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REVOLVING LABORATORY-STEWARDSHIP FOR PHENOTYPIC RESISTANCE PROFILES OF COMBINED ANTIFUNGAL AGENTS ON HUMAN CLINICAL *CANDIDA* SPECIES IN A DEVELOPING COUNTRY AND COVID-19 INDICATIONS

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ABSTRACT: ***Background:*** *Antimicrobial stewardship has always revolved round prudent antimicrobial regimens, including combined antimicrobial therapy, to curb antimicrobial resistance. But there is dearth of local microbiological data on combination antifungal therapy. Materials and Methods: 129 strains of human oral and vulvo-vaginal Candida species were assayed for phenotypic susceptibility / resistance profiles to 12 commonly-available-in-country antifungal drugs and 12 antifungal creams.* ***Results:*** *When tested singly, C. albicans strains were totally resistant to Primpex and Flucamed. Resistance rates of 46.6-95.2% were exhibited to remaining antifungal drugs, with multiple antibiotic resistance (MAR) of 41.7-100%. All C. glabrata strains were resistant to Fesovin; 55.7-96.2% strains exhibited resistance to other antifungals, and MAR was 41.7-100%. C. pseudotropicalis strains were totally resistant to Fesovin, Ketoconazole, Primpex and Flucamed; 36.8-84.2% strains resisted other antifungals, while MAR was 41.7-100%. C. tropicalis, exhibited resistance rates of 37.5-100% and MAR of 50.0-100%. The Candida species were resistant to all antifungal creams. Combined phenotypic assays of five most-resisted antifungal drugs and six most-resisted antifungal creams, on 40 most-resistant Candida strains gave 37.5% and 27.5% resistance (or 62.5% and 72.5% susceptibility) instead of 100% resistance (0.0% susceptibility) recorded during single antifungal drug and cream susceptibility assays.* ***Conclusion:*** *Appropriate laboratory-based direct-antifungal-drugs assay can serve as antimicrobial stewardship intervention, which can enhance ideal antifungal prescriptions that translates to better treatment regimens of mycotic infections, and confirm non-potent antifungals, including adulterated, fake, substandard or expired antifungals, common in some developing countries, including Nigeria.*

KEYWORDS: Combined Antifungal Therapy, Commonly Available-in-Country Antimicrobials, CoviD-19, Mycoses, Laboratory-Derived Antifungal Prescriptions, Substandard Medications.

INTRODUCTION

Many species of *Candida*, once considered as minor pathogens have potentials of causing diseases commonly known as candidiasis, which range from non-life-threatening mucocutaneous illnesses to invasive conditions that may involve virtually any organ.[1-8]

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Globally, fungal-associated mortality is reportedly more than three times that of malaria, with an estimated 1.6 million deaths annually[4.] Even, most recent evidences on emerging clinical reports infer that severe and adverse CoviD-19 complications may be caused by fungal co-infections or secondary infections, such as oral candidasis, lung candidasis, and other respiratory system candidasis, etc., which is another important factor influencing reported mortalities.[9-17] However, in addition to antifungal agents like azoles, polyenes, echinoderms, etc., candidasis have also been treated with various agents, such as, phytotherapeutic products, probiotics, as well as, disinfectants and germicides, especially for surface cleansing.[18-27] Meanwhile, antifungal resistance has become increasingly recognised as a major health concern, and significant increase in resistance to antifungal agents by pathogenic *Candida* species and associated effects have been repeatedly documented.[22, 24, 27-30]

Past few decades have observed sustained medical implications of opportunistic infections, especially due to significant increase in incidence of invasive fungal infections, and world-wide increase in number of immuno-compromised patients, who are highly susceptible to opportunistic fungal infections.[9, 10] But antifungal armamentarium for treatment of serious fungal infections remains limited, mostly in developing countries. It has therefore, been proposed that a possible approach to improve treatment outcomes, and overcome antifungal drug resistance and high mortality rates seen in severe fungal infections is, combination therapy, which can lead to increase in susceptibility and reduce resistance of microbial pathogens.[10, 30-31]

