vitek 2 compact are equally effective at detecting Candida spp. in laboratory samples. However, the PCR technique needs more tools, reagents, and facilities to complete the diagnostic process than the Vitek 2 System and has shortcomings in detecting antifungal susceptibility instantly.

Keywords: Candida Spp., Vitek 2, PCR, non-immuno deficient children, mouth swabs

Introduction:

Candida infections are considered as a serious problem to public health, causing high morbidity and mortality rates worldwide(Pfaller et al., 2014)(Matthaiou, Christodoulopoulou and Dimopoulos, 2015) , the infections by this fungi range from cutaneous to systemic and become severe in immune compromised individuals(Wächtler et al., 2012). Several species within the genus Candida are considered pathogenic to humans, including C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. krusei C. lusitaniae, C. kefyr, C. guilliermondii, and C. dubliniensis(Moran, Coleman and Sullivan, 2011).

The Vitek 2 YST system (BioMerieux, France) is a fully automated system for identifying and antimicrobial susceptibility testing of different microorganisms. Its advantage is the fast detection procedure to species level within 18 hours instead of 48-72 hours for other methods(Melhem et al., 2013)(Sariguzel et al., 2015). The ID-YST database of Vitek 2 YST for yeast identification includes 54 different taxa containing the newly identified species and takes recent developments in taxonomy into account. The Biomerieux Vitek-2 system includes Vitek-2 cards, which enable species identification by comparing the biochemical profile to an extensive database. The system also includes antifungal susceptibility testing (AST) cards(Cuenca-Estrella et al., 2010).

Molecular identification methods for detecting different microorganisms have become more popular for epidemiological analysis(Marol and Yücesoy, 2008). And PCR technique, one of these techniques, exhibited high sensitivity, easiness, rapid, reliable, and applicable in the clinical laboratory for identifying the medically important Candida species compared with other phenotypic techniques such as germ tube and API system (Kadry, El-Ganiny and El-Baz, 2018) .

This study evaluates the Vitek 2 ID YST system from (BioMerieux/France) and PCR techniques using ITs global primers to rapidly detect Candida spp isolated from the mouth of non-Immunodeficient children in Al Najaf city.

Materials and methods:

A total of 34 mouth swabs were collected from non-immunodeficient children in the Al Najaf province, Iraq. The collected swabs were streaked directly on the Sabouraud dextrose agar plates (HiMedia Laboratories Pvt. Ltd., India) containing 0.5 mg\1000 ml chloramphenicol and on CHROMagar Candida plates (Rambach, France) and incubated at 37°C for 48–72 hours; the plates were observed for checking the size, color, and morphology of the colonies(Othman et al., 2018).

Identification by Vitek2 compact system

Vitek 2 Compact (BioMerieux/France) systemwas used for the primery diagnosis and MIC detection of candida isolates. The identification cards were loaded with yeast suspensions of 1.80–2.0 turbidity using DensiChekTM(Hata et al., 2007) . The results contained on the card were compared to an identification database within 18 hr of incubation(Reiss, Shadomy and Lyon, 2012).

Identification by PCR technique.

DNA-Pure Yeast Genomic Kit (bio World, USA) was used to extract genomic DNA according to the manufacturer's instructions. The PCR technique was used to identify study isolates using

universal primers ITS1, ITS3 and ITS4(White et al., 1990). Amplification reactions were performed in 25 µl final volume containing 13 µl Robust HotStart Readymix (Kappabiosystem, South Africa), 1µl (10 pmol) from each primer, 5µl DNA template, and the volume was complete by PCR grade water to 25 µl. The mixture was exposed to the following thermal cycling: initial denaturation at 94°C for 3 minutes followed by 37 cycles of 95°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute, finally one cycle for 5 minutesat 72°C**.**

Results:

Thirty isolates were obtained from normal non-immuno compromised children aged 3-12 years old. The colonies of Candida spp. grow on CHROM agar-Candida medium showed various degrees of green color (Figure1).

