Incidence, Virulence Potential and Genotypic Diversity of Fungi Associated with Ready-to-eat Street-vended Foods in Gaborone, Botswana

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Abstract

Fungi have recently come to the fore as important etiologic agents of infectious diseases, especially in immunocompromised patients. While the role of food as an avenue of spread of foodborne fungal disease remains unclear, the virulence potential and genetic diversity associated with street food fungi remains to be determined. Therefore, the present study sought to investigate the occurrence of fungi in various foods sold by ambulatory and stationary street vendors at three geographical areas of Gaborone (BBS Mall, Train Station and Bus Rank) from October 2018 to March 2019. From a total of 685 ready-to-eat street foods cultured, 480 (70.1%) tested positive for fungi. Statistical analyses revealed that the detection of different fungal species was neither influenced by the three sampling areas studied in Gaborone nor the six food commodities analysed ($p = 0.001$). Out of the 28 strains pooled thereafter, virulence studies by activities of protease and lipase as well as biofilm formation suggested virulence potential of various fungi such as *Candida albicans* and *Aspergillus fumigatus*. Assessment of genetic diversity of the pooled 28 isolates detected some genetic differentiation of the fungi studied herein especially *Candida albicans*, *Aspergillus fumigatus*, *Rhodotorula glutinis* and *Aspergillus niger*. The unprecedented levels of incidence of fungi in food, virulence potential and high genetic diversity warrant further investigations on source tracking of food-borne fungi and evaluation of the reasons of high genetic diversity to protect the vulnerable population consuming these ready-to-eat foods.

Keywords: Street-vended foods; fungi; virulence potential; protease; lipase; biofilm formation; genetic diversity.

Introduction

The World Health Organisation (WHO 1996:2) defines street-vended foods as foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption later without further processing or preparation. Street-vended foods are especially popular among urban dwellers in most developing countries (Mosupye and Van Holley 1999; Ohiokpehai 2003; Rane 2011). The popularity of these ready-to-eat foods has been attributed to their convenience and nutritional value, proximity to consumers as well as their affordability (Tinker 1997).

In Botswana, street-food vending is an important microeconomic activity and forms a significant component of the urban informal sector (Chicho-Matenge and Ongori 2013). However, pathogenic, and potentially pathogenic microorganisms have been isolated from street-vended foods (Murindamombe *et al*., 2004; Loeto *et al*., 2017) and food handlers (Loeto *et al*., 2007) in Botswana. Coupled with this, knowledge on food safety and quality among consumers in Botswana has been reported to be lacking (Kealesitse and Kabama 2012). Taken together, these factors place the consuming populace at risk of food-borne diseases.

It is important to note that previous studies on the microbiological analyses of street-vended foods in

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Botswana have mainly tended to focus on bacteria (Murindamombe *et al*., 2004; Loeto *et al*., 2016; Letsholo *et al*., 2009), and in a few cases where fungi were isolated (Mpuchane and Gashe 1996; Mpuchane and Gashe 1998; Matthews *et al*., 2016), they were not afforded the same prominence as bacteria. Nonetheless, fungi were also found to heavily contaminate street foods in some developing countries such as Nigeria (Nwachukwu *et al*., 2008), India (Das *et al*., 2010) and Ghana (Annan-Prah *et al*., 2011).

The presence of fungi in these ready-to-eat foods is of public health concern because HIV/AIDS is endemic in Botswana, with UNAIDS in 2016 estimating 350,000 people to be infected by AIDS, and an adult positivity rate of 22.2%.[1] Various studies conducted in Botswana have established fungi as opportunistic pathogens in both the healthy and individuals with underlying health conditions (Ansari *et al*., 2002; Bisson *et al*., 2013; Wale *et al*., 2016). They possess virulence factors such as proteases and lipases (Stehr *et al*., 2003) and can produce biofilms (Nunes *et al*., 2013), which are associated with antifungal resistance. In addition, proliferation in foods of fungal genera such as *Penicillium*, *Fusarium* and *Aspergillus* can result in the elaboration of mycotoxins resulting in associated symptoms of mycotoxicoses such as hepatic and nephritic damage, skin necrosis and extreme immunosuppression (Sweeney and Dobson 1998; Rundberget *et al*., 2004). The possible role of consumption of street-vended foods as an avenue of spread of opportunistic fungal infections, especially in the context of developing countries, remains poorly characterised.

Currently, there are no available data on the virulence and genotypic diversity of fungal isolates from ready-to-eat foods in Botswana. Considering the foregoing, the present study aims to analyse the virulence and genetic diversity of fungi associated with street vended foods.

