Fungal Contamination of Home-Made and Factory-Produced Antiseptics and Disinfectants in Lagos, Nigeria

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Abstract
This study was carried out to assess the fungal contamination of commercially available antiseptics and disinfectants in Lagos, Nigeria. Fifty (50) samples were purchased from different vendors and subjected to microbiological analysis. Isolation of fungi was done by enrichment and culturing on Sabouraud Dextrose Agar fortified with chloramphenicol using the spread plate method. Identification was achieved using cultural and morphological characteristics with lactophenol cotton blue stain. Antimicrobial susceptibility test was achieved by the disc diffusion assay using commercially available nystatin (100 units), voriconazole (1 µg) and fluconazole (25 µg) antifungal discs. Thirty-one (31) of the samples analysed were commercially produced in factories whereas nineteen (19) were home-made and commercialised. Twenty one of the stock samples (42%) were contaminated with fungi after incubation for a week. The enriched samples had a fungal contamination rate of 86% (43/50) while 14% (7/50) was free of any form of fungal growth. The fungal species identified were as follows: *Chrysosporium tropicum* (44%) > *Penicillium chrysogenum* (25%) > *Alternaria solani* (13%) > *Aspergillus niger* (10%) > *Aspergillus flavus* (4%) > *Fusarium solani* (2%) > *Penicillium marneffei* (2%). *Penicillium chrysogenum*, *Fusarium solani* and *Penicillium marneffei* were resistant to all the antifungal agents used while *Chrysosporium tropicum* and *Alternaria solani* were susceptible to the three antifungals. This study revealed a high level of fungal contamination of the antiseptics and disinfectants investigated and established the presence of some potentially pathogenic organisms that could have serious medical consequences.

Keywords: antimicrobial susceptibility, antiseptics, contamination, disinfectants, fungi

Introduction

Antiseptics and disinfectants have a long history of use to reduce the microbial load of living tissues and non-living surfaces, respectively. Some examples of antiseptics are alcohols, anilides, chlorhexidine, triclosan, quaternary ammonium compounds, iodine and chloroxylenol (McDonnell & Russel, 1999) while examples of disinfectants include alcohols, aldehydes, hydrogen peroxide, phenols and cresol, choramine and quaternary ammonium compounds (McDonnell & Russel, 1999; Weber *et al.*, 2007).

The application of antiseptics and disinfectants in hospitals, industries, pharmacies and homes has helped reduce the spread of infections from person to person and from non-living surfaces to persons. In hospitals, for example, equipment such as flexible gastrointestinal endoscopy and gronchoscopy that cannot withstand heat sterilisation procedures is decontaminated with antiseptics and disinfectants to prevent the transmission of infections (Kovaleva *et al.*, 2013).

Antiseptics and disinfectants are no exception when it comes to contamination. Contamination may occur in solutions that are in use or during large scale and indiscriminate production due to a drastic increase in demand, as witnessed in Nigeria in 2014 during the Ebola virus crisis. Several authors have documented the bacterial contamination of antiseptics and disinfectants (Oje and Kamiya, 1996; Gajadhar, 2003; Danchaivijitr *et al.*, 2005; El-Mahmood *et al.*, 2009). However, there is a dearth of information on the fungal contamination of antiseptics and disinfectants.

LaFleur *et al.* (2006) reported a biofilm-forming fungal pathogen, *Candida albicans*, which was highly recalcitrant to amphotericin B (a polyene antifungal) and chlorhexidine (an antiseptic). The mechanisms of resistance of fungi to antiseptics and disinfectants (McDonnell and Russell, 1999) could either be intrinsic (Hector, 1993) or acquired (White, 1997). Generally, the hierarchy of resistance of micro-organisms to disinfectants is of the order of bacterial spores > fungi > non-sporulating bacteria (Russell, 2003).

Therefore, this study was undertaken to assess and identify fungal contaminants from factory-produced and home-made antiseptics and disinfectants from vendors within Lagos State in Nigeria.

Materials and Methods

Sample Collection

50 different brands of antiseptics and disinfectants were purchased from different pharmacies, supermarkets and open markets. These samples were obtained randomly and transported to the laboratory for immediate analyses.

