Fungal Load of Medical Wastes Generated from the Federal Medical Centre, Owerri Imo State, Nigeria

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Abstract: Medical wastes generated within the Federal Medical Centre, Owerri between the months of April to July, 2019 were evaluated for fungal load. Material wastes divided into sharps, laboratory, infectious and general wastes were collected from the Physicians' offices, wards and laboratory for a period of 16 weeks. They were weighed using a standard weighing balance and their weights ranged between 0.06kg to 1.92kg. The fungi present in the medical wastes were determined using standard mycological methods. The fungi isolated from the waste samples were *Candida albicans* having a frequency of 13(34.21%), followed by *Aspergillus fumigatus* and *Mucor* with 6(15.79%), each. *Aspergillus flavus* had 5(13.16%), *Penicillium marneffei* and *Candida tropicalis* had 3(7.89%), while *Candida glabrata* had the least occurrence of 2(5.62%). The laboratory wastes had the highest fungal load than any of the other medical wastes analyzed with 34.90% of the total isolates followed closely by the infectious wastes having 31.58%. Sharps contained 26.32% and general wastes had the least percentage of 7.89%. So, adequate care should be taken in the handling of these medical wastes to avoid the spread of these fungal organisms which will go a long way in reducing the spread of nosocomial infections.

Keywords: fungal load, medical wastes, Owerri, Imo State

INTRODUCTION

Humans are by nature almost too careless with wastes with respect to its generation, disposal and implications. People from different walks of life can define wastes based on how their endeavor(s) result in generating and disposing of wastes; which are material remains that are of no immediate or further use, even though some of it can be recyclable. According to Wikipedia (2016), medical wastes were defined as any substance which is discarded after primary use, or that is worthless and of no use. Recently, the reported amount of wastes has drastically increased worldwide due to the related increase in susceptible human population, health care industry and disposable medical kits (Nneka and Ebele, 2005; Hyeonjin *et al.,* 2009; Vieira *et al.,* 2010).

Arora, (2004) reported that about 5.2 million people die each year from waste related diseases and added that by 2025, the rate of waste generation will quadruple globally. More so, the United States Environmental Protection Agency (USEPA, 2013) as at 2012, defined medical wastes as materials generated at health care facilities such as hospitals, clinics, medical research facilities and medical laboratories. These medical wastes include and are not limited to: blood soaked bandages, culture dishes, glass wares, discarded surgical equipment, discarded needles used to give shots or drain blood/aspirates, swabs, removed body organs (tonsils, limbs, appendices, etc) and discarded lancets. The World Health Organization (WHO, 2015), reported medical wastes to have higher potentials for infection than any other type of wastes, hence classified into non-hazardous and bio-hazardous wastes. Reports from (Okot-Okuma, 2012; Hien *et al.,* 2012), stated that medical wastes contain significantly high concentrations of pathogenic organisms, hence the management of medical wastes should be considered as an integral part of hospital hygiene and infectious control.

In this research, medical wastes will be classified into sharp, infectious, laboratory and general wastes. Many literatures have talked about the isolation of bacteria, parasites and viruses from medical wastes, but a marked paucity of records about fungal species associated with medical wastes remains almost unattended to (Geo *et al.,* 2013). Fungi are eukaryotic organisms including unicellular micro-organisms and multi-cellular organisms. They are principal decomposers of the ecological system which explains why they are mostly found in dump sites. Fungal infections otherwise known as mycoses are less than viral and bacterial infections, but pose similar health challenges especially with the increase in immunocompromised conditions (Pruss *et al.,* 1999; Udofia and Nriagu, 2013). The fungi of medical importance include: *Candida* species, *Aspergillus fumigatus, Penicillium* species, etc. Therefore, this study tends to provide handy and substantial pieces of information about these health challenges by focusing on the isolation and identification of associated fungi of medical importance within the chosen health facility.

