Effects of chemically synthesized Azole compounds on Clinical isolates of Vaginal Candidiasis, in comparison with commercially available anti-fungal drugs

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Citation

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Abstract

Candida species is observed as the most common cause of 'Opportunistic Mycoses' worldwide. Candida species is a member of normal flora of skin, mouth, vagina, and stool. Infections caused by Candida species are in general referred to as Candidiasis. Vaginal Candidiasis is a fungal or yeast infection of the vulva and/or vagina, which causes a smelly, thick, white-yellow discharge, which might be accompanied by itching, burning and swelling. It can also can make walking, urinating or sex very painful.

The Genus Candida includes around 154 species. While Candida albicans is the most abundant and significant species; importantly, there has been a recent increase in infections due to non-albicans Candida species along with increasing drug resistance.
The present study includes isolation of Candida species from the patients suffering from vaginal candidiasis followed by identification and differentiation between different Candida species by their morphological studies, microbiological analysis and biochemical activities, which are: C. albicans, C. tropicalis, C. glabrata & C. krusei.

The present study also includes a comparative study of anti-microbial activities of commercially available anti-fungal drugs as well as 22 novel chemically synthesized 1% DMSO soluble compounds (belonging to Azoles) by “Disc Diffusion Method” according to NCCLS Guidelines. Out of which 5 compounds were found to be remarkably effective, especially AntiC-15 was found to be the most potent and excellent among all; which exhibits its inhibitory effects against some drug resistant isolates also. It is sent for further study in order to use it at commercial level.

INTRODUCTION

Candida species is yeast and the most common cause of opportunistic mycoses worldwide. It is also a frequent colonizer of human skin and mucous membranes. Candida species is a member of normal flora of skin, mouth, vagina, and stool. Infections caused by Candida species are in general referred to as Candidiasis. The clinical spectrum of Candidiasis is extremely diverse. Almost any organ or system in the body can be affected. Candidiasis may be superficial and local or deep-seated and disseminated. Candidiasis is mostly an endogenous infection, arising from overgrowth of the fungus inhabiting in the normal flora. However, it may occasionally be acquired from exogenous sources such as catheters or prosthetic devices or by person-to-person transmission such as Oral Candidiasis in neonates of mothers with Vaginal Candidiasis or Endophthalmitis following corneal transplantation from an infected donor.

Vaginal Candidiasis is a fungal or yeast infection of the vulva and/or vagina. It causes a smelly, thick, white-yellow discharge that might be accompanied by itching, burning and swelling. It can also can make walking, urinating or sex very painful.

The Genus Candida includes around 154 species. Among these, very few are most frequently isolated in human infections. While Candida albicans is the most pathogenic and most commonly encountered species among all due to its ability to adhere to host tissues, produce aspartyl proteases & phospholipase enzymes and transformation from yeast to hyphal phase, which are the major determinants of its pathogenicity. Candida tropicalis, Candida glabrata, Candida parapsilosis, Candida krusei and Candida lusitaniae

are also isolated as causative agents of Candidiasis.

Importantly, there has been a recent increase in infections due to non-albicans Candida species along with increasing drug resistance. For the same, the present study also includes a comparative study of anti-microbial activities of commercially available anti-fungal drugs as well as 22 newer chemically synthesized 1% DMSO soluble compounds (belonging to Azoles).

MATERIAL & METHODS

STUDY GROUP

The study group includes different patients suffering from Vaginal Candidiasis with varying degree of severity.

SPECIMEN COLLECTION:

DIRECT EXAMINATION:

ISOLATION, IDENTIFICATION AND DIFFERENTIATION BETWEEN SPECIES:

ISOLATION FROM OTHER YEASTS:

CULTURAL TECHNIQUES:

GERM – TUBE TEST:

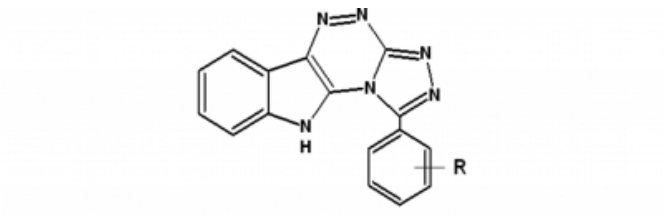
CARBOHYDRATE UTILIZATION PROFILES:

ANTI-MICROBIAL ACTIVITY

In present study, we have analyzed 22 heterocyclic chemical compounds (1% DMSO soluble); synthesized at Chemistry Department, Saurashtra University at Rajkot by preparing discs of these compounds with different concentrations [5µg/disc, 10µg/disc, 15µg/disc] using sterile discs from Hi-Media. It is followed by analyzing their anti-microbial activities on clinical isolates by "Disc Diffusion Method" according to NCCLS guidelines in comparison with commercially available anti-fungal drugs [Anti-fungal antibiotic discs were used]. Prepared discs of Azoles: Fluconazole, Itraconazole, Ketoconazole; Polyenes: Amphotericin B, Nystatin were used.