Fungal diseases affect over a billion people, and kills more than 1.5 million,[4] as most medically important *Candida* species overcome a broad range of host-imposed constraints, to increase their pathogenicity.[32, 33] Thus, there has been increasing interest in the use of combination antifungal therapy, especially if the drugs have different mechanisms of action, and sometimes, combinations of new agents, in order to improve prognosis and treatment of fungal diseases.[34-37] Research data on antifungal combination therapy are however, quite poorly reported in developing countries, including Nigeria, where increased adulterated and sub-standard antifungal agents are also common. The aims of this study therefore, is to investigate inhibitory potentials of combined in-country antifungals, using multi-resistant *Candida* species.

MATERIALS AND METHODS

Collection of Clinical Specimens:

Oral *Candida* species [*Candida glabrata* (52), *Candida albicans* (43), *Candida pseudotropicalis* (19) and *Candida tropicalis* (15)] used in this study were stock strains, originally obtained from human specimens at the Department of Medical Microbiology & Parasitology, University College Hospital (UCH), Ibadan, Nigeria. Stock strains were reactivated in sterile peptone water, and incubated at 27-30^{0}C for 24-48 hours before separately sub-cultured on sterile Sabouraud D extrose A gar (SDA, Lab M, Basingstoke, England), to which ofloxaciline antibiotics was added, to inhibit bacterial contaminations. Culture plates were incubated at 27-30^{0}C for 24-48 hours, and obtained pure culture of each *Candida* strain was then checked to confirm identity.

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Determination of anti-candidal activities of antifungal agents against *Candida* strains (Modified Agar Well-Diffusion method):

Antifungal drugs used for bioassay with their respective active ingredients were Itranox (itraconazole); Fesovin (Griseofulvin); Gyno-Tiocosid (Ticonazole); Medcan (Fluconazole); Vulcan-50 (Fuconazole); Grufin (Griseofulvin); Lucon (Fluconazole); Grisovid-500 (Griseofulvin); Diflucan (Fluconazole); Ketoconazole (Ketoconazole); Primpex (Sulphamethoxazole); Flucamed (Fluconazole). Antifungal creams used for bioassay studies and their respective active ingredients were- Mycoten (Clotrimazole), Skmeal (Ketoconazole), Trosyd (Ticonazole), Lamisil (Terbinafine hydrochloride), caneXcream (Clotrimazole), Funbact-A (Clotrimazole, Betamethasone, Neomycin sulphate, and Clobetasol propionate), Fungur (Miconazole), Nizoral (Ketoconazole), Fungusol (Miconazole Nitrate), Ketofung (Ketoconazole), Whitfield's ointment (Salicylic acid and benzoic acid), Dakatrin (Miconazole nitrate).

Holes measuring 6.00 mm in diameter were aseptically bored and punched out of sterile SDA agar plates, followed by surface flaming of the agar plates. Each SDA agar plate was then seeded with each oral *Candida* strain. Using the modification of Tagg *et al*.[38] method, 500µl suspensions of each antifungal agent were separately dispensed into agar wells in seeded plates, followed by incubation at 30^0C for 24-48 hrs. Inhibitory activities depended on release of diffusible inhibitory metabolites from antifungal agents into assay agar, during incubation. Inhibitory zones surrounding the agar wells were recorded in mm diameter, while absence of zones or zones less than 10.0 mm in diameter were recorded as negative (resistant).

Candida strains that were totally resistant to antifungal drugs and creams were assayed for susceptibility trends to combined antifungal drugs and combined antifungal creams. Holes measuring 6.00 mm in diameter were aseptically bored and punched out of sterile SDA agar plates, followed by surface flaming of the agar plates. Each SDA agar plate was then seeded with each selected oral *Candida* strain, and 500µl suspensions of each set of combined antifungal drugs [afd1+afd2; afd1+afd3; afd1+afd4; afd1+afd5 / afd2+afd3; afd2+afd4; afd2+afd5 / afd3+afd4; afd3+afd5 / afd4+afd5 / afd1+afd2+afd3; afd1+afd2+afd4; afd1+afd2+afd5 / afd1+afd3+afd4; afd1+afd3+afd5; afd2+afd3+afd4; afd2+afd3+afd5 / afd2+afd4+afd5 / afd4+afd5].