Isolation of Fungi

Fungal isolation was done from the stock and enriched samples in Saboraud dextrose broth (Oxoid) fortified with chloramphenicol for 48 h. An aliquot of 0.1 mL of the enriched sample and stock were separately aseptically plated on Saboraud dextrose agar (Oxoid) fortified with chloramphenicol using the spread plate method. The cultures were incubated at room temperature (28 ± 2 ^{o}C) for 3 to 5 days. A pure culture of each colony type was maintained by sub-culturing on Saboraud dextrose agar fortified with chloramphenicol in vials and stored at room temperature.

Identification of Fungal Isolates

The fungal isolates were identified using lactophenol cotton blue wet mount method (Sangeetha and Thangadurai, 2013). Microscopic observation was done at x10 and x40 objective lenses. Micrographs (Olympus CX31 RTSF, Philippines) of the isolates were taken and the species were identified using the Humber (1997) and Watanabe (2002) pictorial atlas.

Antimicrobial Susceptibility Test

The *in-vitro* activities of nystatin (100 units), voriconazole (1µg) and fluconazole (25 µg) were determined using disk diffusion method (Alastruey-Izquierdo *et al.,* 2015). The antifungal susceptibility disks were procured from Oxoid.

A suspension of 0.5 McFarland standard (Ogunledun, 1984) was evenly spread on the surface of Sabouraud dextrose agar plate using sterile cotton swab dipped into the inoculum suspensions. The plates were allowed to dry for 10–15 min before the antifungal discs were placed on them. The plates were then incubated at room temperature (28 ± 2 ^{o}C) for 24 h to 48 h. The diameter of zones of inhibition around the antifungal discs was measured using a transparent plastic meter ruler and recorded. Resistance or susceptibility to antifungal discs was measured against the CLSI (2013) standard.

Results

Description of Product

The description of the antiseptics and disinfectants analysed is shown in Table 1. 31 samples were produced in factories, while 19 were home-made. 13 of the factory-produced samples were hand sanitizers and washes while all the home-made samples were antiseptics and disinfectants. The active compounds in 12 of the samples were not listed on the labels of the products. 10 of these samples were home-made (samples JN11, JN14, JN15, JN16, JN19, JN31, JN34, JN35, JN43 and JN49) and 2 were factory-produced (samples JN21 and JN23). The active compounds in 2 of the handwash samples (samples ^{ah}JN46 and ^{ah}JN47) were detergents and surfactants (sodium laureth and ammonium laureth sulphate).

Fungal Population Density

The fungal population density of the antiseptics and disinfectants in Table 2 showed that 16 (32%) of the stock samples were contaminated after 72 h of incubation. After a week's incubation the number of the stock samples contaminated rose to 21 (42%). A contamination rate of 43 (86%) was observed in the enriched samples while 7 samples (14%) did not show any fungal growth. 3 of the hand sanitiser and wash samples (^{ah}JN 40, ^{ah}JN 41 and ^{ah}JN 46) were among those with no fungal growth. Only one home-made antiseptic and disinfectant (^{c}JN 36) did not show perceptible fungal growth.

Fungal Description and Distribution

The fungal contaminants, their cultural characteristics and distribution in the antiseptics and disinfectants are shown in Figure 1 and Tables 3 and 4. 47 isolates were obtained in all; 10 from the hand sanitisers and washes and 37 from the rest of the antimicrobial agents. The isolates were *Chrysosporium tropicum* (20; 44%), *Penicillium chrysogenum* (12; 25%), *Alternaria solani* (6; 13%), *Aspergillus niger* (5; 10%), *Aspergillus flavus* (2; 4%), *Penicillium marneffei* (1; 2%) and *Fusarium solani* (1; 2%). The percentage frequency of occurrence of the fungal isolates in the antiseptics and disinfectants showed that their abundance was of the following order: *Chrysosporium tropicum* (20; 44%) > *Penicillium chrysogenum* (12; 25%) > *Alternaria solani* (6; 13%) > *Aspergillus niger* (5; 10%) > *Aspergillus flavus* (2; 4%) > *Fusarium solani* (1; 2%) > *Penicillium marneffei* (1; 2%).

