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Mycological Analysis and Potential Health Hazards of Dextrose Intravenous Infusions in Nigeria.

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***Abstract*—One hundred and forty two samples (142) of two brands of commercial intravenous fluids manufactured and marketed in Nigeria were analyzed for fungal contaminants. The pH and osmolarity values were also determined. All samples were analyzed within their expiry dates. The infusions were categorized into the visibly defective samples and the visibly normal samples. Sixty percent (60%) of the samples were contaminated with at least one fungal species. The fungal counts ranged from 1600cfu.ml^{-1} in the visibly defective sample to 50cfu.ml^{-1} in the visibly normal sample. Fourteen viable fungal species belonging to ten genera were isolated. The fungal isolates in the visibly defective samples were *Aspergillus repens, Aspergillus glaucus, Penicillium roqueforti, Chaetomium spp, Paecilomyces variotii , Humicola grisea, Geotrichum candidum* and *Geomyces cretacea,* while the visibly normal samples contained *Aspergillus japonicus, Candida valida, Candida krusei, Candida parapsilosis, Geomyces cretacea, Paecilomyces variotii, Humicola grisea* and *Fusarium oxysporum.* The pH and osmolarity values of contaminated samples were lower than specified for the products. [pH:4.5-5, osmolarity 280mosmol/l for 5% Dextrose infusion; pH 6 , osmolarity 555mosmol/l for 10% Dextrose infusion] The presence of pathogenic fungi particularly in the visibly normal samples before their expiry dates , coupled with the reductions in pH and osmolarity values below the critical safe levels (pH 4, & 306 mosmol l $^{-1}$) render the two brands and two categories of products potentially hazardous to health.**

I. INTRODUCTION

The parenteral route of administration is generally adopted for medicaments that cannot be given orally, either because of patients' intolerance, drug instability, or poor absorption via the enteral route. In the unconscious patient, parenteral administration is the only safe and most effective means of administering medicaments through the intravenous route (The Pharmaceutical Codex, 1994). Sterile intravenous infusion fluids, popularly called "Drips", are large volume parenteral products which consists of single dose injections with a volume of 100 to 1000ml.

It is generally mandatory that microorganisms or their products must not be present in sterile pharmaceutical products throughout its shelf life (PHLS Working Party, 1971; European Pharmacopoeia, 2000). Pharmaceutical preparations are regarded contaminated or spoilt , if , low levels of acutely pathogenic microorganisms or higher levels of opportunist pathogens are present, if toxic microbial metabolites persist even after death or removal of any microorganism originally present, or if chemical changes have occurred in the product(Hugo and Russell, 1992).

Although Pharmaceutical Manufacturers strive under Good Manufacturing Practice (GMP) to ensure quality of their products, occasionally this is not achieved due to inadequate analytical facilities, lack of properly trained personnel, or indeterminate errors during quality control process (Aluoch-Orwa, *et al.*, 1995). Contamination is a recurrent problem and can have fatal consequences particularly with intravenous products. In a Nigerian survey of intravenous products by the National Agency for Food Drug Administration and Control (NAFDAC) in 2004, out of 566 samples comprising 42 brands from 8 manufacturers, 9 brands of Dextrose 5%, Dextrose 4.3% in 0.18% NaCl, Darrows half strength, and Dextrose 50% failed the microbiological specification for intravenous fluids, thereby prompting NAFDAC to instititute recall of the contaminated products (Atata *et al*., 2007).

Microbial contamination of injections and infusions often result from poor sterilization management, obsolete equipment , inappropriate production environment, and poor quality packaging (Caudron *et al*., 2008). Parenteral nutritions and Intravenous fat emulsions can become contaminated during preparation and infusion, with fungal pathogens (Kuwahara, *et al*., 2010) especially *Candida* species which accounts for 20 – 30 % of systemic infections associated with Central venous catheters. *Candida albicans* has the ability to either grow very well or sustain prolonged viability in all nutritional intravenous products. Infusion fluids requiring

compounding or the addition of medications to the fluid container were found to produce 7% of primary bloodstream infections when those fluids were prepared (Macias *et al.*, 2010).

Septicemia arising from the administration of contaminated fluids is a particular complication and concern in intravenous therapy. Organisms which have been associated with infusion septicemia include *Staphylococcus, Klebsiella, Aspergillus,* and *Candida.* The genus *Candida* has been incriminated as one of the notorious contaminants of infusion fluids giving rise to fungal septicemia after infusion therapy(Goldman *et al.*, 1993). *Candida krusei* is a commonly isolated opportunist pathogen responsible for serious septic infections in susceptible patients (McQuillen *et al.*, 1994). In the year 2005 and 2006, administration of *Candida albicans* contaminated parenteral fluid was responsible for a major outbreak of systemic candidosis in India (Kumar *et al*., 2011). Pathogenic agents have been reported transmitted by direct contact or indirectly via contaminated instruments and intravenous fluids with fungal colonization and invasive candidosis in babies at Neonatal Intensive Care Units (Rao *et al*., 2005). For more than two decades, *Candida albicans* and *C. parapsilosis* have been recognized to pose significant danger to preterm infants, causing candidemia, particularly catheter related, bloodstream infections, with a crude mortality rate of 23% to 50%. The risk factors include prematurity, central venous catheters, parenteral nutrition, intravenous fluids and intubation (Polin and Siman, 2003). Recently, Vagna and Henao (2010) described the manifestations of meningitis by *Candida* to include disseminated Candidiasis in premature infants, ventricular drainage devices infections and chronic isolated meningitis which were directly related to access of *Candida albicans, Candida parapsilosis* and *Candida tropicalis* to the Central Nervous System through the bloodstream.