NOVEL HETEROCYCLIC COMPOUNDS

Figure 1



(1-sub.phenyl-10*H*-[1, 2, 4] triazolo [3', 4':3, 4] [1, 2, 4] triazino [5, 6-*b*] indole)

Note: The basic structure remains the same in all 22 compounds; while 'R' is variable.

During the experiment, One disc having 1% DMSO/disc was kept in each plate as a standard to check any inhibitory effect of the solvent as well as one plate kept as a control inoculated with the respective isolate only, without any disc.

OBSERVATION & RESULTS

The aim of the present study was to investigate pathogenic yeast organisms, mainly Candida albicans and other non-albicans group from patients suffering Vaginal Candidiasis.

Total 27 isolates of Candida spp. were analyzed for isolation, identification & differentiation using different media as well as the biochemical activities (like; Germ-tube formation, Urease test, Sugar fermentation tests, Sugar assimilation profile), along with the sensitivity profile of each isolate against commercial drugs as well as synthetic chemical compounds. [All the sets were performed in duplicates for each isolate].

Out of 22 heterocyclic compounds [belonging to Azoles], 5 compounds were found to be remarkably efficient; especially, "AntiC-15" was found to be the most potent and excellent among all, giving anti-microbial activity even at 10µg/disc.

Many isolates were found to be relatively more sensitive to commercial drugs, were also found to be fairly good susceptible to the heterocyclic compounds. Certain isolates have been found to be relatively more resistant to commercially used drugs but still found to be susceptible at least to AntiC-15.

Table: 1 includes identification of clinical isolates of different Candida species, isolated from patients suffering from Vaginal Candidiasis.

Graph: 1 indicates a comparative profile of different Candida species isolated from patient suffering from vaginal candidiasis.

Table: 2.1 & 2.2 includes a comparative analysis of anti-microbial effects of commercially available drugs as well as 1% DMSO soluble synthetic heterocyclic chemical compounds (belonging to Azoles) respectively against clinical isolates of different Candida species from vaginal candidiasis.

Figure 2

Table: 1 indicates identification of clinical isolates of different species, isolated from patients suffering from Vaginal Candidiasis.

No.	Isolate Name	Biochemical characteristics													Candida species
		Morphology			Fermentation					Assimilation					
		PH	GT	Chs.	G	S	L	M	Gal	G	S	L	T	R	C	
1	CanV-1	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
2	CanV-2	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
3	CanV-3	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
4	CanV-4	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
5	CanV-5	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
6	CanV-6	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
7	CanV-7	-	-	-	+	-	-	-	-	+	-	-	+	-	-	C.glabrata
8	CanV-8	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
9	CanV-9	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
10	CanV-10	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
11	CanV-11	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
12	CanV-12	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
13	CanV-13	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
14	CanV-14	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
15	CanV-15	-	-	-	+	-	-	-	-	+	-	-	+	-	-	C.glabrata
16	CanV-16	-	-	-	+	-	-	-	-	+	-	-	+	-	-	C.glabrata
17	CanV-17	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
18	CanV-18	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
19	CanV-19	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
20	CanV-20	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
21	CanV-21	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
22	CanV-22	-	-	-	+	-	-	-	-	+	-	-	+	-	-	C.glabrata
23	CanV-23	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
24	CanV-24	+	+	+	+	-	-	+	√	+	+	-	+	-	+	C.albicans
25	CanV-25	+	-	-	+	√	-	+	+	+	+	-	+	-	+	C.tropicalis
26	CanV-26	+	-	-	+	-	-	-	-	+	-	-	-	-	-	C. krusei
27	CanV-27	-	-	-	+	-	-	-	-	+	-	-	+	-	-	C. glabrata

Abbreviations used in the table:

PH : Pseudohyphae
GT : Germ-tube formation
CHS : Chlamydospore
G : Glucose
S : Sucrose
L : Lactose
M : Maltose
Gal : Galactose
T : Trehlose
R : Reffinose
C : Cellobiose
+ : Positive, - : Negative, : Variable

Figure 3

Graph-1 indicates a comparative profile of different species isolated from patient suffering from vaginal candidiasis.