Bioassay was also determined on antifungal creams [afc1+afc2; afc1+afc3; afc1+afc4; afc1+afc5; afc1+afc6 / afc2+afc3; afc2+afc4; afc2+afc5; afc2+afc6 / afc3+afc4; afc3+afc5; afc3+afc6 / afc4+afc5; afc4+afc6 / afc5+afc6 / afc1+afc2+afc3; afc1+afc2+afc4; afc1+afc2+afc5; afc1+afc2+afc6 / afc1+afc3+afc4; afc1+afc3+afc5; afc1+afc3+afc6 / afc1+afc4+afc5; afc1+afc4+afc6 / afc1+afc5+afc6 / afc2+afc3+afc4; afc2+afc3+afc5; afc2+afc3+acf6; afc2+afc4+afc5; afc2+afc5; afc1+afc2+afc6; / afc1+afc3+afc4; afc1+afc3+afc5; afc1+afc3+afc6 / afc1+afc4+afc5; afc1+afc4+afc6 / afc1+afc5+afc6; afd2+afd3+afd5 / afd2+afd4+afd5 / afd4+afd5] and separately dispensed into wells in seeded agar plates, followed by incubation at 30^0C for 24-48 hrs. Inhibitory zones surrounding the agar wells were recorded in mm diameter, while absence of zones or zones less than 10.0 mm in diameter were recorded as negative (resistant).

RESULTS

As shown in Table 1, all the 42 oral C. albicans strains were totally resistant to Primpex [Sulphamethoxazole] and Flucamed [Fluconazole] drugs, while resistance rates of 38.1-95.2% were exhibited towards the remaining antifungal. All the 52 C. glabrata were resistant to Fesovin [Griseofulvin] drug, while 44.2-98.1% of C. glabrata strains exhibited resistance towards other antifungals but slightly less resistant to Vulcan-50 [Fluconazole] and Gruffin [Griseofulvin] (40.4-44.2%). C. pseudotropicalis strains were totally resistant to Fesovin [Griseofulvin], Ketoconazole, Primpex [Sulphamethoxazole] and Flucamed [Fluconazole]; significantly resisted (36.8-84.2%) by other antifungals, but least recorded resistance were 15.8% and 21.1% for Vulcan-50 [Fluconazole] and Gruffin [Griseofulvin]. Exhibited resistance rates for C. tropicalis were 31.3-100%.

Percentage of multiple antifungal resistance (MAR) exhibited by C. albicans, C. glabrata and C. pseudotropicalis strains were 41.7 - 100%, while MAR for C. tropicalis was 50.0-100%. Most of the Candida species displayed over 50.0% antifungal resistance (Table 2). None of the Candida strains of all the species was inhibited by any of the single antifungal cream (Table 3).

Table 4. shows the results obtained when 40 selected Candida strains that were totally resistant to five antifungal drugs (Gyno-tiocosid [Ticonazole], Medcan [Fluconazole], Primpex [Sulphamethoxazole], Ketoconazole [Ketoconazole] and Fesovin [Friseofulvin]) were screened for phenotypic susceptibility, as combined antifungal drugs, as well as, six combined antifungal creams (Whitfield's ointment, Dakatrin [Salicylic acid and Benzoic acid), Dakatrin [Miconazole nitrate], Nizoral [Ketoconazole], Mycoten [Clotrimazole], Funcbact–A [(Clotrimazole, Betamethasone, Neomycin sulphate, and Clobetasol propionate] and Lamisil [terbinafine hydrochloride]). 100% resistance recorded during single antifungal drug and cream susceptibility testing but 37.5% and 27.5% resistance rates respectively were recorded in the combined antifungal drug and cream susceptibility testing (Figure 1). Nine strains of the oral Candida species were minimally inhibited (11.0-19.0 mm in diameter); 13 strains were moderately inhibited (21.0-29.0 mm in diameter), while three strains were highly inhibited (≥30.0 mm in diameter) by five combined antifungal drugs. Eight strains of the oral Candida species were minimally inhibited (11.0-19.0 mm in diameter); 18 strains were moderately inhibited (21.0-29.0 mm in diameter), while two strains were highly inhibited (≥30.0 mm in diameter) by the six combined antifungal creams (Table 4).