Fungal Antimicrobial Susceptibility Test

The antifungal sensitivity of the various isolates to the three antifungal agents in Table 5 indicated that *Penicillium chrysogenum*, *Fusarium solani* and *Penicillium marneffei* were resistant to all the antifungals used. *Chrysosporium tropicum* and *Alternaria solani* were susceptible to all antifungals while *Aspergillus flavus* and *Aspergillus niger* had a multiple antifungal resistance (MAR) index of 0.67 and 0.33, respectively.

Table 1: Product Description of Antiseptics and Disinfectants

Antiseptic/Disinfectant group	Sample Code	Product Type		Production Date	Expiry Date
		Factory -made	Home-made		
Hand Wash					
Triclosan, cocamide diethylaomide, methyl parabenzene, sodium laureth ether sulfate	[ah]JN1	1			
Sodium laureth, sodium chloride, betaine	[ah]JN42	1		01/11/14	01/10/17
Aqua, sodium laureth, sodium chloride	[ah]JN46	1			
Aqua, ammonium laureth sulphate, calcium chloride, cocamide	[ah]JN47	1			
Hand Sanitiser					
Alcohol	[ah]JN3	1			
Alcohol, aminomethyl, propanol, disodium EDTA	[ah]JN7	1			
Not listed	[ah]JN21	1		01/08/15	01/07/17
Alcohol denat, aqua, propylene glycol	[ah]JN38, [ah]JN40, [ah]JN45	3		01/06/14, 10/01/14	01/08/17, 10/11/17
Ethyl alcohol	[ah]JN41	1			01/09/16
Alcohol, anionic surfactants, water	[ah]JN44	1			01/12/17
Ethanol, acrylates, triethanolamine	[ah]JN48	1			
Antiseptic					
Chlorhexidine gluconate, cetrimide	[a]JN4	1		03/15	03/15
Balsamic acid	[a]JN6	1		07/13	06/18
Methylated spirit	[a]JN9	1		08//14	7/17
Alcohol, wood naphtha	[a]JN10	1		02/14	01/16
Iodine potion	[a]JN22	1		01/10/11	01/09/15
Not listed	[a]JN35		1	01/11/14	01/10/17
Hydrogen peroxide	[a]JN39	1		01/06/14	01/04/17
Disinfectant					
Sodium hypochlorite	[b]JN5, [b]JN8	2		23/01/15, 14/16/14	22/12/15, 02/16
HCl, hexadecyltrimethyl-ammonium chloride	[b]JN25	1		01/02/15	01/01/17
Antiseptic and Disinfectant					
Chloroxylenol, alcohol, terpineol	[c]JN2	1		07/01/13	06/01/16
Not listed	[c]JN11, [c]JN14, [c]JN15, [c]JN16, [c]JN19, [c]JN31, [c]JN34, [c]JN43, [c]JN49		9	01/11/14	01/10/17
Not listed	[c]JN23	1		01/06/14	01/05/17
Tar acid phenol, Cresylic cresol	[c]JN12, [c]JN13, [c]JN17, [c]JN18, [c]JN20, [c]JN36,		6	02/10/12	02/11/16
Tar acid phenol, Cresylic cresol	[c]JN50	1		01/05/14	01/04/16
Dichlorometaxylenol, Ethanol, Castor oil	[c]JN24	1		01/03/14	01/02/17
Phenol, Halogenated Phenols, Sodium salicylate	[c]JN26	1		01/06/14	01/06/19
Chlorhexidine gluconate, Centrimide	[c]JN27	1		01/01/15	01/12/17
Ortho benzyl, chlorophenol, meta para cresol	[c]JN28	1		01/01/14	01/06/17
Isopropyl alcohol, Chloroxylenol, aqua, Oleum pini aromaticum	[c]JN29	1		01/01/15	01/01/18
Parfum, Limonene	[c]JN30	1			
Dichlorometaxylenol, ethanol, tarpenol	[c]JN32, [c]JN33		2		
Chloxylenol, isopropyl alcohol, aqua	[c]JN37		1		
TOTAL	**50**	**31**	**19**		

[a]Antiseptics; [b]Disinfectants; [c]Antiseptics and Disinfectant; [ah]Antiseptics (hand sanitiser and hand wash); NTN, Not Known

Table 2: Fungal Population of various Antiseptics and Disinfectants from Lagos Metropolis