Materials and Methods

Study location and sampling

This study was conducted in Gaborone and the food samples were collected from street vendors at Botswana Building Society (BBS) Mall (Latitude: -24.627747 | Longitude: 25.934311), Gaborone Train Station (Latitude: 24.663513 | Longitude: 25.906507); and Gaborone Bus Rank -24.658557 | 25.904669). Mycological analyses of the food samples were carried out at the Microbiology Laboratory at the Department of Biological Sciences, University of Botswana in Gaborone, Botswana. The study was conducted from October 2018 to March 2019.

Ethical Clearance

This research was conducted after ethical clearance from the Institutional Review Board, Office of Research and Development, University of Botswana. Written consent was received from street food vendors who participated in this study. We maintained confidentiality throughout the course of this study. This study proceeded after a research permit was obtained from the Ministry of Local Government (Research Permit No. CLG 14/14/3/1 II (58).

Sample collection

Samples of cooked food (starch, meat, and salads/vegetables) were bought and aseptically collected from street vendors at the three locations. The foods were collected at the point of sale and transported to the laboratory in a cooler box with ice packs for analysis within two hours of collection. A total of 685 food samples were collected from the three food vending sites consisting of mealie pap (n = 108), sorghum pap (n = 119), rice (n = 110), beef (n = 121), chicken (n = 109) and salads/vegetables (n = 118).

Isolation and identification of fungi

Ten (10) grams of each of the food samples was weighed aseptically into 90ml of peptone water in sterile

stomacher bags to homogenize, making a dilution ratio of 1:10. Further dilutions were made from the stock to obtain 10^{-3} and 10^{-6} and duplicates of each dilution were spread plated on sabouraud dextrose agar (Merck, Darmstadt, Germany) and potato dextrose agar (Merck) and incubated at 25^0C and observed for a period of 5 days. Sub-culturing was done on each media to obtain pure isolates.

Morphology of yeast cells and morphotaxonomic characters of filamentous fungi were studied under a light microscope. Slides of fungal cultures were prepared by gently lifting the mycelial mat with a sterile inoculation pin into a drop of lactophenol cotton blue on a slide, teased, covered with a slip, and observed under microscope. Different characteristic features of the isolated fungi such as availability of septa, branching and type of conidia were observed and used for their preliminary identification.

Identification of yeast cultures was done using morphological characters. These included simple staining of yeast cultures and observation of colonies under the microscope. Simple staining was carried out using aqueous Malachite Green for about one to three minutes. The yeasts were also tested for fermentation of glucose, assimilation of selected carbon and nitrogen sources. The filamentous fungi and yeasts were identified to the genus level using identification keys of Watanabe (2002). After identification of the isolates using conventional methods, 28 strains were pooled and analyzed for subsequent virulence and genotypic studies. The strains were selected based on such factors as cultural characteristics, geographical and food commodity source of isolate.

Virulence Studies

Lipolytic and proteolytic assays were performed according to modified protocols of Bentubo *et al*., (2014).

Lipolytic activity

The assay medium consisted of tryptone (Merck) 10.0g, NaCl 5.0, yeast extract (Merck) 5.0g, distilled water 1L (pH 7.0), 0.5% tributyrin (Sigma Aldrich, Missouri, USA), 1.5% agar (Merck), and homogenizing in a Waring blender (Waring Products Inc., Torrington, CT, USA) at set medium speed for 5min. The autoclaved agar plates were inoculated with each fungal strain and incubated at room temperature for a period of 3 days. The development of a clear zone is an indication of lipolytic activity, and its area is a measure of the extent of activity. The zones of inhibition were measured in millimetres to determine lipolytic activity for each strain.

Proteolylitic activity

An 8% solution of gelatin (Sigma) in water was sterilised separately and was added to the nutrient agar (Merck) at a ratio of 5ml per 100ml nutrient medium and adjusted to pH 6.0. After autoclaving, the gelatin agar plates were inoculated with inoculum of each strain at the center and incubated at 25ºC for 3 days. After incubation, zones of clearance due to complete degradation of the gelatin were measured in millimetres and the results recorded for each strain.

Biofilm formation

The biofilm formation assay was adapted from previously described methods (Melo *et al*., 2011). The strains initially cultured in SDA (Merck) at 25 for 48h were further subcultured into RPMI 1640 broth (Merck) and grown for 24 hours with shaking at 200 rpm at both 25 and 37°C. The cell cultures were harvested, washed twice with phosphate-buffered saline (PBS) (Oxoid, London, UK), and adjusted to a concentration of 107 cells/ml in RPMI 1640 medium. Biofilm formation was tested in sterile 96-well polystyrene flat-bottom plates. For the attachment phase, 100 µl of the cell suspension was transferred to each well of the plates and then incubated at 25 and 37°C for 1 h 30 min at 75 rpm. Unattached cells were removed, the wells were washed twice with 150 µl of PBS, and 150 µl of fresh RPMI 1640 medium was added. The

plates were incubated at both 25 and 37°C for 72 h, with shaking at 75 rpm to allow biofilm growth. A test medium without cells was added to the final column of each plate and used as a negative control.