DISCUSSION

Invasive infections by opportunistic Candida species, and incidence of more virulent clinically important Candida species, which represent infectious agents with higher risk for mortality (due to a variety of virulence factors that make them capable of infectivity, even, in otherwise intact immune system), has increased in recent decades.[8, 20, 39, 40] These occur especially in people with critical underlying co-morbidities.[40, 41] There have also been large numbers of cases that reported co-infection of mycoses with viruses, fungi, and bacteria, some of which originate from oral cavity.[42] Antifungal profiles of Candida strains from CoviD-19 positive subjects and patients were not determined in this study, due to gross lack of biosafety for research purposes, and present biosecurity measures in handling isolations of oral fungal isolates from CoviD-19 patients, Notwithstanding, very high MAR rates [C. albicans, C.

glabrata, C. pseudotropicalis (41.7-100%), and C. tropicalis (50.0-100%)] were recorded for non-CoviD-19 clinical Candida species implicated in human oral candidasis.

Antifungal combination regimens could achieve more rapid antifungal effects, prevent emergence of antifungal resistance, and possibly allow reduction in the dosages of individual antifungal agents.[43-46] But implication of obtained results in this study is that none of commonly-available test antifungal drugs and creams could inhibit at least 50% of just 129 clinical Candida strains of human origin. Relentless increase of invasive fungal infections and poor outcomes associated with available antifungal agents, and sometimes with non-effective actions, has therefore, prompted therapeutic strategies, through combination antifungal therapy, as there seems to be several potential advantages in antifungal combinations, in addition to widening spectrum and potency of drug activity. In this study, instead of 100% resistance rates recorded for the single antifungal drugs and creams susceptibility testing, 37.5% and 27.5% resistance rates were respectively recorded for most-resisted five antifungal drugs and six antifungal creams, combined for susceptibility testing.

Oral candidasis can be treated with antifungals belonging to different classes of drugs that target different cellular processes [47] but it has been reported that after administration, intra-oral concentrations of antifungal drugs tend to be sub-therapeutic and transient, due to diluent effect of saliva and cleansing effect of the oral musculature. Hence, intra-orally, *Candida* may undergo just a brief exposure to antifungal drugs,[26] indicating further reduced potencies of oral antifungals. Furthermore, likelihood of oropharyngeal candidasis development in CoviD-19 patients, with a list of attributable risk factors for oral infections has been investigated and reported.[15] It is thus, of further great concern that in this study, none of the classes of antifungal agents, as single drugs or creams had significant maximal *in vitro* inhibitory effects on most of the *Candida* species, and it was not even, until five and six antifungal drugs and creams were combined in equal proportions that inhibitory effects were observed in most of the antifungals. However, it must be borne in mind that, considering the potential life-threatening drug interactions and adverse side-effects like, kidney or liver toxicity associated with antifungal therapies, it would be clinically hazardous to administer five oral antifungals in treatment of oral candidasis because of such multiple resistance.

There has always been a growing universal concern regarding counterfeit medications, particularly, because counterfeit antimicrobial drugs are considered as threat to overall public health, with many devastating consequences for patients, like, increased mortality and morbidity, long duration of antimicrobial therapy, treatments failures, emergence of drug resistance, etc.[47] Apart from concerns on fake, adulterated or sub-standard antimicrobial drugs,[33, 48] another factor of special concern is the issue of inconsistencies in bacteriostatic and/or bactericidal activities of antifungals produced as different batches.[49-51] These factors additionally contribute to relatively high rates of recorded antifungal resistance in several studies, including the present study. In countries with standard drug regulations, less-quality or adulterated drugs cannot be expressly imported, while fake, adulterated and sub-standard drug batches are rejected and/or recalled from the markets but this is not usually the case in Nigeria's situation. So, it is very difficult to regulate drug batches that do not meet standard quality criteria, and of course, not surprising that significant resistance rates were recorded in this study, even, for some combined antifungals, which is of severe clinical disadvantage in combined antifungal therapy.