S/No.	Sample Code	*Population Density (cfu/mL) 72 hours		1 Week	
		E x 10^3	S x 10^1	E x 10^3	S x 10^1
1	[ah]JN1	1	0	1	0
2	[c]JN2	1	0	1	0
3	[ah]JN3	1	5	1	5
4	[c]JN4	1	0	1	0
5	[b]JN5	1	10	1	0
6	[a]JN6	1	15	1	15
7	[ah]JN7	1	5	1	5
8	[b]JN8	1	0	1	0
9	[a]JN9	1	0	1	0
10	[a]JN10	1	0	1	0
11	[c]JN11	1	5	1	5
12	[c]JN12	1	0	1	5
13	[c]JN13	1	0	1	0
14	[c]JN14	2	10	2	10
15	[c]JN15	1	0	1	0
16	[c]JN16	1	0	1	5
17	[c]JN17	1	0	1	5
18	[c]JN18	1	0	1	10
19	[c]JN19	1	0	1	10
20	[c]JN20	1	0	1	10
21	[ah]JN21	1	0	1	0
22	[a]JN22	1	5	1	5
23	[c]JN23	1	5	1	5
24	[c]JN24	0	0	0	0
25	[b]JN25	1	5	1	5
26	[c]JN26	1	0	1	0
27	[c]JN27	1	5	1	5
28	[c]JN28	1	0	1	0
29	[c]JN29	0	0	0	0
30	[c]JN30	1	0	1	0
31	[c]JN31	1	0	1	0
32	[c]JN32	1	10	1	10
33	[c]JN33	1	10	1	10
34	[c]JN34	2	15	2	15
35	[a]JN35	1	0	1	0
36	[c]JN36	0	0	0	0
37	[c]JN37	3	30	3	25
38	[ah]JN38	1	0	1	0
39	[a]JN39	1	0	1	0
40	[ah]JN40	0	0	0	0
41	[ah]JN41	0	0	0	0
42	[ah]JN42	1	0	1	0
43	[c]JN43	1	0	1	0
44	[ah]JN44	1	0	1	0
45	[ah]JN45	1	5	1	5
46	[ah]JN46	0	0	0	0
47	[ah]JN47	1	0	1	0
48	[ah]JN48	1	5	1	5
49	[c]JN 49	1	0	1	0
50	[c]JN 50	0	0	0	0

*****Values are average of two replicates; **S** = Stock, **E** = Enriched; [a]Antiseptics, [b]Disinfectants, [c]Antiseptics and Disinfectants, [ah]Antiseptics (hand sanitiser and hand wash).

Discussion

Due to the re-emergence of diseases and rise in epidemics world-over, there has been a rapid rise in the use of antiseptics and disinfectants. This has led to the sprouting of many commercially produced and home-made antiseptics and disinfectants, especially in developing nations including Nigeria. Oftentimes, little attention is paid to proper manufacturing and packaging, and some of the products lack valuable details such as: the composition, active compounds, address of manufacturer, production and expiry dates. Thus, the contamination of some of the antimicrobials investigated in this study was not surprising. Similar observations have been reported in other studies (Gajadhar *et al*., 2003; Deress *et al.*, 2014).