Table 1: Classification of medical wastes

WASTE CATEGORY	DESCRIPTION AND EXAMPLES
Hazardous wastes:	
Sharp wastes	Used and unused sharps e.g. hypodermic. intravenous or other needles; auto-disposable syringes with attached needles, infusion sets, scalpels, pipettes, knives, blades, etc
Infectious wastes	Wastes suspected to contain pathogens that poses a risk of disease transmission e.g. wastes with blood and other body fluids, laboratory cultures and microbiological stocks, wastes with excreta and materials from wards
Pathological wastes	Human tissues, organs or fluids, body parts, unused blood products
Pharmaceutical wastes	Expired and no longer needed items, cytostatic and genotoxic chemicals
Chemical wastes	Wastes with chemical substances e.g. laboratory reagents, expired disinfectants, solvents, wastes with heavy metals like batteries, thermometers and blood-pressure gauges
Radioactive wastes	Wastes with radioactive substances e.g. unused liquids from radiotherapy or laboratory research, glass wares, absorbent papers, patients with urine and excreta with unsealed radionuclides
Non-hazardous or general medical wastes	Wastes without any particular biological, radioactive, or physical hazards
Sharp wastes	Sharps are item that could cause cuts or puncture wounds, like needles, scalpels, blades, knives, infusion sets, saws, broken glass and pipettes. Whether infected or not, such items are treated as hazardous

Source: Udofia and Nriagu, 2013.

MATERIALS AND METHODS:

Study area

This study was carried out at the Federal Medical Centre, Owerri in Imo State between the months of April to July, 2016. As at 2006, the National Population Commission (NPC, 2006) reported the Owerri cosmopolitan city to have an estimated human population of 401,873.

Sample collection

An ethical approval with reference number, FMC/OW/HREC/117 was sought from and granted by the Federal Medical Centre, Owerri. The samples were collected at the sites of generation such as the physicians' offices, laboratories, wards, accident and emergency unit on weekly intervals for a period of eight weeks and were collected into sterilized plastic bowls with lids measuring 9.4cm deep and 16.2cm in diameter properly labeled, weighed, recorded and transported in these bowls to the Microbiology laboratory of the Medical facility within one hour of collection. The quantities of samples collected from selected sites varied because they were dependent on frequency of generation and the nature of sample in each unit.

Preparation of culture media

Sabouraud Dextrose Agar supplemented with 0.05g/L of Chloramphenicol (S+C), was prepared according to the manufacturer's instruction. The Chloramphenicol was added to inhibit contaminant bacteria from growing as described by Geo *et al.,* (2013).

Sample processing

An Ohaus Harvard Trip mechanical single beam balance, model 80000003 was used to weigh the collected wastes. To every 0.5kg of waste, 200mL of normal saline was poured into the bowls to wash out the micro-organisms from the wastes. The suspension was allowed to stand for three (3) hours for the fungi to acclimatize in the new environment (normal saline) without losing viability or discharging their spores (Gawad *et al.,* 2016). The suspensions were mechanically shaken to release the fungal elements from the wastes. Five millilitres (5 mL) of each suspension was collected into sterile test tubes and centrifuged at 1500 rpm for 10minutes. The supernatant was discarded and the sediment inoculated unto the prepared medium as described by Pruss *et al*., (1999).

Inoculation of specimen

A wire loop was sterilized both before and after seeding of each of the sample unto the prepared media (S+ C) by streaking to isolate discrete colonies of the present fungi. The plates were incubated at 25^0C for 7 days, but were checked daily for growth. Samples reported as negative were discarded after 14 days of no significant growth.

The colonial morphology was carefully examined for identification of the different fungal organisms according to the method put forth by the American Association of Clinical Chemistry for fungal tests (AACC, 2016).

Identification of fungal species

All identification procedures followed the standard methods described by Astrid, 1999; Cheesbrough, 2009; Parveen, 2013.

Lactophenol cotton blue (LPCB) wet mount: A drop of lactophenol cotton blue was placed on a clean grease-free glass slide and the aerial hyphae were picked from strategic points and emulsified by teasing in the drop of LPCB before mounting for examination under the 10x and 40x objective lenses of a light microscope.