In order to curb the global menace of antifungal resistance, there are suggestions of development of new antifungal agents, and with novel modes of actions.[52] But, even, if newly developed antifungal drugs are effective and safe for treatments of topical and invasive fungal infections, and also active against fungal strains that are resistant to current antifungal drugs or if there is upgrading of antifungal armamentarium, through improvement in existing antifungals and/or novel antifungal strategies are devisied;[7] such therapies are very unlikely to be commonly-unavailable in developing countries. This is due to high costs and deficient healthcare policies, being the reason that most antimicrobials in such countries are generic, and not original brands / trademarks, and their pharmacological effects are mostly not exactly the same as those of their brand-name counterparts. There had also been reports of manufacturing of some poor-quality drugs (which cannot be sold or prescribed in countries of manufacture), particularly prepared for developing countries, as some unscrupulous business merchants, specifically request for production of poor-quality medications, which they import into developing countries like Nigeria.[51] Another peculiar missing link about antimicrobial resistance in Nigeria, and possibly few other developing countries, is deliberate change of expiration dates labels on clinical medications.[53]

Invasive fungal infections have major impacts on human morbidity and mortality, which are responsible for almost over a million deaths yearly, and even, with increasing rates; since many fungal species cause infections, with mortality rates exceeding 50%.[54] Fungal co-infections and their impact on CoviD-19 patients are still understudied, [12, 52, 54] but it is of more recent safety concerns that severe CoviD-19 infection alone can cause damage to multiple organ systems.[54, 55] Thus, adequate antifungal dosing strategies are necessary, to ensure successful mycotic treatments,[15] following antifungal susceptibility testing, which is crucial for ensuring appropriate antifungal therapy for patients' optimal treatment options.[56] In consideration of overall challenges on antimicrobial resistance facing many developing countries, this study tried to apply a standardised, easily-reproducible, and clinically-applicable susceptibility testing method for *Candida* strains, for detection of antifungal resistance, through direct choice of most-potent antifungal agents.

In spite of mycotic co-morbidity, Africa has been quite fortunate to be less affected by the severity of recent emergence of CoviD-19 pandemic, but, this should serve as a stark reminder that, there is a need for continued medical innovation, to ensure availability of safe and effective antifungal drugs, for treatment of patients with serious invasive fungal infections.[13] The fact that fungal infections have been implicated as co-morbidities in CoviD-19 cases should attract much attention on antifungal therapy and resistance. Findings of this study also confirm some earlier submissions,[33, 51] particularly on low quality and substandard/counterfeit antimicrobial drugs in developing countries, which can lead to increased morbidity, mortality, as well as, emergence of antimicrobial resistance, especially by invasive and non-invasive *Candida* strains in the environment.[57] There must also be consideration for emergence of antifungal-resistant *Candida* strains displaying cross-resistance to other clinical antifungals,[58] which are of significant consequences for local and global public health. Mycotic infections have not been attracting as much attention as bacterial and viral infections in Nigeria; and for antibiotic susceptibility tests, antibiotic discs are commonly used, with inhibitory zones usually denoted as, +, ++, +++ and ++++, which are for interpretations of the increasing zones of inhibitions on agar plates. These interpreted values form basis for prescriptions of antibiotics; however, they do not translate to curative potencies by corresponding antibiotic drugs or

creams, as there can be significant differences in the susceptibility / resistance values between antibiotic-discs and corresponding antibiotic drugs.[59, 60]