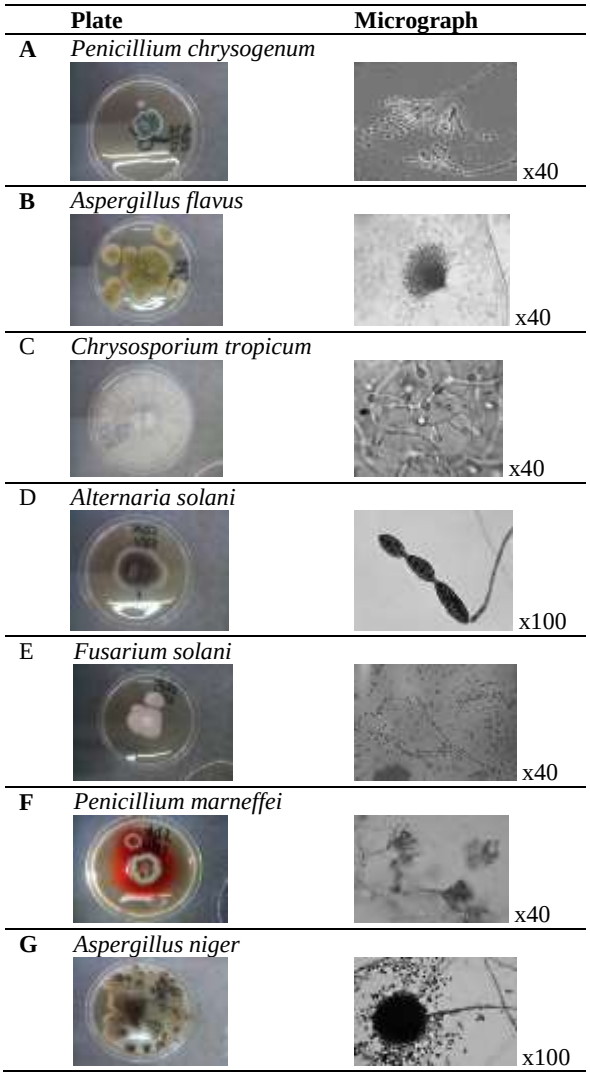

Figure 1: Plates and Micrographs of Fungal Isolates from Antiseptics and Disinfectants

In the present study, the enriched antimicrobials had more fungal growth than the stock solutions. More microbial contaminants from diluted antiseptics and disinfectants were also reported by Oje and Kamiya (1996), Gajadhar *et al*. (2003) and Deress *et al.* (2014). All the fungi isolated in this study were moulds unlike in previous studies where *Saccharomyces cerevisae* and *Candida albicans* were the major fungi identified (de Nobel *et al.*, 1990; Hiom *et al.*, 1996; LaFleur *et al.*, 2006). Moulds are generally more resistant than yeasts and considerably more resistant than non-sporulating bacteria (Russell and Furr, 1996).

Although, most of the fungi isolated in this study do not usually cause infections in humans, they can lead to diseases in immunocompromised individuals. *Penicillium marneffei* causes penicilliosis; a fatal mycosis, particularly in HIV patients (Vanittanakom *et al.,* 2006). *Penicillium chrysogenum* (a rare human pathogen) causes intestinal invasion and disseminated diseases in immunocompromised patients (Barcus *et al.,* 2005) whereas *Aspergillus flavus*, which is a leading cause of invasive aspergillosis, is the most common cause of superficial infections (Hedayati *et al.,* 2007). *Aspergillus niger* rarely causes human diseases but mostly results in serious lung infection when it occurs (Vanittanakom *et al.,* 2006). *Alternaria solani* is a plant pathogen but on rare occasions may cause keratitis and endophthalmitis (Hsiao *et al.,* 2014). *Fusarium solani* causes keratitis and endophthalmitis (Edelstein *et al.,* 2012) while *Chrysosporium tropicum* causes adiaspiromycosis (Nuorva *et al.,* 1997).

The fungal organisms identified were multi-drug resistant to the antifungal agents used. Therefore, the diseases caused by them would be difficult to treat and may lead to chronic infections. LaFleur *et al.* (2006) also reported the occurrence of antifungal resistant organisms in their study.

This study found that there are more substandard antiseptics and disinfectants in circulation as evidenced from the results. The use of antiseptics and disinfectants is important in the control of microorganisms and the spread of infections. However, microbial contaminants from these antimicrobials may contain resistant forms, which may pose great danger to people and the environment.

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Table 3: Cultural Characteristics of Fungi Isolated from Antiseptics and Disinfectants from Lagos Metropolis

S/No.	Organism	Conidial Colour	Mycelial Colour	Reverse Colour of the Plates
1.	*Aspergillus flavus*	Pale Green	Yellow Green	Pale Yellow
2.	*Penicillium chrysogenum*	Blue Green	Graish Green	Yellow White
3.	*Chrysosporium tropicum*	Green	Cream	Brownish Yellow
4.	*Alternaria solani*	Brown	Black	Black
5.	*Fusarium solani*	Brown	White	Brown
6.	*Penicillium marneffei*	Lemon	Light Pink	Pink
7.	*Aspergillus niger*	Pale Brown	Black	White

Table 4: Distribution of Fungal Contaminants in Antiseptics and Disinfectants Sampled within Lagos Metropolis