Gram staining: Smears were made from the cultures, stained according to the Gram staining technique and examined for the presence of large and oval gram-positive budding yeast cells with pseudohyphae.

Germ tube test: A loopful of each isolate was emulsified in 0.5ml of human serum in a clean sterile test tube and incubated at 37^0C for 3 hours. A loopful of the suspension was placed on a clean glass slide, covered with a slip and examined using the 40x objective for germ tube (broad based budding daughter cells) production typical of *Candida albicans.*

Carbohydrate assimilation test: The commercial kit API 20C AUX yeast system was used to identify and characterize the isolates based on the manufacturer's instruction. The sugar impregnated discs were placed and incubated at room temperature for 48 hours and were examined for either the presence or absence of growth around each disc. Disks without growth around indicate that the sugar was not assimilated whereas the disks with zones of growth around them indicate sugar assimilation. An un-inoculated plate with sugar disk was used as negative control and a plate with known *Candida albicans* was used as positive control.

RESULTS:

Table 2 shows the different categories of medical wastes collected from the wards and other hospital departments including the laboratory, kitchen, laundry, theatre, etc. Their weights were measured and recorded in kilogram (kg). Out of the categories of wastes, laboratory wastes weighed more due to the mass of waste generated there while the infectious wastes weighed the least.

Table 2: The different weights of wastes collected for analysis from different wards and departments of FMC, Owerri.

Types of Waste	Wards (kg)	Depts. (kg)	Total (kg)
Sharps	0.56	0.79	1.35
Infectious	0.06	0.05	0.11
Laboratory	1.92	1.86	3.78
General	0.13	0.27	0.40
Total	**2.67**	**2.97**	**5.64**

Table 3 represents the fungi isolated from medical wastes at the Federal Medical Center, Owerri and their percentage frequency of occurrence among all categories of medical waste.

Table 3: Fungi isolated from the medical wastes at the Federal Medical Centre, Owerri

Medical Wastes	**Fungal Organisms Isolated**														**Total**	**(%)**
	C. a	***%***	***C. g***	***%***	***C. t***	***%***	***A. fu***	***%***	***A. fl***	***%***	***Mu***	***%***	***P. m***	***%***		
Sharps	3	23.08	0	-	2	66.67	2	33.33	1	20	2	33.33	0	-	10	26.32
Laboratory	4	30.77	1	50	0	-	2	33.33	1	20	3	50	2	66.67	13	34.90
Infectious	4	30.77	1	50	1	33.33	1	16.67	3	60	1	16.67	1	33.33	12	31.58
General	2	15.38	0	-	0	-	1	16.67	0	-	0	-	0	-	3	7.89
Total	**13**		**2**		**3**		**6**		**5**		**6**		**3**		**38**	

Key;

C. a = Candida albicans *A. fu = Aspergillus fumigatus*

C. t = Candida tropicalis *A. fl = Aspergillus flavus* *P. m = Penicillium marneffei*

Mu = Mucor *C. g = Candida glabrata*

Table 4: Germ tube and sugar assimilation tests of isolated *Candida* species

S/N	***CANDIDA* SPECIES**	**SUGAR ASSIMILATION**											
		G.T.T	**Dex**	**Mal**	**Suc**	**Lac**	**Gal**	**Raf**	**Inos**	**Tre**	**Mel**	**Xyl**	**Cel**
1	***C. albicans***	**+**	**+**	**+**	**+**	**-**	**+**	**-**	**-**	**+**	**_**	**+**	**-**
2	***C. tropicalis***	**-**	**+**	**+**	**+**	**-**	**+**	**-**	**-**	**+**	**-**	**+**	**+**
3	***C. glabrata***	**-**	**+**	**+**	**-**	**-**	**-**	**-**	**-**	**+**	**-**	**-**	**-**
4	**Known *C. albicans***	**+**	**+**	**+**	**+**	**-**	**+**	**-**	**-**	**+**	**-**	**+**	**-**