In 90% of patients with fungemia and endocarditis owing to infection with the genus *Paecilomyces*, the predisposing factors were found to be peritoneal dialysis, contaminated intravenous fluids and medicaments (Marzec and Heron, 1993). The high glucose concentration of both dextrose infusions and dialysate fluids render them optimal media for fungi growth, even in dialysate fluids where *Paecilomyces variotii* was isolated and incriminated as the aetiology of peritonitis in a patient (Elamin *et al*., 2010)

Acute systemic infections and deaths have resulted from the administration of contaminated fluids where invasive infections caused by fungi were the major causes of morbidity and mortality in the immunocompromised patient (Cornelius, *et al*., 1998). The Reuters News reported the death of nine patients in an Alabama hospital as a result of outbreaks of infection from a batch of contaminated intravenous fluids (Peggy, 2011). In addition sixteen pregnant women were reported dead in an India Hospital after the administration of contaminated dextrose intravenous fluids that led to infection, excessive bleeding and multi organ failure (Rajalakshmi, 2011)

In the tropical countries such as Nigeria, pharmaceutical preparations are frequently stored under conditions of high temperature (Av= 31^0C) and high relative humidity (Av=75%); and may be dispensed in non – protective packaging (Blair, *et al.*, 1998). While the presence of a few microbial survivors in an injection after production is often unlikely to induce infection, considerable growth can occur during storage prior to administration thereby yielding highly infective or toxic products(Hugo and Russell, 1992). While these products were intended to mitigate diseases and death, more infections and deaths are rather often initiated worldwide, most especially in developing countries, by such unwholesome drugs. Although Aluoch- Orwa *et al.*, 1995 reported substantial failure in the available content of the active ingredients of some commercial intravenous infusions in Kenya, Atata *et al*., 2007 reported on the microbiological qualities of some intravenous fluids in Nigeria. However, important parameters of pH and osmolarity values of the products were not determined in both studies.

The objective of this research was to evaluate the presence of fungi in commercial 5% and 10% Dextrose intravenous fluids retailed under tropical conditions, and to evaluate the changes in pH and osmolarity values of the products that may have been caused by the fungal contamination.

II. MATERIALS AND METHODS

Samples

A total of 142 units of two brands of 5% (100) and 10% (42) dextrose intravenous fluids manufactured and marketed in Nigeria were randomly purchased from various retail outlets. The samples of each brand was made up of 50% visibly defective units and 50% visibly normal units.

Fungal Content Analyses of the Samples

Each of the visibly defective samples was analyzed employing the pour plate technique following the methods of Scott *et al*. (1985) and adaptation of the British Pharmacopoeia (1988) in consistence with the U.S. Pharmacopoeia (2008). Each sample consist of a 500ml intravenous infusion in a plastic bag. The exterior of the bag was disinfected by swabbing with 70% ethanol. The content was shaken properly and with the aid of a sterile syringe inserted through the wall of the bag, 10ml sample of each product was withdrawn and diluted ten-fold in sterile distilled water. One ml of the aliquot was used to seed replicate plates of sterile Sabouraud Dextrose Agar (SDA) supplemented with 0.025g Streptomycin. The plates were incubated at 27^0C for 5 days.

Under a laminar air flow cabinet, the membrane filtration technique was employed to analyze the visibly normal samples. The whole content of 500ml bag was filtered through a 0.45µm membrane filter, coupled on a sterile 47mm magnetic filter funnel(Gelman Sciences, USA) . After filtration, the membrane filter was cut aseptically into two and incubated in sterile Sabouraud Broth supplemented with 0.025g Streptomycin at 27^0C for 5 days. At the end of the fifth day, 1ml aliquot of the culture was inoculated into replicates sterile plates of SDA supplemented with 0.025g Streptomycin and incubated at 27^0C for 5days. The resultant colonies were enumerated and the cultural characteristics recorded. Pure cultures were developed and stored on SDA slants as stock cultures which were recultured every two weeks until employed for identification.

Stringent aseptic techniques were adopted to prevent adventitious contamination of the work. Negative controls employing sterile distilled water in place of infusions were set up.

PH and osmolarity determination

The pH of the samples were measured using a Pye – Unicam pH meter model 291mkz equipped with a glass electrode (accuracy, $\pm$ 0.01 pH unit).

The osmolarity value of each test sample was determined using an advanced Osmometer 3W11.

Characterization and Identification of the fungal isolates

Typical discrete colonies of each isolate were selected and studied employing colonial and cultural characteristics. Yeast isolates were subjected to a number of biochemical tests and cultured for pseudo mycelium formation (Lodder, 1970). The isolates were further identified based on the microscopic fungal features such as phialides, septate sporangiophores, conidiophores and budding, as previously described (Harrigan and McCance, 1976). Furthermore, typical isolates were confirmed by culturing the pure isolates on 2% Malt Extract Agar to achieve enough conidiation, from which suspensions were prepared and inoculated into the BIOLOG FF Microplate (BIOLOG, CA, USA) and incubated at 26^0C for 5days. Characteristic fingerprints of each isolate were then read using the BIOLOG Microstation and confirmed with the FF Database.

III. RESULTS AND DISCUSSION

The attributes of the samples , the visible defects, the fungal counts and species are summarized in Tables 1a, 1b, 1c and 1d. While the products carry comparable production dates, none had expired prior to analysis. A significant higher number of fungal counts were observed in the visibly defective samples than the visibly normal samples in both categories of products. However, *Aspergillus* and *Penicillium* were not recovered from the visibly normal samples of 10% Dextrose products (Tables 2 and 3).

Table 1a: Attributes and fungal species isolated from Defective 5% Dextrose intravenous fluids.