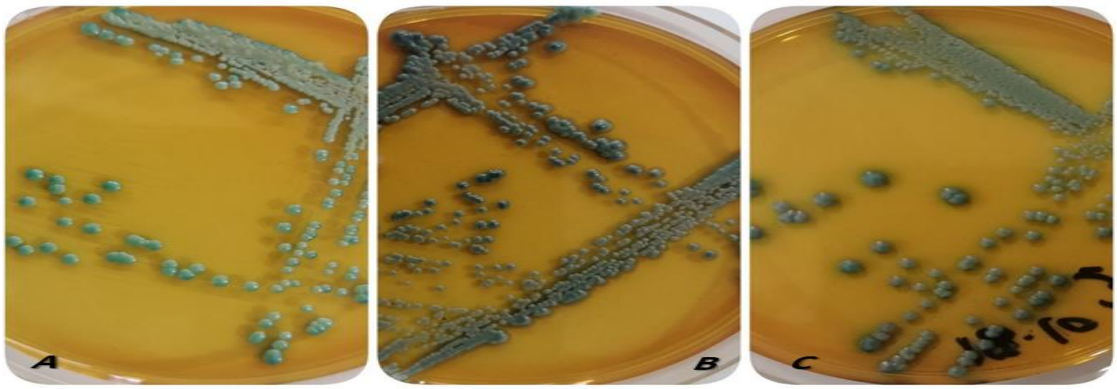


Figure 1: Candida spp. colonies on CHROM agar Candida medium at 37 ◦C for 24 h

Fourteen out of 30(46.6%) isolates formed Germ tubes when the yeast cells were incubated in fresh human serum for 3 hours at 37 °C, and overall, 17 (56.6%) of 30 isolates displayed the typical chlamydospores (Figure 2).

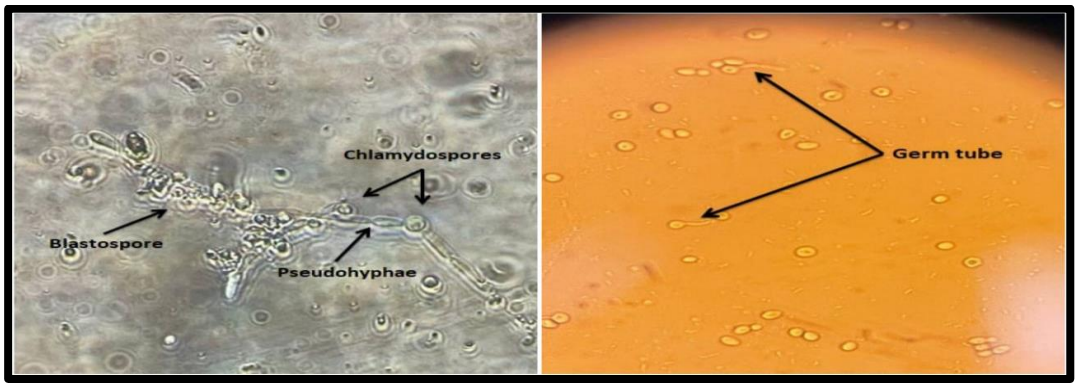


Figure 2: A) chlamydospores formation after 3 days on cornmeal agar (with 1% Tween 80). B) germ tube production in fresh humans serum (100X).

ID_AST by Vitek -2 compact Technique:

Vitek2 compact System data showed 24(80%) as C. albicans, 4(13.3%) isolates as C. dubliniensis and 2(6.6%) isolates as C. tropicalis. Antifungal sensitivity patterns of isolates for a group of

antifungal agents were detected by Vitek2 compact System; all isolates sensitive to antifungal agents were tested according to CLSI and EUCAST (Table 1).