Key;

Glu – Glucose Raf – Raffinose

Dex – dextrose Inos – Inositol

Mal – Maltose Tre – Trehalose

Suc – Sucrose Mel – Melbiose

Lac – Lactose Xyl - Xylose

Cel – Cellulose G.T.T – Germ tube test

DISCUSSION

This study indicates that *Candida* species had the highest rate of occurrence with (47.37%) out of the whole organisms isolated. This agrees with the report of (Vieira *et al.,* 2010). *Candida* species were found to be present in all the categories of medical wastes analyzed. The reason as stated by (Parda *et al.,* 2012) is that, it is commensal and a normal flora which can also be pathogenic in some conditions.

Among the *Candida* species, *Candida albicans* appeared the most with the percentage of (30%) from the sharps, (33.33%) from the infectious wastes, (30.77%) from the laboratory wastes and (66.66%) from the general wastes. Totally, *Candida albicans* occurred 13 times out of the whole (38) organisms isolated with a percentage of (34.21%) and NAC occurred 5 times with a percentage of (13.16%) making a total of 18 times with a percentage of (47.37%) altogether. This finding corresponds with the work of (Gawad *et al.*, 2016), which stated that *Candida albicans* accounted for about 70 – 90 % of isolates recovered from patients while other *Candida* species are rarely isolated from the clinical specimens. This, on the other hand contradicts the work of (Vieira *et al.,* 2010) which found out that *Candida parapsilosis* to be the most prevalent. This could be as a result of geographical location, weather, temperature and some environmental conditions.

Aspergillus species were the second most common isolated organism from the whole wastes collected as seen in Table III, with a percentage of (28.95%). This stands in outright contradiction to the report of (Gawad *et al.,* 2016), which stated that *Aspergillus* has the highest prevalence rate among the fungal world, but this book also helped to establish the fact that among the *Aspergillus* world, *Aspergillus fumigatus* is the most prevalent. The other isolated organisms such as *Mucor* with (5.79%), *Penicillium marneffei* with (7.89%) have low rate of occurrence as compared to Candida *species and Aspergillus* species.

The findings of this study so far has been in consonance with the work of (Savita *et al.,* 2004) which showed that *Aspergillus* species as the most frequently fungal organisms isolated from hospital wastes followed by *Aspergillus* and *Mucor.* The general wastes had the least fungal load. These wastes were collected from offices within the hospital premises and as such have little or no contact with human specimens. The presence of *Aspergillus* species appearing once with the incidence rate of (5.2%) of the total organisms as seen on Table III, can be attributed to its spore dispersal. The occurrence of *Candida albicans* isolated is (2.6%) out of the whole isolates.

More organisms were recovered from the sharps unlike the general wastes. This is because they come in contact with human specimens such as during blood collection, fixing of drip sets, incisions on the body, etc. These organisms grow at the tip of these needles because of their moist nature and during disposition, they can prick the handler. The laboratory and infectious wastes yielded almost the same quantity of organisms and possess the highest quantity of organisms amongst all the wastes analyzed. This is because the wastes with the highest degree of contact with human specimens such as blood, sputum, CSF, urine, etc as seen on Table III. Most of these wastes still retain a little quantity of the specimen upon disposal and serve as breeding sites for these heterotrophic fungal organisms (WHO, 2015).

When these refuses are dumped in open waste bins without coverage, they multiply, release their spores which are carried by the wind as droplets that remain in the hospital environment, hence when inhaled by patients or clients visiting the hospitals, the debilitated ones may develop infections (Tankeshwar, 2015). The organisms are also spread by mechanical means to foods or items used by patients. Another means of spread is through

careless handling by health and medical personnel who come in contact with these wastes.

In conclusion, medical wastes contain a significant quantity of fungi, which were also isolated from among the infectious and laboratory wastes than the other wastes. Disposal of healthcare wastes should be properly done to avoid the spread of these infectious organisms.

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