After biofilm formation, each well was washed twice with 150 µl PBS, and the plate was dried for 20 min at 35°C. The washed biofilms were stained with 110 µl of 0.4% aqueous crystal violet (CV) solution for 45 min. Thereafter, the wells were washed three times with 200 µl of sterile water and de-stained with 200 µl of 95% ethanol. After 45 min, 100 µl of de-staining solution from each sample was transferred to a new plate and measured with a spectrophotometer plate reader (model 680; Bio-Rad) at 570 nm. The absorbance values (A570) of the negative controls (containing no cells) were subtracted from the values of the test wells to minimize background interference. For each strain, biofilm production capacity was reported as the arithmetic means ± standard deviations (SD) of the A570 values after 3 replicate tests. The ability of each strain to form biofilms was compared to *Cryptococcus neoformans* strain B3501 acquired from the American Type Culture Collection (Rockville, MD) and this strain was used in all experiments. This strain forms strong biofilms on polystyrene surfaces such as the 96-well polystyrene flat-bottom plates used in the present study.

Molecular Identification of the Fungal Isolates

Molecular characterisation was performed to identify the filamentous fungal isolates and yeasts that were associated with street food. First, DNA extraction from pure cultures was performed using a Zymo Fungal/Bacterial DNA miniprep kit (Zymo Research, USA), followed by amplification of the internal transcribed spacer (ITS) region using the primers; ITS1 (5′TCC GTA GGT GAA CCT GCG G 3′), which hybridizes at the end of 18S rDNA, and ITS4 (5′TCC TCC GCT TAT TGA TAT GC 3), which hybridizes at the beginning of 28S rDNA (Life Technologies, Barcelona, Spain). PCR amplification was performed in a 25 µL reaction mix containing 12.5 µL of 2X Master Mix (New England Biolabs, Ipswich, MA, USA), 20 ng total DNA, 0.5 µM of each reverse (ITS4) and forward primer (ITS1), and the mixture was made up to 25µl with sterile nuclease-free water.

PCR reactions were carried out in a DNA Thermal cycler (Biorad, Hercules, California, USA) with an initial 8 min denaturation step at 94 °C for 8min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1min and 72 °C for 2min then a final extension step at 72 °C for 12min. The PCR products were then purified and sequenced in both directions using an automated ABI 3500XL sequencer (Applied Biosystems, USA). The sequence data was aligned and edited using BioEdit 7.2.6.1 (BioEdit).[1] The sequence similarity searches were done using the BLAST algorithm that is available from the National Centre for Biotechnology Information (NCBI).[2] A phylogenetic tree was then constructed by using the neighbour-joining method, which produced a unique final tree under the principle of minimum evolution using the MEGA X program (Kumar *et al*., 2018). The evolutionary history was inferred using the unweighted pair group method with arithmetic mean (UPGMA) method, conducted in MEGA-X. The evolutionary distances were computed using the Jukes-Cantor model and bootstrapping values were calculated using 2000 replicates.

Statistical Analyses

The obtained data was analysed using the Statistical Package for Social Sciences (IBM SPSS 21.0, SPSS, Chicago, Illinois). Pearson's correlation coefficient was used to determine differences in means among street vended foods obtained in three geographical areas of Gaborone, Botswana. The level of significant difference between mean values was set at p-value <0.01.

1 http://www.mbio.ncsu.edu/BioEdit/bioedit.html, accessed 8 July 2021.
2 https://www.ncbi.nlm.nih.gov/, accessed 12 July 2021.

Results

Mycological analysis revealed the contamination by fungi in six ready-to-eat food commodities obtained from three food vending sites in Gaborone. The prevalence of fungi at the vending sites ranged from 55.3% in rice at BBS Mall to 77.8% in chicken at the Train station. However, the contamination rates of different dishes at the three street food vending sites were not statistically different ($p = 0.001$). The food commodities were associated with three filamentous fungi (moulds) genera (*Aspergillus*, *Fusarium* and *Aternaria*) and four yeast genera (*Rhodotorula*, *Debaryomyces*, *Yarrowia* and *Candida*), with *Aspergillus* being the most common mould genus and *Rhodotorula* was the most common yeast. Among the yeasts, *Candida* tended to be predominant in meats and salads/vegetables (Table 1).