Product	Age of Sample	Expiry date (Months to expiry)	Visible defect	Fungal Count(cfu/ml)	Fungal Species Isolated
DF1	15 months	Sept.2004(20)	Whitish strands	270	*Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*
DF2	12 months	Jan. 2005(24)	Brown coloration	630	*Aspergillus glaucus, Penicillium roqueforti, Aspergillus repens, Paecilomyces variotii*
DF3	17 months	Jul.2004(19)	Cloudy	320	*Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF4	17 months	Jul. 2004(19)	Wooly strands	300	*Paecilomyces variotii Aspergillus glaucus, Penicillium roqueforti*
DF5	16 months	Aug. 2004(20)	Wooly strands	300	*Paecilomyces variotii Aspergillus glaucus, Geomyces cretacea*
DF6	18 months	Jun. 2004(18)	Tainted wooly strands	160	*Paecilomyces variotii Aspergillus repens, Penicillium roqueforti*
DF7	18 months	Jun. 2004(18)	Brownish strands	330	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*
DF8	18 months	Jun. 2004(18)	Cloudy	350	*Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF9	18 months	Jun 2004(18)	Wooly strands	300	*Aspergillus glaucus, Aspergillus repens, Paecilomyces variotii, Penicillium roqueforti*
DF10	18 months	Jun. 2004(18)	Ropiness	250	*Geomyces cretacea Penicillium*

					roqueforti
DF11	18 months	Jun 2004(18)	Cloudy	248	*Aspergillus repens, Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF12	19 months	Jul. 2004(17)	Brown coloration	358	*Aspergillus glaucus. Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*
DF13	19 months	Jul. 2004(17)	Brown coloration	320	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea*
DF14	19 months	Jul. 2004(17)	Whitish strands	300	*Aspergillus glaucus, Geomyces cretacea, Paecilomyces variotii*
DF15	19 months	Jul. 2004(17)	Brownish slimy strands	350	*Aspergillus repens, Geomyces cretacea, Penicillium roqueforti*
DF16	19 months	Jul. 2004(17)	Whitish strands	248	*Aspergillus glaucus, Geomyces cretacea, Paecilomyces variotii*
DF17	12 months	Feb. 2005(24)	Brownish strands	320	*Aspergillus glaucus, Aspergillus repens*
DF18	20 months	Aug. 2004(16)	Brownish strands	300	*Asperillus glaucus, Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*
DF19	12 months	Feb. 2005(24)	Packaging defect	0	Nil
DF20	20 months	Aug, 2004(16)	Brown coloration	350	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*
DF21	20 months	Aug. 2004(16)	Bloated	0	Nil
DF22	20 months	Aug.2004(16)	Packaging	0	Nil
DF23	20 months	Aug, 2004(16)	Cloudy	330	*Aspergillus glaucus, Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF24	20 months	Aug. 2004(16)	Less content	230	*Aspergillus glaucus, Geomyces cretacea, Paecilomyces variotii*
DF25	12 months	Feb. 2005(24)	Less content	0	Nil
DF26	24 months	Dec 2008(12)	Cloudy	340	*Aspergillus glaucus, Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF27	24 months	Dec.2008(12)	Brownish coloration	360	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea, Penicillium roqueforti*
DF28	24 months	Aug. 2009(12)	Brownish coloration	220	*Aspergillus glaucus, Aspergillus repens, Paecilomyces variotii*
DF29	24 months	Aug. 2009(12)	Tainted strands	180	*Aspergillus repens, Geomyces cretacea*, Paecilomyces variotii
DF30	24 months	Aug.2009(12)	Bloated	0	Nil
DF31	25 months	Sep 2009(11)	Brownish strands	200	*Aspergillus glaucus, Aspergillus repens, Penicilliumroqueforti*
DF32	24 months	Aug. 2009(12)	Whitish strands	240	*Aspergillus glaucus, Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF33	16 months	Oct 2010(20)	Brownish strands	230	*Aspergillus glaucus, Aspergillus repens, Paecilomyces variotii, Penicillium roqueforti*
DF34	14 months	Sep 2010(22)	Tainted speckles	120	*Aspergillus glaucus, Aspergillus repens,*

DF35	17 months	Nov 2010(19)	Cloudy	220	*Aspergillus repens, Geomyces cretacea, Penicillium roqueforti*
DF36	15 months	Aug. 2010(21)	Less content	0	Nil
DF37	15 months	Aug.2010(21)	Cloudy	100	*Paecilomyces variotii, Geomyces cretacea, Penicillium roqueforti*
DF38	16 months	Oct 2010(20)	Whitish strands	180	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea*
DF39	16 months	Oct 2010(20)	Tainted strands	220	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea, Penicillium roqueforti*
DF40	12 months	Oct 2012(24)	Less content	0	Nil
DF41	33 months	Nov 2010(3)	Dark strands	420	*Aspergillus glaucus, Penicillium roqueforti, Paecilomyces variotii*
DF42	28 months	Apr 2011(8)	Whitish strands	300	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*
DF43	31 months	Feb 2011(6)	Faint milky coloration	330	*Aspergillus repens, Geomyces cretacea, Paecilomyces variotii, Penicillium roqueforti*
DF44	29 months	Mar 2011(7)	Whitish strands	320	*Aspergillus glaucus, Aspergillus repens, Paecilomyces variotii, Penicillium roqueforti*
DF45	29 months	Mar 2011(7)	Whitish strands	320	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea*
DF46	31 months	Feb 2011(5)	Whitish strands	330	*Aspergillus repens, Paecilomyces variotii, Penicillium roqueforti*
DF47	31 months	Feb 2011(5)	Whitish strands	300	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea*
DF48	29 months	Mar 2011(7)	Yellowish coloration	320	*Aspergillus glaucus, Aspergillus repens, Geomyces cretacea, Paecilomyces variotii*

DF49	28 months	Apr 2011(8)	Less content	128	*Aspergillus repens, Paecilomyces variotii,*
DF50	29months	Mar 2011(7)	Less content	240	*Aspergillus repens, Geomyces cretacea, Penicillium roqueforti*

Table 1b: Attributes and fungal species isolated from Normal 5% Dextrose intravenous fluids.