Species	Samples Code		Number	Percentage Frequency
	Factory-produced	Home-made		
Penicillium chrysogenum	[ah]JN1, [ah]JN3, [a]JN6, [ah]JN21, [a]JN22, [c]JN23, [c]JN27, [c]JN28, [ah]JN38	[c]JN12, [c]JN14, [c]JN34	12	25%
Aspergillus flavus		[c]JN43, [c]JN49	2	4%
Chrysosporium tropicum	[c]JN2, [c]JN4, [b]JN5, [ah]JN7, [b]JN8, [a]JN9, [a]JN10, [b]JN25, [ah]JN47	[c]JN11, [c]JN13, [c]JN14, [c]JN15, [c]JN16, [c]JN17, [c]JN18, [c]JN19, [c]JN20, [a]JN35, [c]JN37	20	44%
Alternaria solani	[a]JN39, [ah]JN42, [ah]JN44, [ah]JN45, [ah]JN48	[c]JN 37	6	13%
Fusarium solani		[c]JN37	1	2%
Penicillium marneffei	[c]JN26		1	2%
Aspergillus niger	[c]JN30	[c]JN31, [c]JN32, [c]JN33, [c]JN34	5	10%
TOTAL			**47**	**100%**

[a]Antiseptics; [b]Disinfectants; [c]Antiseptics and Disinfectants; [ah]Antiseptics (hand-sanitiser and hand-wash).

Table 5: Antifungal Susceptibility Test and Mar Index of Fungal Isolates

FUNGI ISOLATES	*ANTIFUNGAL AGENTS (Diameter in mm)						MAR INDEX	% MAR
	VOR		NS		FCA			
Time	48 h	72 h	48 h	72 h	48 h	72 h		
Penicillium chrysogenum	R(0)	R(0)	R(0)	R(0)	R(0)	R(0)	1.00	100
Aspergillus flavus	S(25.5)	S(22.66)	R(16.5)	R(14.8)	R(0)	R(0)	0.67	66.67
Chrysosporium tropicum	S(90)	S(90)	S(90)	S(90)	S(90)	S(90)	0.00	0
Alternaria solani	S(24.4)	S(22.5)	S(34)	S(32.2)	S(30)	S(23.5)	0.00	0
Fusarium solani	R(0)	R(0)	I(19.8)	R(13.3)	R(0)	R(0)	1.00	100
Penicillium marneffei	R(0)	R(0)	R(0)	R(0)	R(0)	R(0)	1.00	100
Aspergillus niger	S(21)	I(16.8)	I(19)	R(13.8)	R(0)	R(0)	0.33	33.33

*Values are average of two replicates; **VOR**; voriconazole, **NS**; nystatin, **FCA**; fluconazole, **R**; resistant; **S**; susceptible, **I**; intermediate and **MAR**; multiantibiotic resistance.

References

Alastruey-Izquierdo, A., Melhem, M. S. C., Bonfietti, L. X. and Rodriguez-Tudela, J. L. (2015). Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. *Rev. Inst. Med. Trop. Sao Paulo* **57**(19): 57–64.

Barcus, A. L., Burdette, S. D. and Herchline, T. E. (2005). Intestinal invasion and disseminated disease associated with *Penicilium chrysogenum*. *Ann. Clin. Microbiol.* **4**: 21.

Clinical and Laboratory Standards Institute (CLSI) (2013). Performance standards for antimicrobial susceptibility testing; 23rd informational supplement, Wayne, PA, CLSI document M100-S23.

Danchaivijitr, S., Dhiraputra, C., Rongrungruang, Y., Srihapol, N. and Pumsuwan, V. (2005). Microbial contamination of antiseptics and disinfectants. *J. Med. Assoc. Thai.* **88**: 133–139.

de Nobel, J. G., Klis, F. M., Priem, J., Munnik, T. and Van Den Ende, H. (1990). The glucanase-soluble mannoproteins limit cell porosity in *Saccharomyces cerevisiae*. *Yeast* **6**: 491–499.

Deress, T., Girma, M., Birhan, W., Biadgo, B., G/cherkos, T. and Alemu, A. (2014). Isolation of bacteria from commonly used antiseptic and disinfectant solutions in Gondar University Hospital, North West Ethiopia. *Am. J. Nurs. Res.* **2**(3): 44–49.