Two major indications of CoviD-19 pandemic are that tremendous attention must be focused on fungal co-infections, and qualities of antifungals in the country. Direct antifungal susceptibility method, using in-country antifungal medications can therefore, ensure, not only appropriate guidance, in the choice of antifungal therapy, but also, knowledge of local epidemiology of antifungal resistance in the country. Most importantly, direct antifungal susceptibility method can expose lack of potent activities of fake antifungals, especially, as there are no general clinical and epidemiological surveillance systems in place, to determine prevalence of antifungal resistance, as well as, fake and substandard antimicrobials in the country. Such direct assay can firstly, enhance coordinated optimisation of antimicrobial interventions, referred to as, *Antimicrobial Stewardship*, which promotes selection of optimal antimicrobial drug regimen; and secondly, for assessing the consistency of different batches of antimicrobial drugs, particularly with regards to potency. Complete documented investigations into failure of drug batches that fail to meet expected specifications can also be carried out by the direct assay used in the current study; thereby, aiding in appropriate policies for poor quality antifungals. So, bodies like, National Agency for Foods and Drugs Administration and Control (NAFDAC), and the Federal Competition and Consumer Protection Commission (FCCPC) must put in place, novel regulations for antimicrobials and other medications produced or imported into Nigeria, to protect patients' safety and prevent antimicrobial (antifungal) resistance.

CONCLUSION AND RECOMMENDATIONS

Significant reduction in phenotypic resistance rates were recorded for combined antifungals, compared to single antifungals; indicating that combined antifungal therapy can enhance better treatments of mycotic infections. Direct-antifungal-susceptibility-drug-assay also confirmed lack of bacteriostatic and bactericidal potentials in most of the combined test antifungal drugs and creams. Adulterated, fake, substandard or expired antifungals, as well as, inconsistencies in bacteriostatic and/or bactericidal activities of antifungals produced as different batches, are common in developing countries, including Nigeria. By the findings of this study, it is therefore recommended, a need to constantly implement antimicrobial stewardship; through, standardised, easily-reproducible, and clinically-applicable, direct-antifungal-drug-assay, for detecting phenotypic susceptibility or resistance, which can translate to ideal laboratory-derived antifungal prescribing and therapy, especially because of co-morbidities like, CoviD-19 and fungal infections.

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Competing interests

Authors declare no competing interests.

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APPENDIX

Table 1: Antimycotic susceptibility / resistance patterns and profiles of oral *Candida* species to antifungal drugs

		Antifungal drugs											
	Candida species	**IT**	**FV**	**GT**	**MC**	**VC-50**	**GF**	**LC**	**GS-500**	**DF**	**KZ**	**PX**	**FD**
	C. albicans [42]												
	Total % Resistance →	61.9	95.2	73.8	85.7	38.1	47.6	95.2	71.4	64.3	83.3	100	100
Inhibition zones	1.0-9.0 [+]												
Inhibition zones	10.0-19.0 [++]	7.1	0.0	9.5	9.5	14.3	11.9	4.8	16.7	16.7	7.1	0.0	0.0
Inhibition zones	20.0-29.0 [+++]	23.8	4.8	16.7	4.8	23.8	21.4	0.0	7.1	16.7	9.5	0.0	0.0
Inhibition zones	30.0-39.0 [++++]	7.1	0.0	0.0	0.0	21.4	16.7	0.0	2.4	2.4	0.0	0.0	0.0
Inhibition zones	≥40.0 [+++++]	0.0	0.0	0.0	0.0	2.4	2.4	0.0	0.0	0.0	0.0	0.0	0.0
	C. glabrata [52]												
	Total % Resistance →	61.5	100	73.1	80.8	40.4	44.2	84.6	71.1	65. 3	98.1	92.3	94.2
Inhibition zones	1.0-9.0 [+]												
Inhibition zones	10.0-19.0 [++]	7.7	0.0	19.2	19.2	17.3	15.3	9.6	11.5	19.2	0.0	3.8	5.8
Inhibition zones	20.0-29.0 [+++]	23.1	0.0	7.7	0.0	26.9	32.7	1.9	15.4	13.4	1.9	0.0	0.0
Inhibition zones	30.0-39.0 [++++]	7.7	0.0	0.0	0.0	11.5	7.7	3.8	1.9	1.9	0.0	3.8	0.0
Inhibition zones	≥40.0 [+++++]	0.0	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C. pseudotropicalis [19]												
	Total % Resistance →	36.7	100	63.1	78.9	15.8	21.1	73.6	63.1	68.4	100	100	100
Inhibition zones	1.0-9.0 [+]												
Inhibition zones	10.0-19.0 [++]	21.1	0.0	21.1	5.3	10.5	36.8	15.8	10.5	31.6	0.0	0.0	0.0
Inhibition zones	20.0-29.0 [+++]	21.1	0.0	15.8	10.5	31.6	26.3	5.3	21.1	0.0	0.0	0.0	0.0
Inhibition zones	30.0-39.0 [++++]	21.1	0.0	0.0	5.3	42.1	15.8	5.3	5.3	0.0	0.0	0.0	0.0