Product	**Age of Sample**	**Expiry date (months to expiry)**	**Visible defect**	**Fungal Count(cfu/ml)**	**Fungal Species Isolated**
NF1	15 months	Sept.2004((21)	Nil	150	*A. japonicus, Fusarium oxysporum, Paecilomyces variotii*
NF2	3 months	Sept.2005(33)	Nil	10	*Candida krusei*
NF3	17 months	Jul.2004(19)	Nil	60	*Paecilomyces variotii, A. japonicus, Candida krusei*
NF4	17 months	Jul.2004(19)	Nil	50	*Paecilomyces variotii, A. japonicus*
NF5	15 months	Aug. 2004(21)	Nil	50	*Paecilomyces variotii, Geomyces cretacea*
NF6	18 months	Jun. 2004(18)	Nil	150	*Paecilomyces variotii, A.japonicus, Candida krusei*
NF7	18 months	Jun. 2004(18)	Nil	60	*A. japonicus ,Candida krusei, Paecilomyces variotii*
NF8	18 months	Jun. 2004(18)	Nil	30	*Candida krusei, Paecilomyces variotii*
NF9	17 months	Jul.2004(19)	Nil	30	*Candida krusei, Geomyces cretacea*
NF10	17 months	Jul.2004(19)	Nil	40	*A. japonicus, Candida krusei*
NF11	18 months	Jun.2004(18)	Nil	50	*A. japonicus, Candida krusei*
NF12	19 months	Jul. 2004(17)	Nil	40	*Candida krusei, Geomyces cretacea*
NF13	19 months	Jul.2004(17)	Nil	50	*A. japonicus, Candida krusei*
NF14	19 months	Jul. 2004(17)	Nil	60	*A.japonicus, Candida krusei*
NF15	19 months	Jul. 2004(17)	Nil	70	*A. japonicus, Candida krusei, Fusarium oxysporum*
NF16	19 months	Jul.2004(17)	Nil	70	*A. japonicus, Candida krusei*
NF17	12 months	Feb. 2005(24)	Nil	0	*Nil*
NF18	20 months	Aug. 2005(16)	Nil	70	*A. japonicus, Candida krusei*
NF19	12 months	Feb.2005(24)	Nil	20	*Geomyces cretacea, Paecilomyces variotii*
NF20	20 months	Aug. 2004(16)	Nil	60	*A. japonicus, Candida krusei*
NF21	20months	Aug. 2004(16)	Nil	70	*A. japonicus, Candida krusei*
NF22	20 months	Aug 2004(16)	Nil	60	*A. japonicus, Candida krusei*
NF23	20 months	Aug 2004(16)	Nil	50	*Candida krusei, Geomyces cretacea*
NF24	20 months	Aug 2004(16)	Nil	40	*Geomyces cretacea, Paecilomyces variotii*
NF25	12 months	Feb. 2005(24)	Nil	0	Nil
NF26	24 months	Dec.2008(12)	Nil	80	*A. japonicus, Candida krusei, Fusarium oxysporum*
NF27	24 months	Dec.2008(12)	Nil	80	*A. japonicus, Candida krusei, Geomyces cretacea*
NF28	24 months	Aug 2009(12)	Nil	70	*A. japonicus, Candida krusei, Fusarium oxysporum*

NF29	24 months	Aug 2009(12)	Nil	90	*A. japonicus, Candida krusei, Fusarium oxysporum*
NF30	24 months	Aug 2009(12)	Nil	80	*A. japonicus, Candida krusei, Fusarium oxysporum*
NF31	25 months	Sep.2009(11)	Nil	90	*A. japonicus, Candida krusei, Fusarium oxysporum,*
NF32	24 months	Aug 2009(12)	Nil	80	*A. japonicus, Candida krusei, Fusarium oxysporum*
NF33	16 months	Oct.2010(20)	Nil	60	*A. japonicus, Candida krusei*
NF34	14 months	Dec.2010(22)	Nil	50	*Fusarium oxysporum, Geomyces cretacea*
NF35	17 months	Nov.2010(19)	Nil	60	*A. japonicus, Candida krusei*
NF36	15 months	Aug. 2010(21)	Nil	60	*Geomyces cretacea, Paecilomyces variotii*
NF37	15 months	Aug.2010(21)	Nil	50	*Fusarium oxysporum, Paecilomyces variotii*
NF38	14 months	Dec 2010(22)	Nil	50	*Paecilomyces variotii, Fusarium oxysporum*
NF39	16 months	Oct. 2010(20)	Nil	60	*Fusarium oxysporum, Geomyces cretacea, Paecilomyces variotii*
NF40	13 months	Nov.2012(23)	Nil	50	*Geomyces cretacea, Paecilomyces variotii*
NF41	28 months	Apr.2011(8)	Nil	80	*A. japonicus, Paecilomyces variotii Fusarium oxysporum*
NF42	27 months	May 2011(9)	Nil	70	*A. japonicus, Candida krusei, Paecilomyces variotii*
NF43	26 months	Jun.2011(10)	Nil	70	*A. japonicus, Paecilomyces variotii, Candida krusei*
NF44	25 months	Jul.2011(11)	Nil	60	*A. japonicus, Fusarium oxysporum, Paecilomyces variotii*
NF45	24 months	Aug.2011(12)	Nil	60	*Fusarium oxysporum, Paecilomyces variotii*
NF46	30 months	Mar 2011(6)	Nil	70	*A. japonicusGeomyces cretacea, Paecilomyces variotii*
NF47	3 months	Apr 2013(33)	Nil	0	Nil
NF48	6 months	Jan 2013(30)	Nil	0	Nil
NF49	7 months	Dec. 2012(29)	Nil	20	*Geomyces cretacea, Candida krusei*
NF50	5 months	Sep.2013(31)	Nil	0	Nil

Table 1c: Attributes and fungal species isolated from Defective 10% Dextrose intravenous fluids.