Table 1: Incidence of fungi in street-vended foods at three vending sites in Gaborone, Botswana

Food sample	Vending site	No. sampled	No. (%) positive for fungi	Fungal genera isolated
Mealie pap	BBS Mall	34	24 (70.1)	*Aspergillus*, *Fusarium*, *Debaryomyces*
	Train Station	38	28 (73.7)	*Alternaria, Aspergillus*, *Rhodotorula*
	Bus Rank	36	26 (72.2)	*Aspergillus*, *Fusarium*, *Debaryomyces*
Sorghum pap	BBS Mall	40	30 (75.0)	*Alternaria, Aspergillus*, *Rhodotorula*
	Train Station	40	27 (67.5)	*Aspergillus*, *Fusarium*, *Rhodotorula*
	Bus Rank	39	26 (66.7)	*Aspergillus*, *Fusarium*, *Yarrowia*
Rice	BBS Mall	38	21 (55.3)	*Alternaria*, *Aspergillus*, *Rhodotorula*, *Yarrowia*
	Train Station	37	23 (62.1)	*Aspergillus*, *Fusarium*, *Debaryomyces*, *Rhodotorula*
	Bus Rank	35	26 (74.3)	*Aspergillus*, *Fusarium*, *Candida*
Beef	BBS Mall	42	31 (73.8)	*Alternaria*, *Aspergillus*, *Debaryomyces*
	Train Station	40	29 (72.5)	*Aspergillus*, *Rhodotorula*, *Candida*
	Bus Rank	39	28 (71.8)	*Fusarium*, *Alternaria*, *Candida*
Chicken	BBS Mall	38	25 (65.8)	*Aspergillus*, *Candida*, *Yarrowia*
	Train Station	36	28 (77.8)	*Aspergillus*, *Yarrowia*, *Candida*
	Bus Rank	35	26 (74.3)	*Aspergillus*, *Candida*, *Rhodotorula*
Salads/vegetables	BBS Mall	38	24 (63.2)	*Alternaria*, *Aspergillus*, *Yarrowia*, *Candida*
	Train Station	39	28 (72.0)	*Fusarium*, *Aspergillus*, *Candida*
	Bus Rank	41	30 (73.2)	*Aspergillus*, *Fusarium*, *Candida, Rhodotorula*

In this study, 28 isolates identified to the genus level using macroscopic, cultural, and microscopic features were pooled and their identity confirmed using ITS-PCR sequencing, and there was a general agreement between the two methods, but with sequencing showing higher discriminatory power. The strains could be identified to the species level using ITS-PCR sequencing, revealing the presence of pathogenic and potentially pathogenic fungi such as *Aspergillus fumigatus*, *A. flavus*, *Candida albicans* and *Candida glabrata*. The virulence and/or potential virulence of the strains was evaluated by measuring their proteolytic and lipolylitic activities. Primary fungal pathogens such as *Aspergillus fumigatus* and *Candida albicans* showed strong lipolytic and proteolytic activities, while those that are considered saprophytes (e.g., *Alternaria* sp.) showed weak or no activity (Table 2).

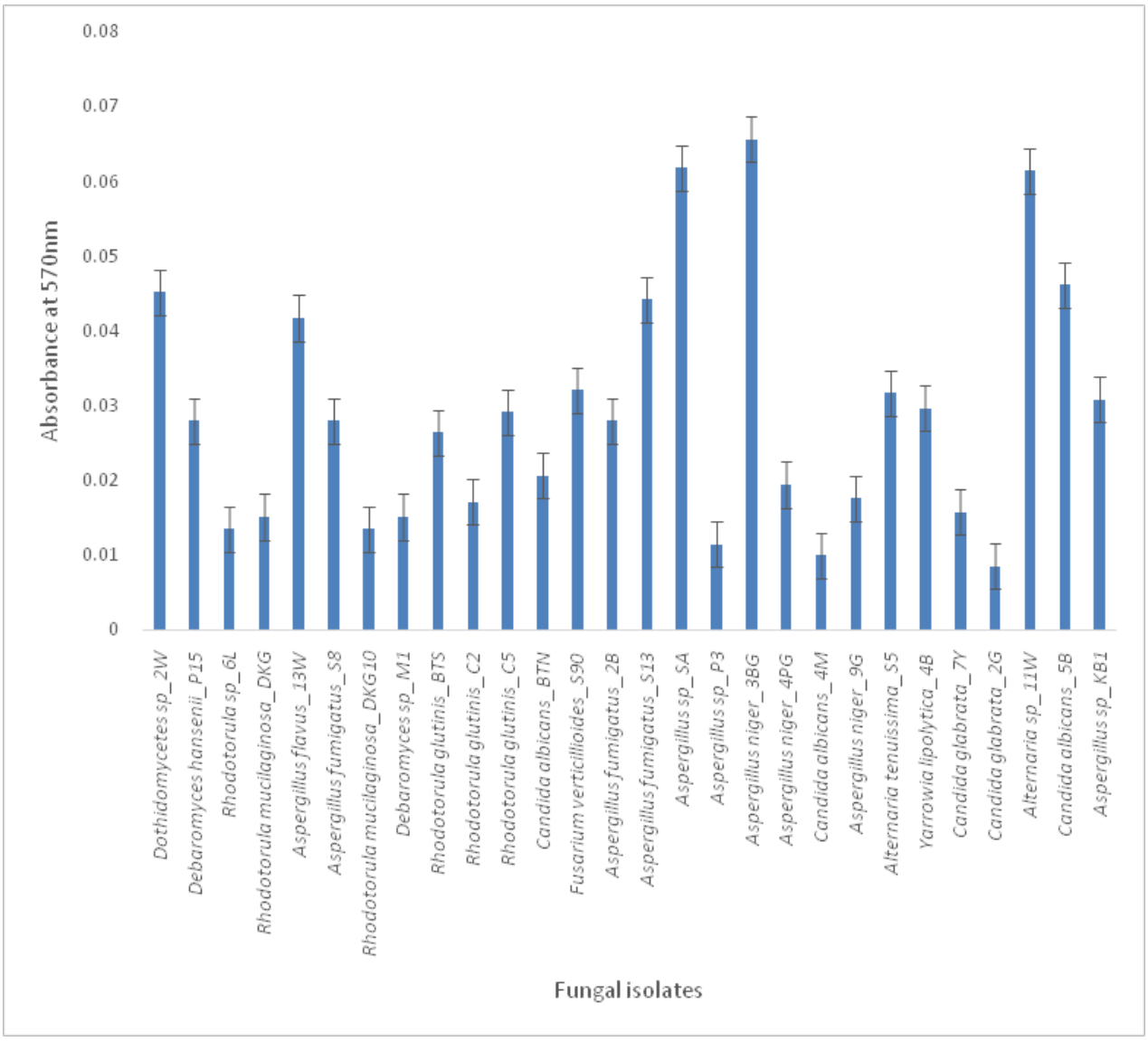
Table 2: ITS_PCR Sequence-identified Fungi from Street -vended Foods and their Virulence (Proteolytic and Lipolytic Activity)