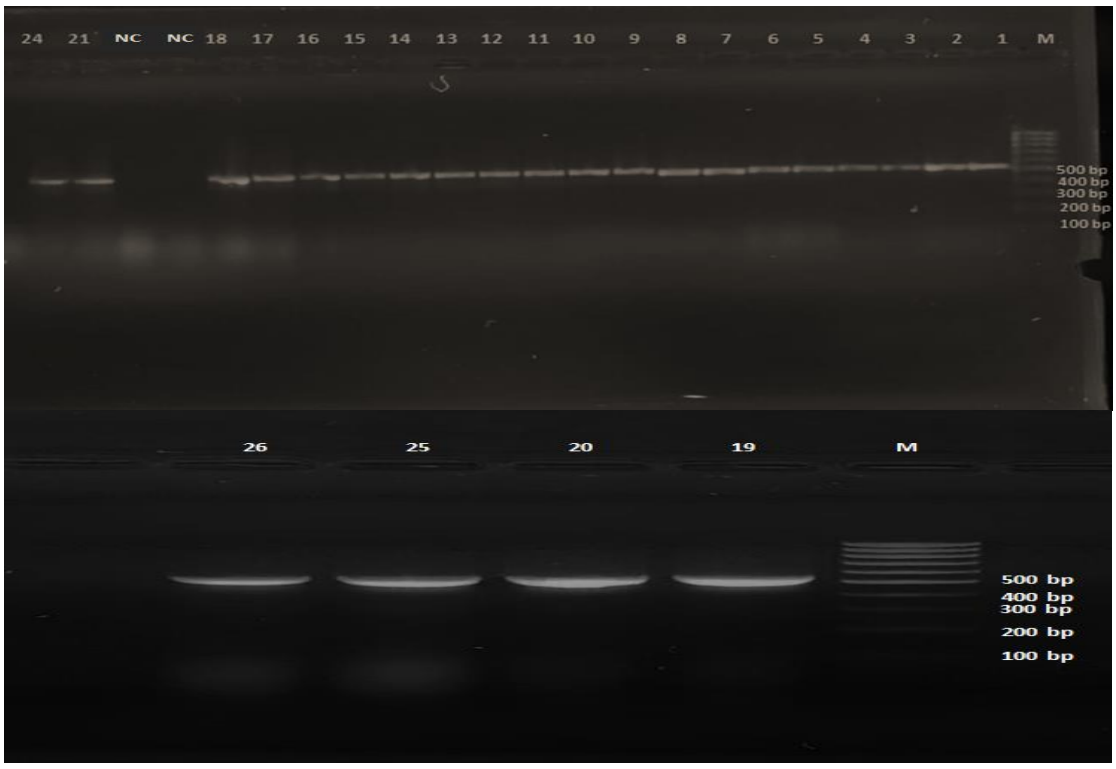
Table 1: in vitro susceptibility profile ofCandida albicans,Candida dubliniensis and C. tropicalisto antifungal using Vitek 2

Genus	MIC (µg/ml)							
	Numbers of Isolates	Fluco	Vorico	Caspo	Micaf	Flucy	Amph	Interpretation
C. albicans	**24**	≤0. 5	≤0.12	≤0.12	≤0.06	≤1	0.5	**S**
C . dubliniensis	**4**	≤1	≤0.12	≤0.12	≤0.06	≤1	≤0.25	**S**
C. tropicalis	**2**	≤1	≤0.12	≤0.12	≤0.06	≤1	≤0.25	**S**

The study isolates were identified according to ITs region; PCR data showed that from these 30 isolates, 24(80%) isolates identified as C. albicans, 4(13.3%) isolates as C. dubliniensis, and 2(6.6%) isolates as C. tropicalis,(Table 2)

Table 2: Candida spp. detection by CHROM-agar medium, Vitek 2 compact, and PCR

NO. isolates	Candida spp.	CHROM-agar	Vitek 2	PCR
30	+Ve	30(100%)	30(100%)	30(100%)
	-Ve	0(0%)	0(0%)	0(0%)