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C. tropicalis [16]												
Total % Resistance →	62.5	93.8	75.0	75.0	37.5	31.3	81.3	56.3	87.5	93.8	100	93.8
Inhibition zones												
1.0-9.0 [+]												
10.0-19.0 [++]	6.2	6.2	12.5	18.8	6.2	12.5	6.2	12.5	12.5	6.2	0.0	6.2
20.0-29.0 [+++]	25.0	0.0	12.5	6.2	37.5	43.7	6.2	25.0	0.0	0.0	0.0	0.0
30.0-39.0 [++++]	6.2	0.0	0.0	0.0	18.8	12.5	6.2	6.2	0.0	0.0	0.0	0.0

Keys: IT = Itranox; GF = Gruffin; FV = Fesovin; LC = Lucon; GT = Gyno-Tiocosid; DF = Diflucan; MC = Medcan; KZ = Ketoconazole; VC-50 = Vulcan-50; PX = Primpex; GS-50 = Grisovid; FD = Flucamed

Table 2: Multiple antimycotic resistance profiles of *Candida* species to antifungal drugs

Candida species	% multiple antimycotic resistance profiles
C. albicans [42]	41.7 [2 (4.8%)], 50.0 [3 (7.1%)], 58.3 [6 (14.3%)], 66.7 [6 (14.3%)], 75.0 [4 (9.5%)], 83.3 [8 (19.0%)], 91.7 [3 (7.1%)], 100 [10 (23.8%)]
C. glabrata [52]	41.7 [2 (3.8%)], 50.0 [4 (7.7%)], 58.3 [4 (7.7%)], 66.7 [11 (21.2%)], 75.0 [11 (21.2%)], 83.3 [7 (13.5%)], 91.7 [5 (9.6%)], 100 [8 (15.4%)]
C. pseudotropicalis [19]	41.7 [1 (5.2%)], 50.0 [1 (5.2%)], 58.3 [7 (36.8%)], 66.7 [1 (5.2%)], 75.0 [4 (21.0%)], 83.3 [1 (5.2%)], 91.7 [2 (10.5%)], 100 [2 (10.5%)]
C. tropicalis [16]	50.0 [2 (12.5%)], 58.3 [1 (6.2%)], 66.7 [4 (25.0%)], 75.0 [3 (18.8%)], 83.3 [2 (12.5%)], 91.7 [3 (18.8%)], 100 [1 (6.2%)]

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Table 3: Trends of antimycotic susceptibility / resistance patterns of *Candida albicans* to topical antifungal creams

	Antifungal creams												
Overall resistance	**MY**	**SN**	**TY**	**LS**	**CC**	**FA**	**NZ**	**FG**	**KC**	**FC**	**WO**	**DR**	**%MAR**
Total % resistance	100	100	100	100	100	100	100	100	100	100	100	100	100
Total % resistance	100	100	100	100	100	100	100	100	100	100	100	100	100
Total % resistance	100	100	100	100	100	100	100	100	100	100	100	100	100
Total % resistance	100	100	100	100	100	100	100	100	100	100	100	100	100

