Use of the biomerieux id 32c fungal identification system for fungal isolated from mother & child hospital dump site in akure, Ondo State Nigeria

Abstract

The purpose of this research work is to isolate and identify Using ID 32C API kit and to determine the antifungal sensitivity profile of fungi isolated from the mother and child hospital dump site Akure, Ondo state, Nigeria. Ten isolates (Yeasts, Aspergillus flavus, Aspergillus niger, Fusarium solani, Candida albucan, Candida krusei, Phytophtera, Rhizopus strohinfer, Trichoderm horizianum, Fusarium vortezelium) were used for this research work. Potato Dextrose Agar was used to isolate and prepared for enumerated count for fungi colonies. Identification of isolates was performed using API KIT ID 32C. Antibiotics susceptibility testing were observed, to determine the antibiogram profile of the isolated fungi, it was observed that zone of inhibition is higher by 70% resistance to fluconazole and lower by 10% of the fungi susceptibility to texbinazole and miconazole respectively. Also the average total count range from 1.6×10⁴cfu/g to 4.6×10⁵cfu/g. These finding reinforce the potential risk of waste handling and point out the need for safe management of solid waste, to minimize the spread of these infectious agent in the environment. Filamentous fungi fungus isolation in almost all sample from the environment indicate that a periodic monitoring of airborne microbiota in the health care service environment is required. This result showed that all fungi isolated from the solid hospital waste were resistant to more than one antibiotic during the course of the research work.

Keywords: id 32c API kit, isolation, identification, antibiogram, antifungal sensitivity test

Introduction

Hospital solid waste is any waste which is generated in the diagnosis, treatment or immunization of human beings or animals or in research in a hospital. Despite the enormous volumes of waste produced by health care systems and the increasing concern among scientists, waste generation has received little attention in clinical literature. The isolation of fungal species in health care solid waste is rarely mentioned in the literature described an outbreak of candiduria arising from improper disposal of infectious waste detected by molecular techniques. The health care sector includes a diverse range of health care facilities and activities, ranging in size from large general and specialist hospitals to small medical and dental offices and clinics. Ancillary facilities in this sector include medical laboratories and research facilities, mortuary centers and blood banks and collection services. All of these facilities present common environmental, health and safety issues that need to be addressed at a scale appropriate to the size of the facility and its activities.

Maintenance of sanitary conditions, use of appropriate disinfection and sterilization techniques, provision of potable water and clean air for all operations, and nosocomial infection control are the basic infrastructure requirements for health care facilities. These elements are mandatory to improve the health of patients, prevent transmission of infections among patients and staff, and reduce hazards for employees and the host community.

Solid wastes pose numerous hazards and must be appropriately managed to avoid damage to the environment and human health. Disposal of medical wastes is a growing environmental concern in the developing world. The problem is growing with an ever-increasing number of hospitals, clinics, and diagnostic laboratories universally. Medical waste is infectious and hazardous; posing serious threats to environmental health and requires specific treatment and management prior to its final disposal. Until recently, the management of medical wastes has received little attention despite their potential environmental hazards and public health risks. Although medical waste constitutes a small fraction of the municipal solid waste, the potential environmental and health hazards could be dangerous if not properly handled, the worst scenario being in developing countries. In recent years, medical waste disposal has posed more difficulties with the appearance of disposable needles, syringes, and other similar items. Wastes generated in a hospital are too hazardous to be treated, and carelessness in the management of these wastes tends to spread infections and contaminate the entire living environment prevailing in a hospital. However, since the late 1980’s, the spreading trend of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and other agents associated with blood borne diseases has raised public awareness and concern of the disposition of medical waste. As a result, medical waste is required to be treated in a special way and not to be mixed with municipal waste.