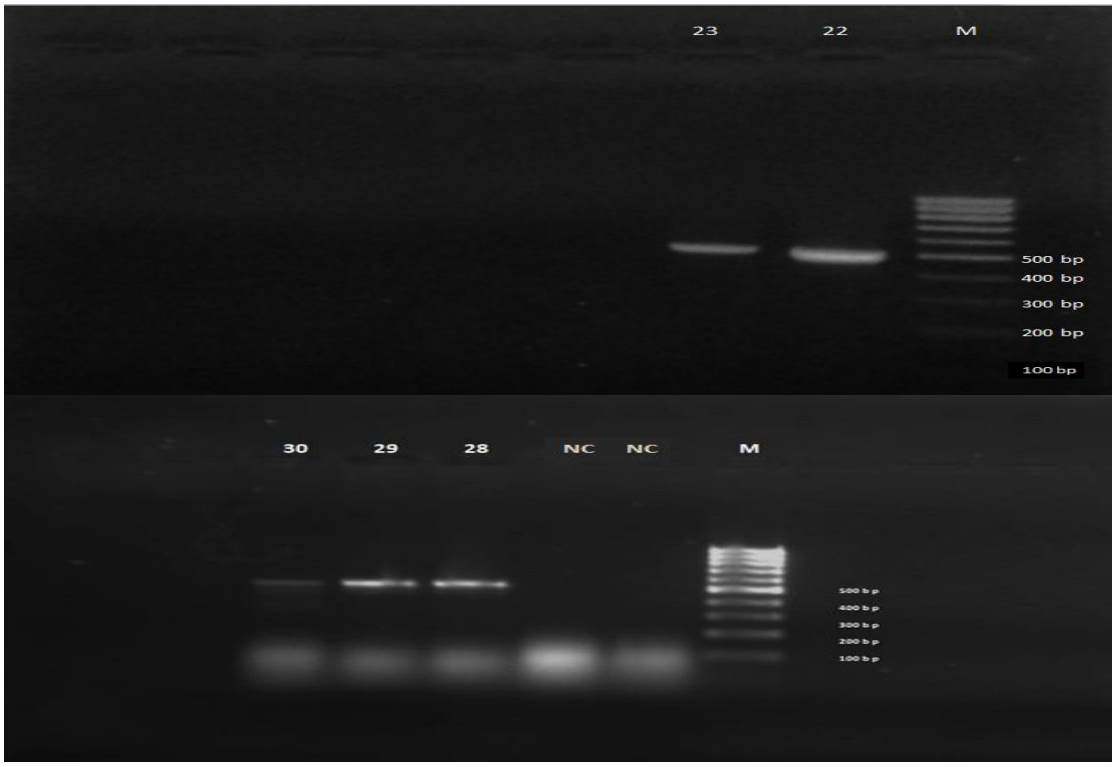
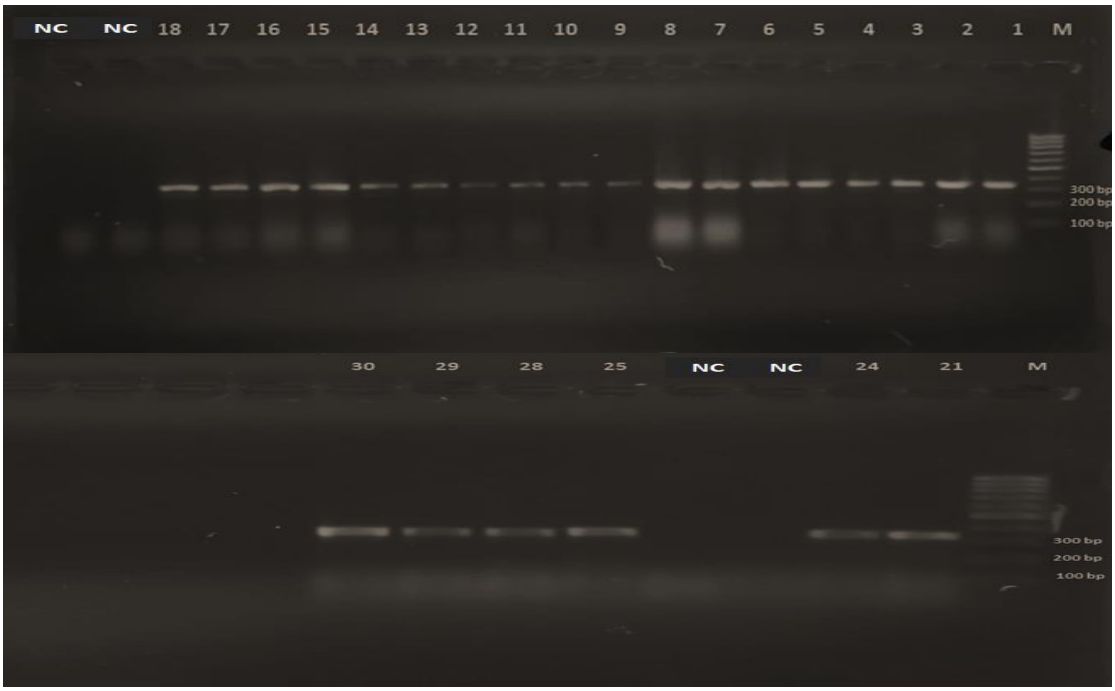


Figure 3A: Amplification profiles of PCR products of ITS1and ITS4 region, Lane M: Molecular marker (100bp), lane NC: negative control, lane 1-18,21,24,27-30 C. albicans, lane 19-20,25-26 C.dubliniensis and lane 22-23 C. tropicalis.