Keys: *MY = Mycoten cream; NZ = Nizoral cream; SN = Skineal cream; FG = Fungusol cream; TY = Troyd cream; KC = Ketofung cream; LS = Lamisil cream; WO = Whitefield's ointment; CC = Care-cream; DR = Daktarin cream; FA = Funbact A cream; FG = Fungur cream*

Table 4: Trends of antimycotic susceptibility / resistance patterns of *Candida* species to combined antifungal drugs and topical creams

***Candida* species**	**Single drugs (5)**	**5 combined drugs**	**Single creams (12)**	**6 combined creams**
C. albicans [9]	R [9 (100%)]	R [3 (33.3%)] S 20.0, 22.0, 23.0, 25.0, 28.0, **31.0**	R [9 (100%)]	R [3 (33.3%)] S 19.0, 23.0, 25.0, 27.0, 28.0, **30.0**
C. glabrata [19]	R [19 (100%)]	R [6 (31.6%)] S 15.0, 16.0, 17.0, 17.0, 17.0, 18.0, 18.0, 19.0, 20.0, 24.0, 27.0, 28.0, 28.0	R [19 (100%)]	R [4 (21.0%)] S 13.0, 16.0, 17.0, 17.0, 18.0, 20.0, 22.0, 23.0, 26.0, 26.0, 27.0, 27.0, 28.0, 29.0
C. pseudotropicalis [6]	R [6 (100%)]	R [3 (50.0%)] S 13.0, 23.0, 28.0	R [6 (100%)]	R [2 (33.3%)] S 15.0, 19.0, 20.0, 29.0
C. tropicalis [6]	R [6 (100%)]	R [3 (50.0%)] S 28.0, **30.0**, **32.0**	R [6 (100%)]	R [2 (33.3%)] S 22.0, 25.0, 27.0, **33.0**
Overall % susceptibility	0.0%	62.5%	0.0%	72.5%

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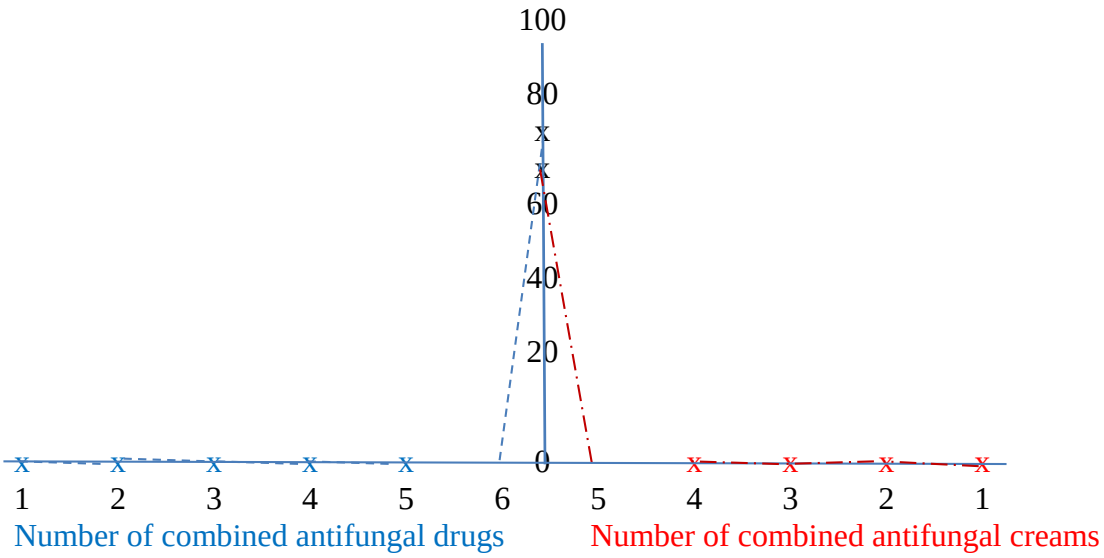

Figure 1: Susceptibility rates of combined antifungal drugs and creams

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