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Antimicrobial Activity of Local Isolated *Streptomyces* against *Candida* Species

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Abstract

Introduction: The incidence of fungal infections which is caused by an increase in antimicrobial resistance, contributes to morbidity and mortality. *Candida* species are normally a virulent in healthy people but could be disseminated to deep tissue and cause fatal disease in unhealthy people. The *Streptococcus* is a heterogeneous group of Gram-positive bacteria which has broad significance in medicine and industry.

Aim: To investigate the antimicrobial activity of the *Streptomyces* isolated from Sudanese soil against some of the *Candida* species (C. species) which included: *C. albicans, C. tropicalis, C. glabrata* and *C. krusei*.

Methods: A total of 50 clinical samples were collected from individuals consisted of 10 (20%) high vaginal swabs, 20 (40%) urine and 20 (40%) sputum. Identification of the *Candida* species was performed using growth characteristics, the germ tube test and the CHROMagar *Candida*.

Results: Results show that 27 out of 50 samples (54%) were *C. albicans* whereas 23 (46%) were non *C. albicans*. Non albicans *Candida* included: *C. tropicalis* 12 (24%), *C. krusei* 6 (12%), *C. glabrata* 5 (10%). Four of the *Streptomyces* isolates succeeded to inhibit the growth of most of the tested *Candida* species. The highest activities were shown by isolate SB4 against *C. albicans* (23 mm diameters), *C. tropicalis* (21 mm), *C. glabrata* (21 mm) and 19 mm against *C. krusei*. The study indicated that *C. albicans* was the most frequently isolated species (54%) followed by (46%) non albicans *Candida* isolates as confirmed by CHROMagar *Candida.* SB4 was found to be very effective in inhibition of the growth of certain C. species such as *C. albicans, C. tropicalis, C. glabrata* and *C. krusei*.

Conclusions: The isolation of *Streptomyces* from diverse geographical locations in Sudan may present significant capacity for antifungal agents production. Therefore a long term screening programme is recommended to discover a novel antibiotic.

Keywords: *Antifungal activity; Streptomyces; CHROMagar Candida; Aspergillus*

Introduction

Until the end of the 19th century fungi have been considered to be plants, though they are heterotrophic eukaryotic organisms that are more closely related to human's than bacteria at cellular level. Today, fungi are grouped in their own taxonomic kingdom, which is estimated to consist of more than one million species. More than 100,000 species have been described, Only a very small fraction of approximately 400 species have been identified as human pathogens but the numbers are rising [1].

Most species grow as multicellular filaments called hyphae-forming mycelium such as molds; some species also grow as single cells like yeasts.

Fungi can cause significant number of human diseases represented by pathogens such as *Trichophyton* species, *Epidermophyton* species, *Histoplasma* species, *Blastomyces* species, *Sporothrix* species, *Coccidioides* species, and *Paracoccidioides* species, capable of infecting healthy people, or opportunistic invaders such as *Aspergillus* species, *Candida* species, *Cryptococcus* species, *Fusarium* species, and *Rhizopus* species, which are normally a virulent in healthy people but could be disseminated to deep tissue and cause fatal disease in unhealthy people [2]. The morbidity and mortality rates caused by fungal species such as *Candida, Aspergillus, Fusarium*, and *Trichosporum* are relatively higher [3].

The incidence of fungal infections has increased significantly, so contributing to morbidity and mortality. This is caused by an increase in antimicrobial resistance and the restricted number of antifungal drugs, which retain many side effects. *Candida* species are major human fungal pathogens that cause both mucosal and deep tissue infections [4].

Streptococcus species are bacteria belonging to the Firmicutes phylum under the order of Lactobacillales and the family of Streptococcaceae [5]. *Streptococcus* species are found mostly in the oral cavity and nasopharynx and form a significant portion of the normal microbiota of humans and animals [6-8]. The genus *Streptococcus* is a perplexing group causing an extensive variety of illnesses such as: rheumatic fever, impetigo, pharyngitis, laryngitis, lethal stun disorder, scarlet fever, and endocarditis. In this study, 10 isolates of *Streptomyces* were recovered from soil samples collected from different locations in Khartoum city, Sudan. The objective of the study was to investigate the activity of the *Streptomyces* isolates against *C. albicans, C. tropicalis, C. glabrata* and *C. krusei*.