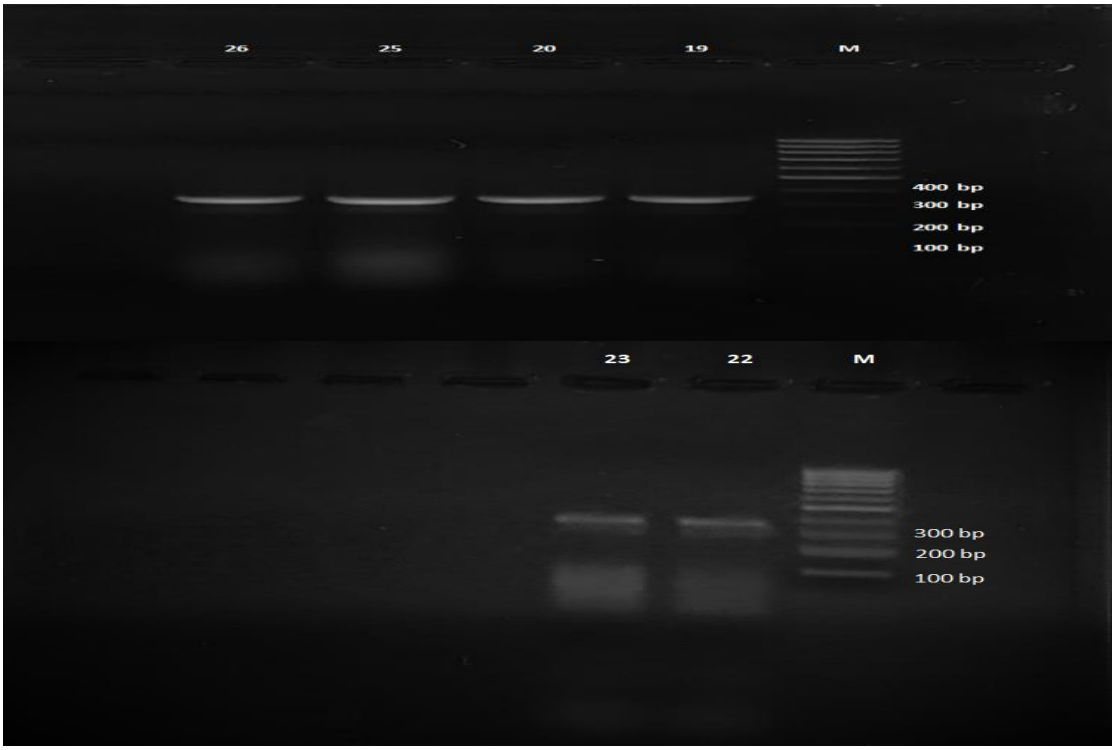


Figure 3B: Amplification profiles of PCR products of ITS3 and ITS4 region**;** Lane M: Molecular marker (100bp), lane NC: negative control, lane 1-18,21,24,27-30 C. albicans, lane 19-20,25-26 C.dubliniensis and lane 22-23 C. tropicalis

Discussion

In our current study, Fourteen out of 30(46.6%) isolates formed germ tube as elongated structure from the mother cell in the absence of reduction at their root, the positive result were referred to rapid and presumptive identification technique, which differentiated C. albicans/ C. dubliniensis from other Candida species this corresponds to other researchers such as(Alrubayae et al., 2020).The present study pointed out that all 30 isolates could grow on cornmeal agar(Al-Dabbagh, 2020), but not all of them could produce Chlamydospore, whereas 17 (56.6%) of 30 isolates displayed the typical chlamydospores , The result was identical to the result of (Mugale et al., 2015).all thirty isolates demonstrated sufficient growth at 15 h of incubation, and the VITEK 2 system could determine the species at different incubation periods. It showed that 24(80%) as C. albicans, 4(13.3%) isolates as C. dubliniensis and 2(6.6%) isolates as C. tropicalis, shows that C. albicans, C. dubliniensis, and C. tropicalis exhibited sensitivity to all antifungal used in this study shown in Table 1, and this corresponds to other researchers such as(Alrubayae et al., 2020).In our current study,molecular identification according to universal primers ITS1, ITS3, and ITS4 were used (White et al., 1990)showed the same data as VITEK 2 system, 24 isolates C. albicans showed (338\ 535 bp), 2 isolates C.tropicalis (327\524 bp)(Khodadadi et al., 2017)(Mohammadi et al., 2016)and4 isolates C. dubliniensis (500\342 bp)(Khodadadi et al., 2017)(Aldossary et al., 2018).

Conclusion

PCR and Vitek 2 compact both have the same efficiency in identifying Candida spp. from laboratory samples. However, the PCR technique still needs several types of equipment, reagents,

and facilities more than the Vitek 2 System to complete the diagnostic procedure and have limitations in detecting antifungal sensitivity instantly**.**

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