Product	**Age of Sample**	**Expiry date(months to expiry)**	**Visible defect**	**Fungal Count(cfu/ml)**	**Fungal Species Isolated**
DT1	32 months	Apr.2003(4)	Dark Wooly strands	1600	*Exophiala dermantitidis, Geomyces cretacea, Chaetomium sp, Geotrichum candidum*
DT2	32 months	Aug. 2003(4)	Wooly strands	250	*Candida krusei, Chaetomium sp, Exophiala dermantitidis, Geotrichum candidum*
DT3	30 months	Apr. 2003(6)	Cloudy	110	*Geomyces cretacea, Chaetomium sp, Geotrichum candidum*
DT4	32 months	Jul.2004(4)	Whitish strands	120	*Humicola grisea, Chaetomium sp, Geomyces cretacea, Geotrichum candidum*

DT5	29 months	Jul..2004(7)	Cloudy	100	*Geotrichum candidum, Geomyces cretacea*
DT6	28 months	Aug.2004(8)	Wooly strands	210	*Candida krusei, Exophiala dermantitidis, Geomyces cretacea, Geotrichum candidum*
DT7	28 months	Sept 2004(8)	Ropiness	90	*Candida krusei, Exophiala dermantitidis*
DT8	28 months	Oct.2004(8)	Slimy strands	100	*Candida krusei, Exophiala dermantitidis*
DT9	28 months	Nov 2008(8)	Wooly strands	110	*Geomyces cretacea, Chaetomium sp, Exophiala dermantitidis, Geotrichum candidum*
DT10	24 months	Aug.2008(12)	Cloudy	108	*Candida krusei, Geomyces cretacea*
DT11	24 months	Aug.2008(12)	Cloudy	100	*Candida krusei, Geomyces cretacea, Geotrichum candidum*
DT12	18 months	Aug.2008(18)	Cloudy	80	*Candida krusei, Exophiala dermantitidis*
DT13	18 months	Aug.2009(18)	Cloudy	120	*Candida krusei, Geomyces cretacea, Geotrichum candidum*
DT14	16 months	Sep.2009(20)	Ropiness	80	*Candida krusei, Exophiala dermantitidis*
DT15	14 months	Sep.2009(22)	Whitish strands	120	*Humicola grisea, Chaetomium sp, Geomyces cretacea, Geotrichum candidum*
DT16	14 months	Sep.2009(22)	Cloudy	100	*Candida krusei, Geomyces cretacea*
DT17	14 months	Sep.2011(22)	Slimy strands	110	*Candida krusei, Exophiala dermantitidis*
DT18	14 months	Sep.2011(22)	Whitish strands	110	*Chaetomium sp, Geomyces cretacea, Geotrichum candidum*
DT19	12months	Sep.2011(24)	Wooly strands	130	*Geomyces cretacea, Candida krusei, Exophiala dermantitidis, Geotrichum candidum*
DT20	10 months	Oct.2011(26)	Cloudy	80	*Candida krusei, Chaetomium sp, Exophiala dermantitidis*
DT21	12months	Oct. 2011(24)	Dark strands	128	*Geomyces cretacea Exophiala dermantitidis, Geotrichum candidum*

Table 1d: Attributes and fungal species isolated from Normal 10% Dextrose intravenous fluids.

product	**Age of Sample**	**Expiry date (months to expiry)**	**Visible defect**	**Fungal Count(cfu/ml)**	**Fungal Species Isolated**
NT1	32 months	Apr. 2003(4)	Nil	80	*Candida valida, Candida krusei, Fusarium oxysporum, Humicola grisea*
NT2	30 months	Aug. 2003(6)	Nil	100	*Candida krusei, Fusarium oxysporum,C. parapsilosis, Humicola grisea*
NT3	32 months	Apr.2003(4)	Nil	110	*Candida valida, Candida krusei, C. parapsilosis, Humicola grisea*

NT4	32 months	Jul.2004(4)	Nil	60	*Humicola grisea*, C. *valida Fusarium oxysporum*
NT5	29 months	Jul.2004(7)	Nil	50	*Candida valida, Candida krusei,* C. *parapsilosis*
NT6	28 months	Aug.2004(8)	Nil	50	*Candida valida, Candida krusei, C. parapsilosis*
NT7	27 months	Sep.2004(9)	Nil	80	*Fusarium oxysporum, Candida valida, C. krusei*
NT8	26 months	Oct.2004(10)	Nil	80	*Candida valida, Candida krusei, C.parapsilosis*
NT9	8 months	Nov 2009(28)	Nil	0	Nil
NT10	24 months	Aug.2008(12)	Nil	90	*Fusarium oxysporum, Candida krusei,C.parapsilosis*
NT11	6months	Sep..2010(30)	Nil	0	Nil
NT12	24 months	Aug.2008(12)	Nil	80	*Fusarium oxysporum, Humicola grisea, C. valida*
NT13	6 months	Aug.2010(30)	Nil	0	Nil
NT14	12 months	Sep.2009(24)	Nil	60	*Candida krusei, Candida valida, Fusarium oxysporum*
NT15	8 months	Sep.2009(28)	Nil	50	*Fusarium oxysporum, Candida krusei, C. parapsilosis*
NT16	8 months	Sep.2009(28)	Nil	50	*Candida krusei, Candida valida,Candida parapsilosis*
NT17	23 months	Oct.2011(13)	Nil	90	*Fusarium oxysporum, Humicola grisea, Candida parapsilosis*
NT18	24 months	Sep.2011(12)	Nil	100	*Fusarium oxysporum, Candida valida,C.parapsilosis*
NT19	24 months	Sep.2011(12)	Nil	80	*Fusarium oxysporum, C. krusei, C.parapsilosis*
NT20	24 months	Oct.2011(12)	Nil	80	*Fusarium oxysporum, Candida valida, C. krusei*
NT21	25 months	Oct.2011(11)	Nil	90	*Candida krusei,C.parapsilosis Fusarium oxysporum*

Table 2: Percentage occurrence of fungal isolates from 5% Dextrose intravenous fluids.