Fungal isolate	Vending site (food source)	Protease[a]	Lipase[b]
Dothidiomycetes sp. _2W	BBS Mall (Mealie pap)	+	+
Aspergillus flavus _13W	Bus Rank (Mealie pap)	++	+
A. fumigatus _S8	Bus Rank (Sorghum pap)	+++	+++
F. verticilloides _S90	Train Station (Salads/vegetables)	++	++
A. fumigatus _2B	Bus Rank	+++	++
A. fumigatus _S13	BBS Mall (Rice)	+++	+++
A. oryzae _SA	Train Station (Beef)	+	+
Aspergillus sp. _P3	Train Station (Salads/vegetables)	-	-
Aspergillus sp. _KB1	Bus Rank (Rice)	-	+
A. niger _3BG	BBS Mall (Chicken)	-	+
A. niger _4PG	Train Station (Mealie pap)	+	+
A. niger _9G	Bus Rank (Sorghum pap)	+	+
*Alternaria temissima*_S5	BBS Mall (Salads/vegetables)	-	-
Alternaria sp._11W	Bus Rank (Beef)	+	-
Debaryomyces hansenii _P15	Train Station (Rice)	+	++
*Rhodotorula mucilaginosa*_DKG10	BBS Mall (Sorghum pap)	+	+
Rhodotorula sp. _6L	Train Station (Mealie pap)	+	+
Debaryomyces sp. _M1	Bus Rank (Mealie pap)	++	-
*R. mucilaginosa*_DKG	Bus Rank (Chicken)	+	+
R. glutinis _BTS	Bus Rank (Salads/vegetables)	-	-
R. glutinis _C2	BBS Mall (Rice)	-	+
R. glutinis _C5	Train Station (Beef)	-	+
Candida albicans _BTN	BBS Mall (Chicken)	++	++
C. albicans _4M	Bus Rank (Rice)	+++	+++
Yarrowia lipolytica _4B	Train Station (Sorghum pap)	+	++
C. glabrata _7Y	BBS Mall (Chicken)	++	++
C. glabrata _2G	Train Station (Salads/vegetables)	++	+
C. albicans _5B	Bus Rank (Beef)	+++	++

[a, b] +++, high activity; ++, moderate activity; +, low activity, -, no activity.

Furthermore, the virulence potential of the 28 strains was determined by their capacity to produce biofilms. The results indicated that the fungi studied herein had absorbance values at 570nm (A570), ranging from 0.0085 to 0.0656 (Figure 1). When moulds were compared to yeasts, the former had more biofilm production capability than yeasts. Among the moulds, *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus fumigatus* showed the greatest capacity to form biofilms. While *Candida albicans* showed the greatest biofilm production ability among the yeasts. The remaining species were considered low/weak biofilm producers.