Materials and Methods

A total of 50 clinical samples were collected during June 2015 to October 2016. Samples collected from individuals consisted of 10 (20%) high vaginal swabs, 20 (40%) urine samples and 20 (40%) sputum samples. Present study was carried out in the Department of Microbiology, International University of Africa.

Isolation of *Streptomyces* Species

A total of 10 soil samples were collected in sterile plastic bags from different locations in Khartoum Sudan. These locations include: (Shambat, Tuti, El kadaro). The samples were then transferred to labeled screw-capped bottles after air- drying for a week. For oven drying one gram of calcium carbonate was added to each 10 gram of the air-dried soil sample, the mixture was then dried in an oven a t 50°C for 6 - 10 hours. For the isolation of *Streptomyces* one gram of the dried mixture was suspended in 10 ml of sterilized distilled water. Serial dilution was made by the addition of nine ml of sterile distilled water to one ml of the soil suspension in test tubes. Dilution process was continued until a 10^{-4} dilution was obtained. One ml from this dilution was transferred and plated on Starch Casein KNO_3 Agar ($SCKNO_3A$) medium. The $SCKNO_3$ agar medium was prepared by dissolving: starch, 10g; KNO_3, 2.0g; KH_2PO_4, 2.0g; NaCl, 2.0; casein, 0.3; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 2.0; $FeSO_4.7H_2O$, 0.001g and 18g agar in one liter of distilled water. The medium was then divided equally into four 500 ml Erlenmeyer flasks and was then plugged tightly with cotton and autoclaved for 15 minutes at 121°C and 15 1bs/square inch.

The inoculated petri-dishes were incubated at 28°C for 5 - 7 days. Colonies, with *Streptomyces* morphological characteristics that appeared in the incubated plates, were repeatedly sub-cultured for purification.

In Vitro Screening of *Streptomyces* Candida spp.

In vitro antifungal activity of the *Streptomyces* isolate was tested against *Candida* species, for screening test, the fungal cultures which were maintained on SDA slants were spreaded on the SDA plates. The *Streptomyces*'s growth which were incubated for 7 days at 28°C were made with a sterile cork borer and placed on SDA plates seeded with the fungal culture. The plates were then incubated at 28°C and observed for antibiosis after 24h. The inhibition zone made by the *Streptomyces* isolate against *Candida* species indicated the production of an antifungal.

Test Organisms

Four species of *Candida* (*C. albicans, C. tropicalis, C. glabrata, C. krusei*) were used to determine the antimicrobial activity of the isolated *Streptomyces* strains. The above mentioned *Candida* were cultured in Sabouraund Dextrose Agar (SDA) (Difco) at 28 ± 0.1°C for 48 hour. The active *Streptomyces* isolates (having inhibition zone diameters of more than 10 mm) were selected.

Identification of the *Candida* isolates

All isolated *Candida* were identified to the species level using the germ tube test and CHROMagar *Candida*.

Germ Tube Test

Using a sterile inoculating loop, a colony of yeasts was transferred into the serum in the labeled test tubes. The colony was emulsified in the serum. The set up was incubated at 37°C for 3 hours. Using a Pasteur pipette, a drop of the suspension taken from the test tube after incubation was placed on a clean dry slide. The suspension was covered with a clean cover glass. The slide was examined under a microscope for germ tubes on the yeasts using the 10X objective lens. A germ tube is a tube-like outgrowth that arises from the yeast cell. The 40X objective was used to confirm the presence or absence of germ tubes. When yeasts with germ tubes were seen, the culture was reported as *Candida albicans* isolated. When the yeast cells do not show germ tubes, the culture was reported as yeasts isolated.

CHROMagar *Candida*

Chromogenic media was prepared according to manufacture instruction and the organism inoculated in the media, then incubated at 37°C for 48 hours. After that the growth of C. spp observed by the change in the colour of the colonies according to the pigment, as a result of reaction between chromogenic substrate and enzymes that secreted by different C. spp, allowing organisms to be identified to the species level by their color and colony characteristics. CHROM agar *Candida* has been shown to allow differentiation of *Candida* yeast by color and morphology. The result was as the following: the product identifies *C. albicans* by growth as light to medium green and wet colonies, *C. tropicalis* by growth as steel blue and wet colonies, *C. glabrata* dark pink and wet colonies, *C. krusei* light pink and dry colonies, and other *Candida* spp. give white color [9].

Growth at 37°C and 45°C

All yeast isolate were tested for growth at 37°C and 45°C on SDA plates held for 3 days. A visible growth was regarded as positive in case of weak growth, the test was repeated.