Visibly defective samples		**Visibly Normal samples**	
Isolate	**% occurrence**	**Isolate**	**% occurrence**
Aspergillus glaucus	55%	*Aspergillus japonicus*	60%
Aspergillus repens	60%	*Candida krusei*	60%
Geomyces cretacea	60%	*Fusarium oxysporum*	30%
Paecilomyces variotii	60%	*Geomyces cretacea*	25%
Penicillium roqueforti	45%	*Paecilomyces variotii*	40%

Table 3: Percentage occurrence of fungal isolates from 10% Dextrose intravenous fluids

Visibly defective samples		**Visibly Normal samples**	
Isolate	**% occurrence**	**Isolate**	**% occurrence**
Candida krusei	60%	*Candida krusei*	65%
Chaetomium spp	40%	*Candida parapsilosis*	60%

Exophiala dermantitidis	55%	*Candida valida*	55%
Geomyces cretacea	65%	*Fusarium oxysporum*	60%
Geotrichum candidum	60%	*Humicola grisea*	30%
Humicola grisea	55%		

Table 4: The pH and osmolarity values of the 5% Dextrose intravenous fluids.

Visibly Defective	p H	Osmolarity (mosmol l^{-1})	Visibly Normal	p H	Osmolarity (mosmol l^{-1})
DF1	3.4	250	NF1	4.0	265
DF2	3.3	245	NF2	3.7	260
DF3	3.5	255	NF3	4.2	268
DF4	3.6	255	NF4	3.8	270
DF5	3.5	250	NF5	4.2	265
DF6	3.8	260	NF6	3.8	260
DF7	3.3	245	NF7	3.7	260
DF8	3.4	250	NF8	3.7	260
DF9	3.5	256	NF9	3.8	262
DF10	3.6	256	NF10	3.8	262
DF11	3.7	256	NF11	4.0	266
DF12	3.8	260	NF12	3.6	258
DF13	3.5	255	NF13	4.1	268
DF14	3.6	256	NF14	3.6	258
DF15	3.7	256	NF15	3.8	270
DF16	3.8	260	NF16	4.1	268
DF17	3.3	245	NF17	3.7	260
DF18	3.4	251	NF18	4.0	265
DF19	3.3	245	NF19	4.2	268
DF20	3.6	256	NF20	3.6	258
DF21	4.4	279	NF21	4.5	280
DF22	4.6	280	NF22	4.5	280
DF23	4.5	280	NF23	4.6	280
DF24	4.6	279	NF24	4.5	280
DF25	4.6	279	NF25	4.5	279
DF26	3.8	262	NF26	4.2	268
DF27	3.7	257	NF27	3.9	267
DF28	3.8	264	NF28	4.1	268
DF29	3.9	266	NF29	4.2	268
DF30	4.4	278	NF30	3.9	268
DF31	3.8	265	NF31	4.0	266
DF32	3.8	264	NF32	4.1	266
DF33	3.8	264	NF33	4.1	268
DF34	3.9	268	NF34	4.1	267
DF35	3.8	265	NF35	4.2	272
DF36	4.5	280	NF36	4.1	268
DF37	4.0	270	NF37	4.1	268
DF38	3.9	267	NF38	4.0	267
DF39	3.8	265	NF39	3.8	265

DF40	4.5	279	NF40	4.1	270
DF41	3.7	256	NF41	3.9	268
DF42	3.7	260	NF42	4.1	270
DF43	3.6	255	NF43	3.8	265
DF44	3.6	255	NF44	4.0	268
DF45	3.6	255	NF45	4.1	270
DF46	3.6	254	NF46	3.9	270
DF47	3.7	259	NF47	4.0	268
DF48	3.6	255	NF48	3.8	266
DF49	3.9	268	NF49	4.5	280
DF50	3.8	264	NF50	4.6	281

Detected Range (pH: 3.3 - 4.6; Osmolarity: 245 – 280 mosmol/l)
Normal Values (pH:4.5 – 5; Osmolarity 280 mosmol/l)

Table 5: The pH and Osmolarity values of the 10 % Dextrose intravenous fluids.

Visibly defective	p H	Osmolarity (mosmol l^{-1})	Visibly Normal	p H	Osmolarity (mosmol l^{-1})
DT1	3.8	480	NT1	3.9	525
DT2	3.8	482	NT2	4.3	530
DT3	3.8	485	NT3	4.2	535
DT4	4.2	534	NT4	4.2	545
DT5	3.8	482	NT5	4.2	545
DT6	4.0	525	NT6	4.2	545
DT7	4.0	525	NT7	3.9	525
DT8	3.8	480	NT8	3.9	525
DT9	3.8	480	NT9	4.3	530
DT10	4.2	535	NT10	4.3	530
DT11	4.2	535	NT11	4.1	528
DT12	4.0	525	NT12	4.2	545
DT13	3.8	480	NT13	4.1	528
DT14	3.9	520	NT14	4.3	530
DT15	3.8	480	NT15	4.1	528
DT16	3.8	480	NT16	3.9	525
DT17	4.0	525	NT17	4.2	535
DT18	3.8	482	NT18	3.9	525
DT19	4.2	534	NT19	4.1	528
DT20	3.9	520	NT20	4.3	530
DT21	3.8	482	NT21	4.2	545