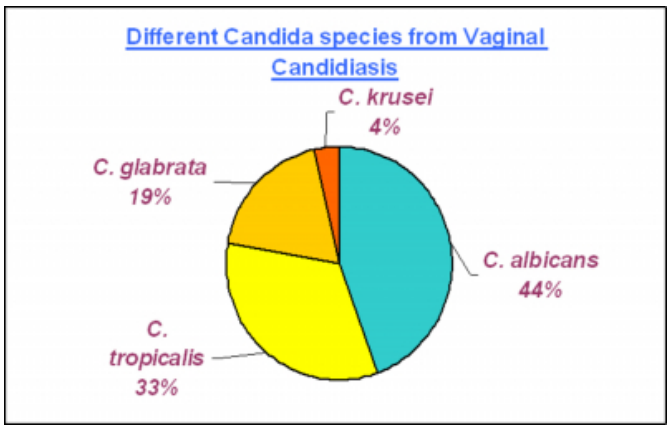


Figure 4

Table: 2.1 include anti-microbial effects of commercially available drugs against clinical isolates of different species from vaginal candidiasis.

No.	Isolate Name	Standard Anti-fungal antibiotic Discs [20 µg/disc]				
		Fluco	Itra	Keto	Amp-B	Nys
		Sensitivity Profiles				
1	CanV-1	S	S	S	S	S
2	CanV-2	R	S	S	S	S
3	CanV-3	S	S	R	S	I
4	CanV-4	S	I	R	I	S
5	CanV-5	I	S	R	S	I
6	CanV-6	R	R	I	S	R
7	CanV-7	S	S	R	S	S
8	CanV-8	S	S	S	S	S
9	CanV-9	S	S	I	S	S
10	CanV-10	I	S	I	I	R
11	CanV-11	S	I	S	R	I
12	CanV-12	S	S	S	S	S
13	CanV-13	S	S	R	S	I
14	CanV-14	R	R	R	S	S
15	CanV-15	S	S	S	R	R
16	CanV-16	I	I	R	I	I
17	CanV-17	R	I	I	I	S
18	CanV-18	S	S	S	S	S
19	CanV-19	S	I	R	I	S
20	CanV-20	S	S	I	R	R
21	CanV-21	R	R	R	I	S
22	CanV-22	S	S	R	S	S
23	CanV-23	I	R	I	S	I
24	CanV-24	I	I	R	I	R
25	CanV-25	R	R	I	S	S
26	CanV-26	R	R	R	R	R
27	CanV-27	I	I	I	I	I

Abbreviation used:

Figure 5

Code of Anti-fungal antibiotic	Name of Anti-fungal antibiotic
Fluco	Fluconazole
Itra	Itraconazole
Keto	Ketoconazole
Amp-B	Amphotericin B
Nys	Nystatin

Figure 6

Table: 2.2 include anti-microbial effects of 1% DMSO soluble synthetic heterocyclic chemical compounds (belonging to Azoles) against clinical isolates of different species from vaginal candidiasis.

S.	Isolate Name	Heterocyclic Chemical Compounds [5, 10, 15 µg/disc]														
		Com-1			Com-2			Com-3			Com-4			Com-5		
		5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
		Zone Diameter (mm)														
1	*CanV-1*	R	6	15	R	5	9	R	7	13	R	4	9	R	3	7
2	*CanV-2*	R	5	8	R	R	5	R	8	15	R	5	10	R	4	9
3	*CanV-3*	R	5	11	R	3	8	R	9	16	R	4	9	R	R	5
4	*CanV-4*	R	3	7	R	6	10	R	6	15	R	R	7	R	2	5
5	*CanV-5*	R	6	10	R	R	3	R	10	19	R	5	9	R	4	9
6	*CanV-6*	R	R	R	R	3	7	R	7	11	R	6	13	R	3	9
7	*CanV-7*	R	6	11	R	5	11	R	7	15	R	4	9	R	3	8
8	*CanV-8*	R	5	11	R	4	10	R	9	20	R	7	15	R	4	11
9	*CanV-9*	R	6	15	R	R	6	R	8	16	R	4	9	R	5	12
10	*CanV-10*	R	2	6	R	5	9	R	4	9	R	3	5	R	R	R
11	*CanV-11*	R	6	14	R	5	13	R	8	15	R	R	3	R	3	8
12	*CanV-12*	R	5	14	R	5	11	R	6	17	R	4	9	R	3	7
13	*CanV-13*	R	7	15	R	6	11	R	8	17	R	5	12	R	4	9
14	*CanV-14*	R	4	7	R	5	9	R	7	15	R	5	8	R	6	15
15	*CanV-15*	R	R	6	R	4	9	R	6	15	R	5	13	R	4	7
16	*CanV-16*	R	5	9	R	R	3	R	7	11	R	6	9	R	R	R
17	*CanV-17*	R	R	5	R	3	5	R	7	11	R	4	9	R	R	R
18	*CanV-18*	R	7	15	R	5	9	R	10	22	R	6	13	R	7	12
19	*CanV-19*	R	R	2	R	3	7	R	6	10	R	5	9	R	R	R
20	*CanV-20*	R	6	11	R	4	9	R	6	15	R	3	5	R	5	9
21	*CanV-21*	R	6	13	R	5	9	R	8	17	R	3	7	R	R	6
22	*CanV-22*	R	3	7	R	5	11	R	6	12	R	R	3	R	2	5
23	*CanV-23*	R	6	14	R	5	11	R	8	17	R	5	9	R	3	5
24	*CanV-24*	R	2	5	R	R	4	R	5	9	R	2	6	R	R	R
25	*CanV-25*	R	5	14	R	4	9	R	6	14	R	2	6	R	R	6
26	*CanV-26*	R	R	R	R	R	3	R	4	9	R	R	2	R	R	R
27	*CanV-27*	R	5	8	R	2	4	R	7	15	R	3	7	R	5	9