Materials and methods

Collection of sample

The samples for microbiological analysis were collected in clean polythene bags. The solid wastes were collected from Mother and Child Hospital, Akure, Ondo State, Nigeria.
Microbiological analysis sterilization

Glassware such as beakers, test tubes, petri dishes, conical flasks were washed with detergent and rinsed with water. The rinsed glassware was sterilized using the oven at 160°C for 2 hours and were subsequently allowed to cool before use. All media and distilled water were sterilized at 121°C for 15 minutes in the autoclave. Working surfaces were also sterilized by the application of 70% ethanol.

Medium preparation

Potato dextrose agar

According to manufacturer’s specification, potato dextrose agar was weighed out on a clean foil paper placed on a beam balance. The amount was dissolved in 100 mL of distilled water in a conical flask, shaken and brought to boil in a water bath until it dissolved. The conical flask was then plugged with clean cotton wool wrapped in aluminum foil and autoclaved at 121°C for 15 minutes. The medium was left to cool up to 37°C.

Fungal isolation procedure

The hospital solid waste samples were processed. One gram of samples was suspended in 99 mL of sterile distilled water and shaken vigorously for 2 minutes then the suspension was serially diluted. Serial dilution of each sample was carried out by first weighing 10 grams of each sample into a sterile beaker containing 100 mL of sterile distilled water. The beaker was properly shaken and then 1 mL of the liquid from the mixture was aseptically pipetted into a test tube containing 9 mL of sterile distilled water, which served as the stock culture. One mL of the dilution was aseptically pipetted into a test tube containing 9 mL of sterile distilled water and the same procedure continues up to the tenth test tube. From the fifth test tube, 1 mL of the dilution was aseptically pipetted into a sterile petri dish, same for the sixth, seventh, and the ninth test tubes.

Potato dextrose agar that had been sterilized by autoclaving and allowed to cool to about 45°C was poured aseptically into the petri dishes containing the dilutions, and then the selected dilution was streaked onto a potato dextrose agar for even distribution of the inoculums in medium and was allowed to solidify. The plates were incubated at 25°C for 3 days (72 hours) and the colonies were isolated and plated out. The isolate were observed for their morphological features such as colony shape and coloration. Various fungal colonies were sub cultured by repeated streaking on agar plate in order to obtain pure culture. The fungi isolates were then transferred into agar slant and stored in the refrigerator.

Preparation and sterilization of culture media

Potato dextrose agar

Potato dextrose agar powder (52 g) was weighed and poured into 1 liter of water. This was homogenized using a magnetic stirrer and hot plate. Chloramphenicol was incorporated into the medium and sterilized in an autoclave for 121°C for 15 min. The agar was cooled up to 37°C on the work bench before utilization.

Preparation and inoculation of samples

The pour plate method of Collins and Lyne was used. The pour plate method was used for culture. About 1 grams of the food samples was weighed by taken aseptically with a sterile forceps and transferred carefully into each of the test tubes containing 9.0 mL of cooled sterilized water, each food samples in different test tubes were mixed thoroughly to ensure dislodgement and even distribution of microorganisms into the suspended sterile water. A ten-fold serial dilution of each 1 mL homogenate prepared. The 1 mL of dilution factor 104 and 106 were inoculated into the sterile Petri dishes for culturing. Incubation was carried out at 25°C for 2 - 4 days for fungi growth. Spores were counted in order to obtain the total viable unit, discrete colonies were purified by sub-culturing into new prepared agar media and growth was observed under the microscope and then characterized using standard method.

Enumeration of microbial colonies

Colonial counting was carried out visually by counting the number of visible colonies that appeared on the plates. Calculation of colony forming unit (CFU) per g for the bacteria and the spore forming unit (SFU) per g for the fungi was based on the formula:

CFU/mL or SFU/g = Number of colonies × dilution factor of sample suspension

Identification of isolates

Identification of isolates from the solid hospital waste samples was obtained from the incubated potatoes dextrose agar using cultural, morphological and biochemical characteristics USING API KIT ID 32C.