Detected range (pH: 3.8 – 4.3; Osmolarity: 480- 545 mosmol /l)
Normal values (pH: 6; Osmolarity: 555mosmol/ l)

Parenteral dosage form must be sterile right from the point of leaving the factory to the moment of administration to the patient. This inherent property of parenteral pharmaceuticals is imparted into the product during the manufacturing process by rigidly adhering to sound and validated procedures at every steps of the preparation (Levchuk, 1991). However, this is not obtained in some cases thereby causing economic losses to manufacturers and health hazards to users. In this work, fourteen (14) fungal species belonging to Ten (10) genera were detected. Statutory pharmaceutical authorities stated that no living microorganism or their products must be present in sterile pharmaceutical products. However, ninety percent (90%) of the total samples failed to comply with the test for sterility. While the highest fungal count of 1600 cfu / ml was obtained in the defective sample DT1 , the least count was 10 cfu / ml in the visibly normal NF2 while NF17 and NF19 had 20cfu / ml; NF8 and NF9 had 30 cfu / ml , NF10 and NF12 had 40 cfu/ml each (Table 1b). The total fungal counts and diversity in the visibly defective samples exceeded the visibly normal samples of all products tested and this may be conclusive of the visible defects in the defective samples (Table 1a,1b,1c,1d).

In a previous study, Atata *et* al., 2007 reported *Aspergillus* spp and *Penicillium* spp in some Dextrose intravenous fluids in Nigeria. However, in this study, the predominant fungal contaminants were *Aspergillus repens*, *Candida krusei*, *Geomyces cretacea*, *Aspergillus glaucus*, *Penicillium roqueforti*, *Chaetomium* spp, *Aspergillus japonicus*, *Exophiala dermantitidis*, *Fusarium oxysporium*, *Candida valida*, *Candida parapsilosis*, *Paecilomyces variotii* and *Geotrichum candidum.* While spoilage organisms were recovered more from the defective samples, pathogenic strains were recovered from the seemingly normal samples of both the 5% and 10% Dextrose intravenous fluids (Tables 2 and 3).

Considering the degree of proliferation and degradation that may occur in a product, the relationship between the fungal counts and the shelf life of such product remains an important factor. With respect to the period of stay on the shelf and the fungal population, samples DF41> DF27 > DF12> DF8> DF7> DF3> DF5> DF35> DF34 (Table 1a); NF31>NF32>NF21> NF14> NF7>NF36>NF34>NF49> NF48> NF47 (Table 1b); DT1> DT2>DT4> DT3>DT6>DT13> DT18>DT20 (Table 1c); NT3>NT2> NT18> NT17> NT14> and NT15> NT13 (Table 1d) ; these indicate that the longer the product was kept before use , the higher the fungal counts. Though none was expired prior to analysis, it is apparent that long storage periods allowed the initially undetected viable spores to multiply, proliferate and become detectable. Therefore the closer the time of use to the manufacturing date the better; but the closer the time of use to the expiry date the worse for the drug and the user.

The microbial contaminants in a pharmaceutical preparation may induce disease in the consumer without necessarily producing spoilage of the product. There are two possible mechanisms for this; either infection by the pathogenic organism or through toxins liberated into the product (Ringertz and Ringertz,1982).The risk of microorganism producing an infection is dependent on the species, dose administered, route of administration and susceptibility of the host. However, irrespective of the innoculum density, in as much as the products are administered directly into the blood circulation, the presence of any microorganism or their product is crucial. It should also be noted that an organism does not need to proliferate in the product in order to induce disease, it just needs to survive (Hugo and Russell, 1992). Acute systemic infections and death have resulted from the administration of microbially contaminated intravenous infusions. The organisms most frequently causing septicemia are *Candida*, *Aspergillus* and *Paecilomyces* (Goldman *et al*., 1993; McQuillen *et al*., 1994). In this work , species of these genera, namely, *Candida valida*, *Candida krusei*, *Candida parapsilosis, Paecilomyces variotii*, and *Aspergillus japonicus* were recovered from the visibly normal samples. Therefore, the use of these infusions has the potential of being injurious to health.

In addition to fungemia and septicemia, these fungi may liberate some pyrogenic substances in the intravenous infusions. Absorbtion and dissemination by the blood of pyrogens can be accompanied by severe clinical symptoms such as low fibrinogen level, hypotension, acute shock and eventual death. Metabolites produced during fungal growth include mycotoxins which are associated with a number of diseases in man and animals. Mycotoxins are produced by a number of species such as *Penicillium* and *Fusarium* which were typically identified in this research. Several *Fusarium* species produce fumonisins and trichothecenes while several *Aspergillus* and *Penicillium* species produce ochratoxins. Aflatoxin by species of *Aspergillus* is known to be a potent hepatocarcinogen (Nigam *et al*., 1994) . *Candida krusei*, *Aspergillus* and *Paecilomyces* constituted the highest proportion in both the defective or normal samples. *Exophiala, Geotrichum* and *Chaetomium* were only found in the defective 10% Dextrose injections while *Fusarium* and *Candida* were recovered from the normal samples of both category of products. The growth of these organisms may have been favored by their capability to withstand high osmotic tension of the high sugar concentration of the fluids; and the high temperature (25^0C-40^0C) of storage of the fluids.