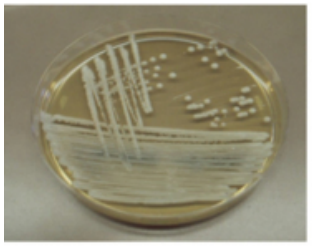
Abbreviations used:

Figure 7

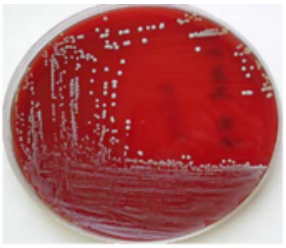
Heterocyclic Chemical Compounds		
Compound No.	*Compound Code*	*Substituents (R)*
Com-1	AntiC-11	2-NO_2
Com-2	AntiC-12	3-OC_6H_5
Com-3	**AntiC-15**	**2-OCH_3**
Com-4	AntiC-16	3-Cl
Com-5	AntiC-21	9-Anthryl

Photographs

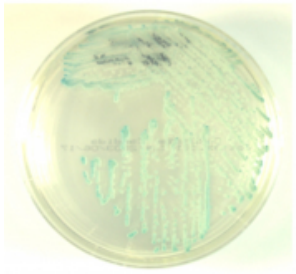
Figure 8



CanV-24 on SDA



CanV-24 on Blood Agar



CanV-24 on Hi-Chrom Agar

Germ-tube formation by *CanV-24*

Sugar Fermentation Profile of *CanV-27*

Sugar Fermentation Profile of *CanV-24*

DISCUSSION & CONCLUSION

Out of 27 isolates; C. albicans constitutes 44.44%, C. tropicalis constitutes 33.33%, C. glabrata constitutes 18.51%, C. krusei constitutes 3.70%; with slightly increasing non-albicans species [normally, C. albicans constitutes 60-70% and C. krusei constitutes only >1%].

Along with increasing number of non-albicans species; interestingly, the drug resistance to commercially available drugs also increases as well as each drug is having certain mild to severe side effects; so, now there is extreme need to evaluate and to use newer and more potent drugs.

Among all isolates, the overall general sensitivity gradation is as follows:

C. albicans > C. tropicalis > C. glabrata > C. krusei

However, among the anti-fungal drugs, the overall

sensitivity gradation is as follows:

Amphotericin-B > Itraconazole > Fluconazole > Nystatin > Ketoconazole

Out of 22 chemical compounds, 5 compounds were found to be remarkably efficient; especially, AntiC-15 was found to be the most potential and excellent among all.

CanV-16 (C. glabrata), CanV-24 (C. albicans) and CanV-27 (C. glabrata) were found to be not sensitive to all the anti-fungal drugs; still found to be sensitive to the chemical compounds, at least to AntiC-15. However, CanV-26 (C. krusei) was found to be resistant to all; i. e., commercially available drugs as well as heterocyclic compounds; still, AntiC-15 exhibited slight inhibition even at 10µg/disc (4 mm) and 15µg/disc (9 mm).

AntiC-15 is sent for further analysis in order to use it at commercial level against candidiasis.

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