Isolation and characterization of fungal isolates

The fungi colonies that were isolated from the different food samples and grown on the Potato dextrose agar plates were sub cultured by teasing out the mycelium with a sterile needle and then placing it on a freshly prepared Potato dextrose agar plate which was incubated at 28°C for 48-72 hours. This procedure was repeated until a pure culture was obtained. The fungal isolates were examined, after staining the smear with two drops of lacto-phenol-in cotton blue dye under the light microscope using medium and high power objectives.

Based on micro-morphological features of colour, types and shapes of spores, conidia and hyphae, the isolated fungi were identified. The fungi characterization was based on micro morphological features which include the following nature of conidia shape, spores head, rhizoid and hyphae (septate or non-septate).

Antibiotics susceptibility testing

Susceptibility to clinically relevant antibiotics was determined following the BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing Version 9.1. This test is carried out to determine the antibiotic drug that will inhibit the growth of the bacteria isolate already detected from culturing. The plate diffusion technique of 0° was used for the antibiotic sensitivity test. Overnight cultures of the organisms were swabbed on sterile Muller Hilton solidified Agar plates using sterile swab sticks. The multiple antibiotic discs was then placed on the agar surface and pressed using sterile forceps to ensure complete contact with agar. All the plates were incubated at 37°C to 24 hours. The zones of inhibition generated by the antibiotics were measured to the nearest millimetres (mm) and interpreted as sensitive (S), Intermediate (I) and resistant (R). The zones of inhibition were measured and interpreted according to (NCCLS, 2000). The antibiotics used and their corresponding concentrations are as follows: it iraconazole, tebinazone, fluconazole, paromycin, miconazole.

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Measuring zone of inhibition

The zone of inhibition was measured at the point which an obvious demarcation between growth and no growth can be seen using a meter rule. Zones of inhibition was measured from the back of the plate using reflected light: the plates were hold back a few inches above a black nonreflecting surface, and was measured to the nearest millimeter with a meter rule.11 The zones of inhibition generated by the antibiotics were measured to the nearest millimetres (mm) and interpreted as sensitive (S), Intermediate (I) and resistant (R).

Total fungal count

One milliliter (1mL) aliquots were transferred from specific dilution level into sterile plates for pour plating using Potato dextrose agar. Incubation was done at room temperature for 3-5 days. Average numbers of colonies per dilution were multiplied by dilution to get the total viable count.12

Antimicrobial susceptibility assays

The yeast samples recovered from solid waste were submitted to antifungal susceptibility testing that was performed according to the Clinical and Laboratory Standards Institute (CLSI) Broth Micro dilution reference method M27-A2.13 Fluconazole, itraconazole, terbinaconole, paromycin and miconazole were obtained as reagent grade powders from their respective manufacturers. The final concentration of tested antifungals was 0.0-2.0μgL for fluconazole, 0.0-6.9μgL for itraconazole, 0-1-2.1μgL for terbinaconole, 0-0-2.4μgL for paromycin and 0-0-2.0μgL for miconazole. Measuring Zone of Inhibition (MZI) was read as the lowest antifungal concentration with substantially lower turbidity (decrease of 80% in turbidity) compared to the growth of the antifungal-free growth control well for all agents except for miconazole and paromycin. For those drugs, MZI results were read as the minimal antifungal concentration with complete inhibition of growth. Candida albicans ATCC 18.804 was also included as the control organism.14

API ID 32C aux system

The inoculated ampule of API C medium was homogenized and 135µl of the suspension was dispensed into each cupule of the strip using the ATB electronic pipette. The lid was then placed on the strip. It was then incubated at 29°C±2°C for 24-48 hours.15

Results

Table 1 is the Average total fungal count in the solid hospital waste samples in spore forming unit per mL (sfu/g) respectively. The table showed that the average total fungi count ranges from 1.6×10⁶ - 3.5×10⁶ cfu/g. The average total fungi count was highest in samples collected from outpatient department and the least count was observed in medical records department.