Figure 1: Biofilm production of 28 fungal isolates from street-vended food. The bar graphs represent the means ± standard error of the absorbance values at 570 nm (A_{570}) obtained from 3 replicate tests for each sample.



Based on phylogenetic analysis as shown in Figures 2A and 2B, the 28 fungal strains showed interesting genetic relatedness amongst themselves and global isolates in NCBI. The global isolates were very useful in discerning street-vended foods studied herein. Both yeasts and filamentous fungi had very strong support (bootstrap = 99% and 98%, respectively) to claim close relatedness amongst respective specimens in the present study. However, interesting observations were made when individual fungal taxa were examined closely. For example, phylogenetic analysis placed *Candida albicans* and *Rhodotorula glutinis* strains in different sub-phyla. The same trend was observed with *Aspergilllus fumigatus*, *Aspergillus niger* and *Aspergillus flavus* strains. Taken altogether, these data suggested high genetic diversity among street food fungal isolates in Botswana that may be associated with food specificity and sampling location.

Figure 2A: The phylogenetic analysis of the ITS sequences of yeast isolates obtained in this study (indicated by asterisks) along with other similar selected sequences from NCBI database. The analysis was conducted using Neighbour-Joining method in MEGA-X.

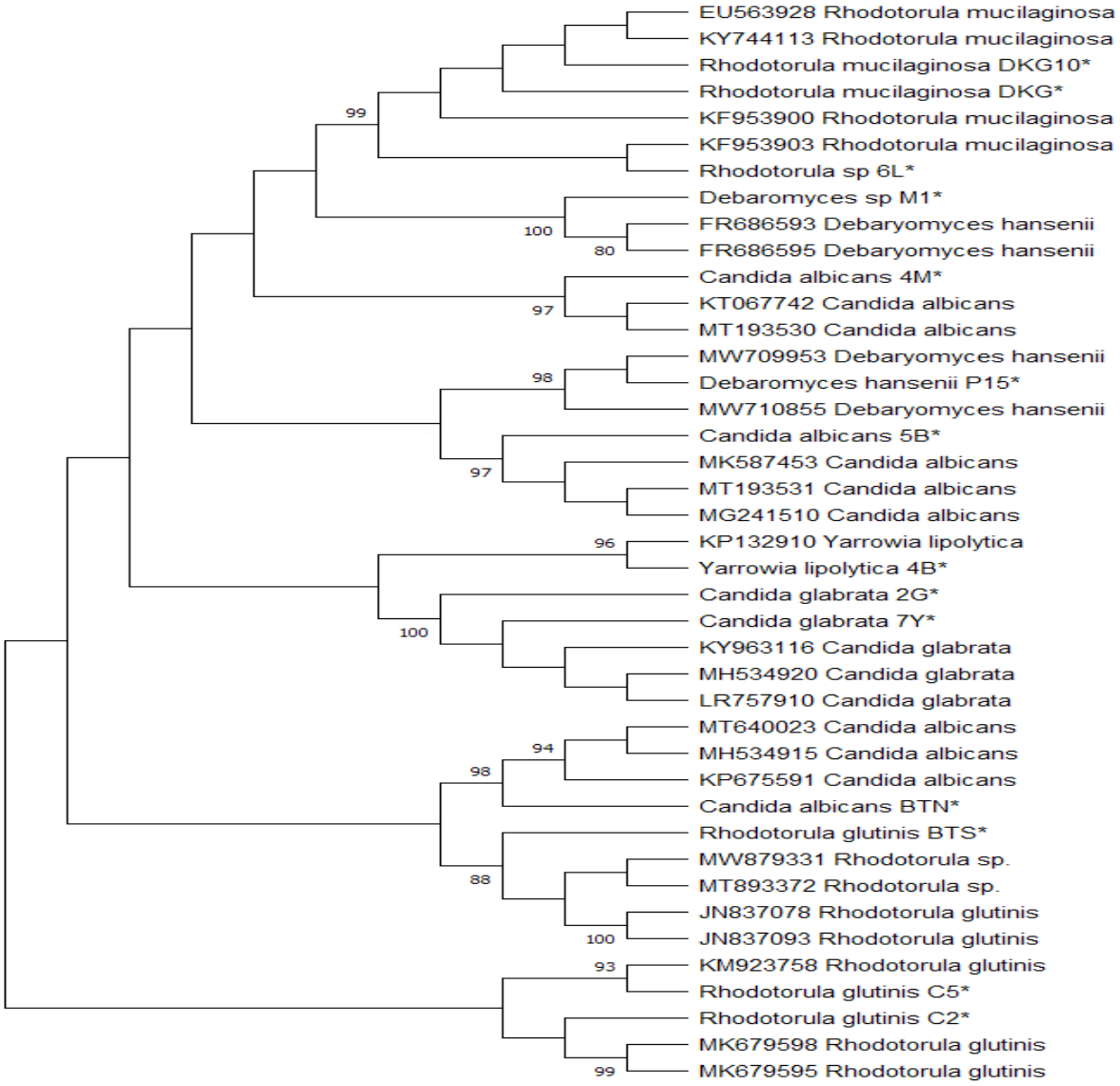
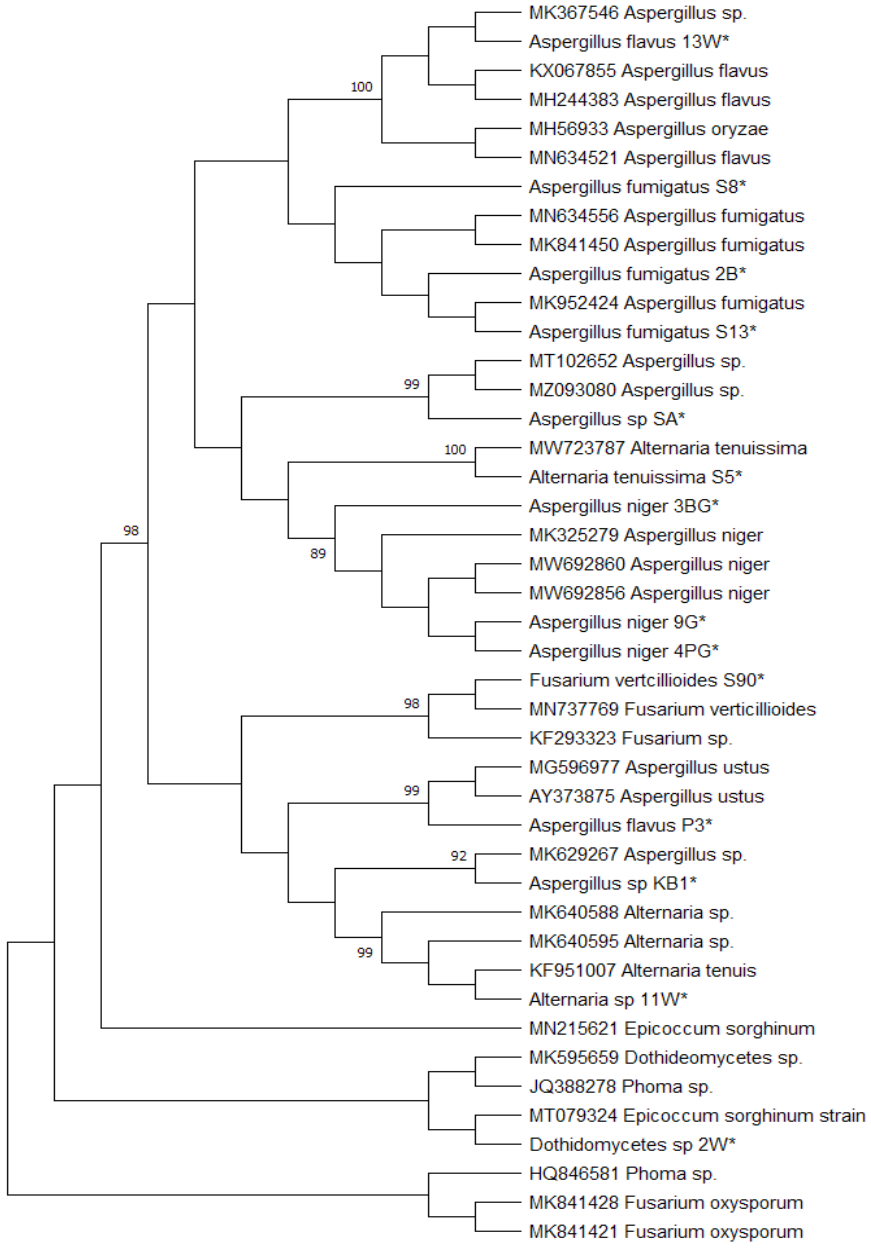


Figure 2B: The phylogenetic analysis of the ITS sequences of the filamentous fungi isolates obtained in this study (indicated by asterisks) along with other similar selected sequences from NCBI database. The analysis was conducted using Neighbour-Joining method in MEGA-X.



Discussion

Fungi have emerged as important causes of primary and opportunistic infections, especially in immunocompromised humans (Armstrong *et al*., 2014). Despite the threat of food as an avenue of spread of opportunistic fungal infections, only a few studies have used molecular methods for the characterisation of foodborne fungi in Botswana. In the present study, the internal transcribed spacer (ITS) sequencing was used as a gold standard for the phylogenetic analysis of a collection of fungi associated with street-vended foods in Botswana. Interspersed between the coding regions of nuclear rRNA genes, the ITS regions have

been used to identify fungal species accurately and reliably (Chen *et al*., 2000).