Results and Discussion

Results

Isolation of *Streptomyces* Species of *Streptomyces* were reported to occur predominantly in soil. In the present study 10 isolates were recovered from samples of soil collected from different locations in Khartoum Sudan. Each isolate was given a number prefixed with the abbreviation (S), three isolates were recovered from the Bank of the Blue Nile (opposite to the University of Khartoum), two isolates from Shambat, one isolates from El-gadaro, and four isolates from Tuti Island. All the isolates were found to be *Streptomyces* depending on their distinct morphological and microscopical characteristics figure 1. Isolation was done on Starch Casein Potassium Nitrate Agar which is a semi- selective medium for *Streptomyces* [10]. Moreover, isolation was enhanced by the addition of Calcium Carbonate to the soil samples (to reduce the number of fungal spores) and heating at 40°C for 6 - 10 hours in order to decrease the number of non- filamentous bacteria [11].

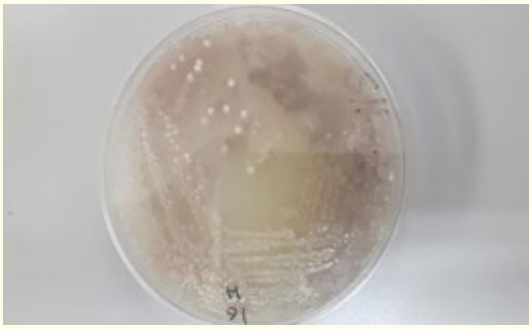

Figure 1: *Morphological features of different Streptomyces isolates grown on Starch Casein Agar.*

Characterization of *Streptomyces* Isolates

Characterization of the obtained *Streptomyces* isolates was carried out according to the directions given by the International *Streptomyces* project [11,12] as follows:

Cultural Characteristics

After three days of incubation all of the isolate colonies were opaque and dry. The colors observed were: white (2 isolate); black with white (one isolate); red (one isolate); gray (1 isolate); White with gray margin (2 isolate); and red with white (3 isolate). The cultural characteristics were confirmed by the growth on Starch Casein Agar medium. The results obtained were in agreement with the description of Felicitas and Hans [11] and Lacey [13]. Based on the results obtained all the isolates were considered as typical Streptomycetes.

Identification of *Candida* spp.

Candida was identified depending on the morphological features on culture medium and germ tube formation. The identity of non-albicans C. spp. was confirmed by CHROMagar *Candida* (focusbiotech.com.my). Four species were identified, *C. albicans, C. glabrata, C. tropicalis* and *C. krusei*, they were identified as follows:

Cultural Characteristics

The morphology of *Candida* species colonies on Sabouraud dextrose agar were white to cream, round, curved, soft and smooth to wrinkled with a characteristic yeast odor, it was grew rapidly and matured in 3 days. These results are agreed with Larone [14]; Bhavan., *et al* [15].

Germ Tube Formation Test

The germ tubes were formed within two hours of incubation and this is a unique diagnosis characteristic of *C. albicans* differentiates it from other fungi table 1. Other yeasts generally do not form germ tubes within this 3 hour timeframe, (neither *C. glabrata* nor *C. tropicalis* that germ tubes was diagnostic and pathogenic character in the same time, The results of this study all *Candida albicans* were positive and were agreement with that in Sheppared., *et al*. [16], they mentioned that "All *C. albicans* strains were germ tubes test positive when tested directly from the colony, and all non-albicans species were germ tubes test negative when tested directly from the colony.

Growth on CHROMagar *Candida*

Nearly all isolates of *Candida* species tested gave colonies with colors ranging from white through pink, pinkish purple, blue and purple after 48 hours of incubation on CHROMagar *Candida* at 37°C (Table 2), and (Figure 2). Of the 50 isolates 27 yielded several shades of green colonies after 48 hours of incubation in CHROMagar *Candida*. They were identified as *C. albicans*. 12 isolates developed a distinctive dark blue or blue-gray color after 48h of incubation on CHROMagar and were identified as *C. tropicalis*. 6 isolates developed a pink, fuzzy color

and were identified as *C. krusei*. And 4 isolates developed a mauve-brown color and were identified as *C. glabrata*. Nearly all isolates of *Candida* species tested gave colonies with colors described as ranging from white, through pink, pinkish purple, and purple after 48h of incubation on CHROMagar at 37°C. The observed morphological characteristics were compared with those in the Dalmau morphology identification chart [17].