Table 2 shows the Colony (morphological and physiological) characteristics of fungi isolated from solid hospital samples in Potato Dextrose agar (PDA) media. The range is from isolate A to isolate E. Table 3 & 4 show the biochemical characteristics of bacteria isolated from solid hospital waste samples using API KIT ID 32C. The results identified the isolates as Yeast, Fusarium solani, Aspergillus niger, Trichoderma horizianum, Candida albucan, Rhizopus stolonifer, Fusarium vortezellum, Aspergillus flavus, Candida krusei and Phytophthora.

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Table 3 Biochemical characteristics of fungi isolated from solid hospital waste samples USING API KIT ID 32C

| Organism | SAC | ARA | CEL | RAF | MAL | TRH | SOR | RHA | MEL | MAN | GLU | INO |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Yeast    | -ve | +ve | -ve | -ve | +ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve |
| Fusarium solani | -ve | -ve | +ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Aspergillus niger | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve |
| Trichoderma horizianum | +ve | +ve | -ve | -ve | +ve | -ve | -ve | -ve | +ve | +ve | +ve | +ve |
| Candida albucan | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve |
| Rhizopus stolonifer | -ve | -ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve |
| Fusarium vertezelium | -ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve |
| Aspergillus flavus | +ve | +ve | -ve | -ve | +ve | -ve | +ve | +ve | +ve | +ve | +ve | +ve |
| Candida kruise | -ve | +ve | -ve | -ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve |
| Phytophthora | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve | +ve |

API KIT ID 32 C

SAC, D - saccharose; ARA, L - arabinose; CEL, D - cellobiose; RAF, D - rafimose; MAL, D - maltose; TRF, D - trhalose; SOR, D - sorbitol; RHA, L - rhamnose; MEL, D - mellobiose; MAN, D - manitol; GLU, D - glucose; INO, inositol; GAL, galactose; NAG, N- acetyl glucosamine; LAT, lactic acid; 2KG, pottasium (II) ketoglucose; MDG, methyl - alpha D - gluporanosidase; XYL, D - xylose; RIB, R-ribose, GLY, glycerol, PLT, palatinose; ERY, erythrotol; GLT, sodium glucaromate; mLZ, D - mellobiose; GLU, D - glucose; INO, inositol

Table 4 Locally isolated fungi from dumpSite and fungal profile from ID.32C

| S/N | Organisms | ID 32C KIT |
|-----|-----------|------------|
| Identification Profile | |
| 1) | Aspergillus niger | 47674763770 |
| 2) | Fusarium solani | 47677757700 |
| 3) | Yeast | 17427207770 |
| 4) | Aspergillus flavus | 07630577140 |
| 5) | Phytophthora megakarya | 57553146710 |
| 6) | Candida kruise | 77071703110 |
| 7) | Rhizopus stonifer | 03434235050 |
| 8) | Trichoderma horizianum | 61412162770 |
| 9) | Fusarium vertezelium | 13431522210 |
| 10) | Syncphalaslarum racemus | 13331225210 |

Table 5 shows the various zones of inhibition exhibited by the different bacteria isolated from the hospital solid waste. The table further showed that all the antifungal drugs caused zones of inhibition on selected isolates.

Discussion

In this project, the microbial quality of the selected solid hospital waste samples were studied. A total of six samples (samples A-F) were collected from Mother and Child Hospital and were tested quantitatively and qualitatively. From Table 1, the total average of fungal spore count in outpatient department of the hospital. It was observed and reported that the highest number of microbial load with 9.2×10^{6}cfu/g were found in the outpatient department of the hospital. The average fungal count was highest in the outpatient department of the hospital (9.2×10^{6}cfu/g) and was lowest in the Medical Records unit (4.6×10^{6}cfu/g) (Table 1). It was observed that the outpatient department has the highest fungal count due higher accessibility to patients and visitors which may introduce more fungi into the section of the hospital and the lowest fungal count in the medical record department was due to less accessibility to patients and visitors which may introduce fewer fungi into the section for the Medical Records unit. The organisms suspected include Fusarium sp, Aspergillus sp, Phytophthora sp, Candida yeast, Rhzyzopus sp (Table 2).