The pH of blood is 7.4 and blood plasma osmolarity is 306 mosmol l^{-1} . It is mandatory that parenteral intravenous injections must be in this range. Five percent (5%) Dextrose intravenous injection has a pH range between 4.5 and 5 . As a result of growth and metabolic activities of the fungal contaminants, there were corresponding reductions in pH of the products emanating from production of acids by the fungi (Table 3).
The pH was reduced to 3.3 in samples DF2, DF7, DF17, and DF19; 3.4 in samples DF1, DF8, and DF18; 3.5 in samples DF3, DF5, DF9, and DF13; 3.6 in samples DF4, DF10, DF14, DF20, NF12, NF14, and NF20; 3.8 in samples NT1, NT2,NT3 and DF6 respectively. Any reduction below the critical safe level of pH 4 as in samples DF1, DF2, DT1, NF2, NF4, NF6, NF7, NF8, NF9, NF10, NF12, NF15, NF14, NF20, NF17, NT1, NT7, NT8, NT16 and NT18 (Tables 4 and 5) will potentially cause complications. The complications may include severe pain at the site of injection, tissue necrosis (De Lucia and Rapp, 1982), damage of venous endothelium and infusion phlebitis. Small changes in pH can signify large changes in H+ ion concentration. Most importantly, the continuous infusion of hypotonic and acidic injections may overtax the buffering capacity of the blood. The incidence of phlebitis increases as infusate pH and osmolarity differs from that of blood (Marc , 2005).

In addition, as a result of microbial metabolism of the nutrients, the 5 % dextrose injections were rendered hypotonic . There were corresponding reductions in osmolarity as the pH also reduced. Five percent

(5 %) dextrose intravenous injections are isotonic solutions having osmolarity of 280 mosmol l^{-1} , used for replacement of lost fluid and electrolyte imbalance in patients. Ten percent (10 %) dextrose injections are hypertonic solutions of osmolarity 555mosmol l^{-1} for treatment of patients in hypoglycaemic coma, for energy supplementation and parenteral nutrition (The Pharmaceutical Codex, 1994). Current USP recommendations for labeling of intravenous fluids require that osmolarity be stated on the package.

The result shows that the osmolarity of the 5% Dextrose injections had been reduced to 250 in sample DF1; 245 in samples DF2, DF7, DF17 andDF19; 265 in sample NF1, 260 in NF2 and 270 in NF4. In the 10% Dextrose injection, the osmolarity had been reduced to 480 in defective samples DT1, DT8, DT9, DT13, DT15 and DT16; 482 in DT2, DT5, DT18, and DT21; 525 in Normal samples NT1, NT7, NT8, NT16, and NT18; 528 in NT11, NT13, NT15 and NT19; 530 in NT2,NT9, NT10, NT14 and NT20 (Tables 4 and 5). Therefore, the level of constituents and attributes as claimed by the product labels are often considerably lower than the normal range as at the time of presentation to users.

The intravenous administration of hypotonic solutions such as in the visibly normal samples NF1, NF2, NF4, NF6, NF7, NF8, NF9, NF10, NF12, NF14, NF17, NF18, NF19, and NF20, will result in fluid movement into the more concentrated venous endothelial cells and blood cells, elicit swelling of erythrocytes and haemolysis, thereby potentiating haemolytic anaemia.

Fungal infection are a particular concern in patients receiving a high concentration of Glucose in intravenous hyperalimentation (Schulhof, 2009) such as in the 10% Dextrose injection in this study, which yielded *Fusarium oxysporum*, *Candida parapsilosis* and *Candida krusei*. Furthermore, the findings of this present study corroborates the earlier findings of Gupta *et al*., 2000 who detected *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans* from dextrose intravenous infusions and concluded that the administered contaminated fluids in a rural setting was found to be a risk factor for development of endogenous fungal endophthalmitis.

Intraluminal spread of infection may result from intrinsic contamination of the infusion fluids. The apparent occurrence of the pathogenic fungi such as *Aspergillus*, *Candida*, *Fusarium* and *Paecilomyces* especially in the visibly normal samples coupled with significant reductions in pH and osmolarity values, suggest that these products are of potential health hazards. The manifestation of such hazards is highly probable especially in developing countries where "commercially sterile " products are often assumed to be actually sterile and are therefore consumed or administered without adequate precautions in respect of debilitated patients whose immunity are already compromised by illness. The patients may experience severe life-threatening complications.

These factory produced pharmaceuticals may constitute reservoirs for disseminating potentially dangerous infections to the community. Consequently, morbimortality and cost of healthcare will increase.
This research is a contribution to post- marketing surveillance, which Drug Regulatory Authorities have recently expressed frustration at not being able to dedicate more resources (WHO, 2006). The results are indices of substandard medicines which represent a far larger risk to public health than counterfeit medicines.
Most importantly, mortality in patients receiving intravenous infusion / therapy are not usually investigated in developing countries but commonly attributed to the underlying ailment or circulatory overload. For example, the fever often observed after post – surgery administration of intravenous fluids is usually interpreted to reflect a general depreciation of the patient's condition rather than possible presence of pyrogens or endogenously contaminated fluids.

The use of contaminated intravenous fluid predisposes patients to increased risk of Hospital acquired infections and extended hospitalization. In Africa, research on intravenous therapy cannot be over-emphasized, considering the developing nature of our economies, scientific technologies, and the enormity of the potential hazards associated with contaminated intravenous fluids. Currently, there are no technologies yet to detect endogenous contamination of fluids either post marketing or at the point of administration, apart from the visual detection of defects in the aesthetic qualities of these products. Therefore, the presence of any form of microorganism in intravenous fluid is clearly a threat to life. This investigation, in addition to other reports, suggest that the current design and / or protocol for sterilization of these products may not be safe or adequate for patients' use. The recommended use of in – line filters will neither filter out the pyrogens, nor be selective for the reduced osmolarity and pH. Consequently, existing standards and procedures should be reviewed and amended accordingly in order to produce a perfectly safe, wholesome and improved products in developing countries.

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