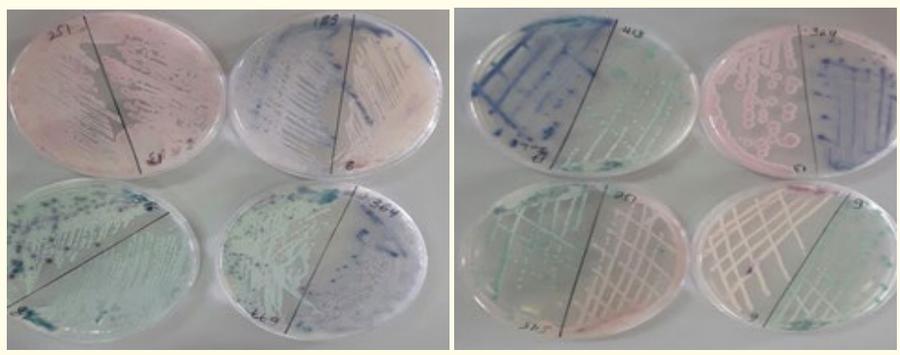

Figure 2: *Different Candida species on CHROMagar Candida after incubation for 48 hours.*

C. species	Number of isolates	*C. albicans*	*C. krusei*	*C. glabrata*	*C. tropicals*
C. species	20 (40)	10 (50)	3 (15)	1 (5)	6 (30)
C. species	20 (40)	12 (60)	2 (10)	3 (15)	3 (15)
C. species	10 (20)	5 (50)	1 (10)	1 (10)	3 (30)
C. species	50	27	6	5	12
C. species	%	54%	12%	10%	24%

Table 2: *Distribution of Candida spp. among various samples.*

Antifungal Activity off *Streptomyces*

Streptomyces isolates were screened for their abilities to inhibit the growth of the isolated *Candida* species. Screening was performed by agar well method and the diameters of growth inhibition zones were measured in millimetres for each of the *Streptomyces* isolates; the results are shown in (Table 3-5).

Isolate No.	C. species	SA4	SB4	SC4	SD3	SD5
1	*C. albicans*	11	10	14	11	13
2	*C. tropicalis*	15	13	11	14	12
3	*C. krusei*	13	19	11	10	11
4	*C. albicans*	10	12	13	14	10
5	*C. albicans*	17	14	12	15	14
6	*C. glabrat*	10	17	11	18	10
7	*C. albicans*	16	10	10	16	11
8	*C. glabrata*	11	19	10	12	14
9	*C. albicans*	18	10	11	11	13
10	*C. albicans*	10	15	12	14	11
11	*C. albicans*	13	11	12	18	12
12	*C. albicans*	19	10	10	11	12
13	*C. krusei*	13	12	11	13	12
14	*C. albicans*	17	11	13	17	13
15	*C. glabrata*	15	21	14	14	12
16	*C. tropicalis*	15	22	12	11	12
17	*C. albicans*	18	12	11	10	15
18	*C. albicans*	10	13	13	14	11
19	*C. albicans*	17	10	14	13	14
20	*C. tropicalis*	11	12	10	12	11

Table 3: *Diameter of growth inhibition zone in (mm) shown by Streptomyces isolates against Candida species isolated from Urine Samples.*

Isolate No.	C. species	SA4	SB4	SC4	SD3	SD5
251	*C. krusei*	15	10	10	11	10
109	*C. tropicalis*	10	10	10	13	10
413	*C. albicans*	10	23	10	17	18
572	*C. albicans*	10	19	10	10	10
659	*C. albicans*	18	10	10	12	13
71	*C. albicans*	10	10	10	10	10
347	*C. tropicalis*	10	11	10	16	18
280	*C. albicans*	16	10	10	10	16
689	*C. albicans*	10	14	10	17	10
675	*C. krusei*	18	10	10	18	19
545	*C. albicans*	16	13	10	10	10
370	*C. glabrata*	10	10	10	10	10
577	*C. krusei*	10	12	10	17	15
227	*C. albicans*	10	15	10	10	10
315	*C. albicans*	17	11	11	14	12
364	*C. tropicalis*	12	12	13	12	15
314	*C. tropicalis*	12	21	14	12	11
677	*C. albicans*	14	10	12	17	00
74	*C. tropicalis*	16	18	11	13	13
437	*C. tropicalis*	17	16	10	15	13