The high microbial load in the solid hospital waste samples depicts a deplorable state of poor hygienic and sanitary practices employed in the generating and disposal of waste in the hospital. These solid hospital samples contain Yeast, Fusarium solani, Aspergillus niger, Trichoderma horizianum, Candida albucan, Rhizopus stonifer, Fusarium vertezelium, Aspergillus flavus, Candida kruise and phytophthora. Fusarium vertezelium was the most resistant strain. It showed reduced susceptibility to terbinafine, paromycin and miconazole with zones of inhibition to be 0.0mm in all. It also showed reduced susceptibility to itraconazole and fluconazole with zones of inhibition to be 2.0mm in both antifungals (Table 5).

Table 5 Various zone of inhibition exhibited by different fungi isolated from solid hospital waste

| Organism | ITK | TER | FLU | PAR | MIC |
|----------|-----|-----|-----|-----|-----|
| Yeast    | 12  | 16  | 14  | 16  | 12  |
| Fusarium solani | 10  | 11  | 13  | 15  | 17  |
| Aspergillus niger | 23  | 19  | 20  | 22  | 15  |
| Trichoderma horizianum | 69  | 10  | 8   | 10  | 12  |
| Candida albucan | 20  | 15  | 8   | 19  | 20  |
| Rhizopus stonifer | 20  | 18  | 17  | 21  | 10  |
| Fusarium vertezelium | 2   | 0   | 2   | 0   | 0   |
| Aspergillus flavus | 17  | 15  | 12  | 10  | 9   |
| Candida kruise | 30  | 21  | 26  | 24  | 18  |
| Phytophthora | 0   | 1   | 0   | 9   | 0   |

ITK, Itraconazole; TER, terbinafine; FLU, fluconazole; PAR, paromycin; MIC, miconazole

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All pathogenic strains and may cause different types of diseases in the human body. Organisms isolated from surgical, medical lab department, and nurse station had moderate growth, around 8.8 × 10^5 sfu/g, 6.0 × 10^5 sfu/g and 5.5 × 10^5 sfu/g respectively on average in agar media.14

The organism isolated from medical record department shows the lowest growth of microorganism in the agar plates with an average of 4.6 × 10^5 sfu/g. due to the fact that solid waste are usually aseptically collected and disposed properly, this may be why microbial count was low. Therefore, the microbial counts in the solid hospital waste samples were within the standards or limits for acceptable environmental waste disposal method.15,16 Therefore if this multi-drug resistant strain isolated from this section of the hospital, i.e., Out patient, medical record etc, it may not succumb to treatment with terbinafine, paromycin, miconzole, itraconazole or fluconazole. Similarly, Phyphthora showed resistance to multiple antifungals in itraconazole, fluconazole and miconzole with 0.0mm as zones of inhibition in all. It also displayed reduced sensitivity terbinafine and paromycin with values of zone to inhibition as 1.0mm and 9.0mm respectively. Therefore if this multi-drug resistant strain is isolated, it may not inhibited by the treatment with terbinafine, paromycin, miconzole, itraconazole or fluconazole.

However, Aspergillus niger showed highest sensitivity with zones of inhibition to be 23.0, 19.0, 20.0, 22.0 and 15.0 in itraconazole, terbinafine, fluconazole, paromycin and miconzole respectively (Table 5). During the course of this research work, it was observed that all organisms were more sensitive to itraconazole, 5 isolates and 50% sensitivity (Table 6) and were least sensitive to fluconazole with 70% resistance (Table 6). The implication being itraconazole would be better in managing fungal infections in the hospital facility.