In this study, a sub-sample of fungi that were initially identified by conventional phenotypic tests was confirmed by ITS-PCR sequencing, which was found to be more accurate. Our study showed that different street-vended foods analysed were contaminated with fungi. Among the fungi detected were medically important species such as *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*, which are the major causes of mycoses (Armstrong *et al*., 2014). Other species such as *Aspergillus niger*, *Rhodotorula mucilaginosa*, and *Rhodotorula glutinis* are considered emerging pathogens that have recently come to the fore especially due to increases globally in people with immunosuppression (as in HIV/AIDS), broad-spectrum antimicrobial therapy, organ transplantation and the use of indwelling catheters (Nunens *et al*., 2013). Because HIV/AIDS pandemic remains unabated in Botswana, the findings of the present study suggest that the consumers of street-vended foods in Botswana may be at risk of opportunistic diseases due to the consumption of food contaminated with opportunistic and mycotoxigenic fungi.

Of further public health concern also, is the isolation of fungal species that are known to proliferate in food and elaborate mycotoxins. These include *Fusarium verticilloides, Aspergilus flavus* and *Aspergillus niger* which are known to produce fumonisins, aflatoxins and orchratoxins (Sweeny and Dobson 1998). Consumption of food containing these toxins has been linked to the development of debilitating conditions such as liver and kidney diseases, cancer, and down-regulation of the immune system (Rundberget *et al*., 2004). This exposure places an additional burden on the consuming populace due to possible mycotoxicoses because of the poor regulatory framework in mycoxotoxin levels in street-vended foods in Botswana. The source of fungi in street-vended foods in this study was not investigated, but previous reports have linked them to factors such as improper handling of food, use of contaminated water and cross contamination from the use of dirty processing utensils and equipment (Das *et al*., 2010). In most of the cases, street-vended foods are sold by unlicensed vendors, most of them with poor education levels and they also lack basic training in food hygiene (Das *et al*., 2010).

This study also evaluated virulence potential and biofilm formation of fungi associated with street-vended foods in Botswana. The study revealed differential virulence potential by monitoring proteolytic and lipolytic activities, which are important markers of virulence (Stehr *et al*., 2003). Proteases and lipases are exoenzymes produced in the cycle of infection of fungi. They play important roles in protein catabolism and may act in degradative pathways, peptide hormone release, blood coagulation, cell death, and tissue differentiation (Calderone and Fonzi 2001). *C. albicans* and *A. fumigatus* were shown by this study to be particularly virulent, findings which were not surprising as both species are established etiologic agents of various mycoses (Vogel *et al*., 2018). In the present study, we also investigated the biofilm formation ability of different fungal species isolated from street vended foods in Botswana. Using CV staining, it was found that *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus fumigatus* and *Candida albicans* were better at forming biofilms than other isolates. Biofilm formation is an established mediator of virulence, known to impart the fungi with the ability to withstand many harmful environmental and host stimuli (Song *et al*., 2021). Biofilms are also linked to the rise in antifungal resistance (Nunes *et al*., 2013).

A sub-sample of the fungi from street-vended food in the present study were also studied for phylogenetic relationships and showed a strong relationship with their relative species from the NCBI database (Figures 2A and 2B). Understanding the genetic diversity of pathogens is important in the control and management of the disease, especially during outbreaks of disease. In the present study, fungi from street-vended food at the three vending sites in Gaborone were found to show high levels of genetic variability, especially in opportunistic pathogens such as *Candida albicans* and *Aspergillus fumigatus*, as well as emerging infectious species such as *Rhodotorula glutinis* and *Aspergillus niger*. The source of the high genetic diversity in the present study is unknown and warrants further investigations in the future. Previous studies have ascribed the high genetic differentiation in fungi to recombination and parasexuality

events (Pekarek *et al*., 2006). The unprecedented level of genetic diversity observed in some species in this study suggests that for effective control of food-borne diseases, this diversity must be considered.

Conclusion

The present study aimed to study the prevalence, virulence potential and genetic diversity associated with fungi isolated from street food at three vending sites in Gaborone Botswana. The results indicated high levels of contamination of food by fungi at the three sites. Furthermore, the 28 strains analysed suggested that fungi isolated from street-vended food were associated with potential for virulence and high genetic diversity.

The potential for virulence of the isolates monitored by lipolytic and proteolytic activities showed that fungal isolates such as *C. albicans*, *A. fumigatus* and *F. verticilloides* had high pathogenic indices while *A. niger* and *A. fumigatus* were shown to be strong biofilm formers. Phylogenetic analysis detected significant diversity among fungi isolated from street-vended foods such as *C. albicans* and *A. fumigatus*. Thus, the findings suggest a need to track the source of fungi in food as well as to analyse the plausible reasons for the high genetic diversity associated with street food fungi in this study.

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