Table 4: *Diameter of growth inhibition zone in (mm) shown by Streptomyces isolates against Candida species isolated from Urine Samples.*

Isolate No. isolate	*C. species*	SA4	SB4	SC4	SD3	SD5
420	*C. albicans*	10	12	11	10	12
90	*C. tropicalis*	15	11	11	12	13
421	*C. albicans*	16	13	10	16	10
189	*C. tropicalis*	10	11	12	14	12
17	*C. tropicalis*	11	10	10	12	15
63	*C. glabrata*	12	12	13	10	11
117	*C. albicans*	18	11	11	14	13
432	*C. albicans*	15	10	10	12	11
172	*C. krusei*	10	12	14	11	14
528	*C. albicans*	10	11	10	16	12

Table 5: *Diameter of growth inhibition zone in (mm) shown by Streptomyces isolates against Candida species isolated from vaginal swab.*

Discussion

Streptomyces is the largest antibiotic producing genus in the microbial world discovered so far. In this study, soils were specifically collected from different locations in Sudan. Most of the soil samples collected were from different agricultural locations. In present study, 10

isolates were obtained. Five of them produced inhibitory substances of certain *Candida* species, these included: *C. albicans, C. tropicalis, C. glabrata* and *C. krusei*.

Also a total of 50 clinical samples were collected during June 2015 to October 2016. Samples collected from individuals consisted of 10 (20%) high vaginal swabs, 20 (40%) urine samples and 20 (40%) sputum samples. The samples were cultured on sabouraud dextrose agar. Identification of the *Candida* species was performed using growth characteristics, the germ tube test and the CHROMagar *Candida.* Antifungal drug susceptibility to Fluconazole, itraconazole was performed by this study.

All the *C. albicans* were positive for germ tube test. They all showed distinct growth at 37°C and 45°C temperatures. Non albicans *Candida* did not form any germ tube test, therefore CHROMagar was used to establish their identity. 27 out of 50 samples (54%) were *Candida albicans* whereas 23 (46%) were non albicans as confirmed by CHROMagar *Candida*. Non albicans *Candida* included; *C. tropicalis* 12 (24%), *C. krusei* 6 (12%), *C. glabrata* 5 (10%). This finding was consistent with the findings of other workers who reported that the incidence of *C. albicans* was 61.3% [18], 49.3% [19]. However, Babin *et al*. [20] found that *C. tropicalis* was the most prevalent species accounted for 22.9% followed by *C. albicans* (35.5%). *C. tropicalis* (24%) was the second most common species reported in the present study. This finding was comparable with other workers, 26.4% [20] and 21% [21]. However, *C. glabrata* was reported as second most common species by 13.7% [22] and 11.9% [23]. *C. krusei* was reported 14% [24] and 10.78% [25]. The pathogenicity of *Candida* species is credited to certain destructiveness variables, such as the ability to evade host defences, adherence, biofilm formation (on host tissue and on restorative gadgets), furthermore, the generation of tissue-harming hydrolytic compounds for example, proteases, phospholipases [26].

The *Streptomyces* isolate succeeded to inhibit the growth of more of the tested *Candida* species. The highest activities were shown by isolate SB4 against *C. albicans* (23 mm diameters), *C. tropicalis* (21 mm), *C. glabrata* (21 mm) and 19 mm against *C. krusei*. It is also evident in table 3-5 that isolates SA4 and SD3 have shown moderate activities against *Candida* species with inhibition zone diameters ranging between 14 and 18 mm. The isolate SD5 have shown low inhibitory effect against *Candida* species with inhibition zones diameters. In the range of 11 - 14 mm. the isolates SC4 have shown weak inhibitory effect against more of the tested C. species with inhibition zone diameters ranging 10 mm.

Conclusions

The results of this study indicated that SB4 was found to be very effective inhibitor for the growth of certain *Candida* species, these included: *C. albicans, C. tropicalis, C. glabrata* and *C. krusei*. This study showed *C. albicans* was the most frequently isolated species (54%) followed by (46%) non albicans *Candida* isolates. Results have also demonstrated that the isolation of *Streptomyces* isolates from diverse geographical locations in Sudan may present significant capacity for antifungal agent's production. Therefore, a long term screening programme is recommend in order to discover a novel antibiotic. It is essential to increase the awareness of the public regarding *Candida* infection and this can be achieved through especially educational designed program.

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