**Table 6** Shows the number(s) and percentage of fungal isolated from hospital solid waste in relation to the zone of inhibition of the various antifungal used

| Antibiotics | ITK | TER | FLU | PAR | MIC |
|-------------|-----|-----|-----|-----|-----|
| N % S | 5 (50%) | 1 (10%) | 2 (20%) | 3 (30%) | 1 (10%) |
| N % I | 1 (10%) | 5 (50%) | 1 (10%) | 3 (30%) | 3 (30%) |
| N % R | 4 (40%) | 4 (40%) | 7 (70%) | 4 (40%) | 6 (60%) |

ITK, itraconazole; TER, terbinafine; FLU, fluconazole; PAR, paromycin; MIC, miconzole; N%, number(s) and percentage of susceptible fungi isolates; N I, number(s) and percentage of fungi with intermediate zone of inhibition; N R, number(s) and percentage of resistance fungi isolates

However, from the results of standard biochemical test for identification of all the ten (10) isolates from the solid hospital waste samples it has been seen that the solid hospital waste samples contains Aspergilus spp (30%), Fusarium spp. (17.4%), Yeast (17.4%), which are the predominant organisms, followed by candida (13%), Rhizopus spp. (8.7%) and Tricodermer spp. (8.7%) and Phytophora spp. (4.3%), respectively in all solid waste samples. These strains are the resident and transient fungi, respectively on hands and are associated with poor hygiene practice.17

Contamination with Aspergilus spp. which has higher percentage of occurrence in all solid hospital waste sample of 30% have resulted from pre and post handling of the solid hospital waste.18 It is normal skin flora of 25% waste site and it does not cause diseases unless it gets into the human system which is significant among Aspergilus genus as it can produce different types of toxins which are heat resistant. According to Lasker et al.,19 Aspergillosis infection could cause blood poisoning, caused by enterotoxin-producing strains of Aspergilus spp, was the most common illness borne. Toxin production strains of Staphylococcus are the leading cause of gastroenteritis following handling of hospital materials, drugs, dressing materials etc by the person who carries the microorganism in their nose and skin. It was suggested that the solid waste contamination in the hospital is mainly due to poor quality and hygiene in the handling of hospital materials and equipment and it was also observed that the minimum percentage of resistant fungi isolate was 40% which may show a high degree of multi-drug resistant fungi across board in the hospital.20

The presence of candida spp. indicates poor practice sanitary conditions during handling, and transportation of hospital materials. The organisms gaining access to patients thereby cause nosocomial infection or disease outbreaks. Manye21 has also reported Candida spp as a well-known cause of illness that is not commonly reported because of its usually mild symptoms. However, he described a fatal family outbreak due to liver failure after the consumption of Candida spp. spores adhere to many surfaces and survive normal washing and disinfection (except for hypochlorite and UVC) procedures, which can be accountable for its prevalence in hospital environment. Candida spp are also a common inhabitant of the human gut and a urinary tract pathogen, widely distributed in the environment and have been isolated from the intestinal tract of mammals, animals and birds (chicken and turkey).22

Also in this study, the antimicrobial sensitivity test was carried out on the fungi isolated and were found to be of public health significance, and were tested against the following antibiotics namely Itraconazole, Texbinacole, Fluconazole, Paromycin and Micanzole respectively. The result shows the resistance profile of the bacteria isolated from the solid hospital samples.

**Conclusion**

In conclusion, Filamentous fungi isolation in almost all sample from the environment indicate that a periodic monitoring of airborne microbiota in the health care service environment is required. We can imply from the result that all fungi isolated from the solid hospital waste were resistant to more than one antibiotic during the course of the research work, further studies are needed to establish the relationship between health risk level and Hospital solid waste. Despite the lack of data in the literature concerning airborne fungi in health services, this results suggest that these environments are ecologically similar to other health care environments. This makes them potentially critical regarding cross-infection control. Airborne fungi measured inside clinical/surgical environment indicate that storage rooms may provide very favourable conditions for survival/dispersion of airborne fungal spores. Considering the biological risk, all health care institutions should establish a safety programme for health care workers in the attempt to prevent accidents and cross infection.

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**Conflict of interest**

The author declares no conflict of interest.

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