Edelstein, S. L., Akduman, L., Durham, B. H., Fothergil, A. W. and Hsu, H. Y. (2012). Resistant *Fusarium* keratitis progressing to endophthalmitis. *Eye contact lens* **38**(5): 331–335.

El-Mahmood, A. M. and Doughari, J. H. (2009). Bacteriological examination of some diluted disinfectants routinely used in the Specialist Hospital Yola, Nigeria. *Afr. J. Pharm. Pharmacol.* **3**(5): 185–190.

Gajadhar, T., Lara A., Sealy, P. and Adesiyun, A. A. (2003). Microbial contamination of disinfectants and antiseptics in four major hospitals in Trinidad. *Rev. Panam. Salud Pública* **4**(3): 193–200.

Hector, R. F. (1993). Compounds active against cell walls of medically important fungi. *Clin. Microbiol. Rev.* **6**: 1–21.

Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P. and Denning, D. W. (2007). *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiol.* **153**: 1677–1692.

Hiom, S. J., Furr, J. R., Russell, A. D. and Hann, A. C. (1996). The possible role of yeast cell walls in modifying cellular response to chlorhexidine diacetate. *Cytobios* **86**:123–135.

Hsiao, C. H., Yeh, L. K., Chen, H. C., Lin, H. C., Chen, P. Y. F., Ma, D. H. K. and Tan, H. Y. (2014). Clinical Characteristics of *Alternaria keratitis*. *J. Ophthamol.* **79**(5): 881–882.

Humber, R. A. (1997). Chapter V-1 Fungi: Identification. In: Manual of techniques in insect pathology, Lacey, L. A. (Ed.). Academic Press, San Diego, California, p. 153–185.

Kovaleva, J., Peters, F. T. M., van der Mei, H. C. and Degener, J. E. (2013). Transmission of infection by flexible gastrointestinal endoscopy and bronchoscopy. *Clin. Microbiol. Rev.* **26**(2): 231–254.

LaFleur, M. D., Kumamoto, C. A. and Lewis, K. (2006). *Candida albicans* biofilms produce antifungal-tolerant persister cells. *Antimicrob. Agents Chemother.* **50**: 3839–3846.

McDonnell, G. and Russell, D. A. (1999). Antiseptics and disinfectants: Activity, action, and resistance. *Clin. Microbiol. Rev.* **12**(1): 147–179.

Nuorva, K., Pitkanen, R., Issakainen, J., Huttunen N. P. and Juhola, M. (1997). Pulmonary adiaspiromycosis in a two year old girl. *J. Clin. Pathol.* **50**: 82–85.

Ogunledun, A. (1984). Comparative yield and biologic evaluation of non-polar toxins produced by an *Aspergillus fumigatus* isolate cultured in two different synthetic media. MS Thesis, Texas Southern University, Houston-Texas, USA.

Oje, S. I. and Kamiya, A. (1996). Microbial contamination of antiseptics and disinfectants. *Am. J. Infect. Control* **24**(5): 389–395.

Russell, A. D. and Furr, J. R. (1996). Biocides: mechanisms of antifungal action and fungal resistance. *Sci. Progr.* **79**: 27–48.

Russell, A. D. (2003). Similarities and differences in the responses of microorganisms to biocides. *J. antimicrob. Chemother.* **52**: 750–763.

Sangeetha, J. and Thangadurai, D. (2013). Staining techniques and biochemical methods for the identification of fungi. In: Laboratory protocols in fungal biology: Current methods in fungal biology, Gupta, V. K., Tuohy, M. G., Ayyachamy, M., Turner, K. M., O'Donovan, A. (Eds.). Springer, New York, p. 237–257.

Vanittanakom, N., Cooper, Jr. C. R., Fisher, M. C. and Sirisanthana, T. (2006). *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin. Microbiol. Rev.* **19**(1): 95–110.

Watanabe, T. (2002). Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species, 2nd edn., CRC Press Washington, D.C., 486pp.

Weber, D. J, Rutala, W. A. and Sick-Bennet, E. E. (2007). Outbreaks associated with contaminated antiseptics and disinfectants. *Antimicrob. Agents Chemother.* **51**(12): 4271–4224.

White, D. C. 1997. Antifungal drug resistance in *Candida albicans*. *ASM News* **63**:427–433.