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Original article

Metagenomic Analysis of Medical Wastes from Secondary and Tertiary Hospitals in Benin City, Nigeria

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ABSTRACT

A wealth of information has been uncovered by metagenomics, such as microbial diversity, vast swathes of uncharacterized metabolism, and increased complexity of biogeochemical pathways and it promises to provide new enzymes and molecules with diverse applications. This study was conducted to determine the metagenomic study of medical waste in a tertiary and secondary hospital in Benin City, Nigeria. This study was conducted in a tertiary and secondary health facilities in Edo State namely University of Benin Teaching Hospital and Central Hospital in Benin City. Clinical waste specimens were collected from labour wards, surgery rooms, medical wards, children's wards, and pathology laboratories at the University of Benin Teaching Hospital and Central Hospital, Benin City. The medical waste specimen was collected into a sterile bacterial transport medium and labeled appropriately. Microbial DNA extraction was conducted on medical wastes, DNA products were amplified and sequenced. The sequence data for this project have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database with the Accession number PRJNA907864. A total of 80 microbial sequence genera were recovered in the medical waste in this study. Sequences of microbial genera recovered were Ruminofilibacter xylanolyticum Georgfuchsia toluolica, Paeniabacillus, Phaeospirilum fulvum, Novosphingobium stygium, Clostridium stercorarium, P. carboxydohydrogena, P. balearica, P. thermotolerans, and P. citronellolis, Brevibacillus thermoruber, Bacillus koreensis, Dysgonomosa species, and Sphaerochaeta species. Measures to reduce infections from medical waste among healthcare workers are advocated.

Introduction

According to WHO, medical waste has been described as healthcare waste or biomedical waste generated within healthcare facilities, research centers, and laboratories during medical procedures as well as those generated from other sources [1]. Medical waste is of importance due to its potential environmental hazards and public health risks with the tendency of resulting in epidemics [2]. Healthcare activities are capable of generating different kinds of hazardous wastes of which mismanagement can result in environmental and occupational health risks [3]. Due to technological, economic, and social challenges, developing countries are resource-constrained, which results in poor training of staff handling waste [4]. In Nigeria, most people are unaware that medical waste contributes substantially to environmental pollution and hazards[5]. Medical waste raises questions about how important human, animal, and environmental health are since poor waste management can have a big impact on public health [6, 7]. Diseases have spread based on the incorrect disposal of medical waste in municipal waste bins, open places, and water bodies [7]. The idea that the whole environmental microbiome can be explored and analyzed together has revolutionized the understanding of the ecology around man. It has opened new horizons in the exploitation of uncultivated microbial species as the vast majority of microorganisms are unculturable[8]. The advent of metagenomics has resulted in discoveries that remained hidden from traditional culturing techniques[9].A wealth of information has been uncovered by metagenomics, such as microbial diversity, vast swathes of uncharacterized metabolism, and increased complexity of biogeochemical pathways and it promises to provide new enzymes and molecules with diverse applications[2].

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In Nigeria, there is no enforcement of policies governing the proper treatment of medical waste at the national, state, and municipal levels. In order to improve operationalization by creating appropriate and affordable infectious waste management, it is imperative to demonstrate and promote best practices and procedures for the management of medical waste in Nigeria that has not yet been fully adopted. There is a dearth of information on the metagenomic study of medical waste in healthcare facilities in Benin City, Edo State, Nigeria. Against this background, this study was conducted to determine the metagenomic analysis of medical waste from secondary and tertiary hospitals in Benin City, Nigeria.

Materials And Methods

Study area: This study was conducted in a secondary and tertiary health facilities in Benin City, Edo State namely Central Hospital and University of Benin Teaching Hospital. These hospitals serve the populace in Edo State and other neighbouring states.

Ethical approval

The Ethics and Research Committees at both healthcare facilities respectively, approved the protocol for this study (Reference number HA-737/37 and Adm/E22/A/Vol. VII/483020). The authors have observed all ethical points including informed consent good behavior, non-plagiarism, dual publication, data distortion and data making in this article.

Collection of specimens

Medical waste specimen was collected from labour wards, surgery rooms, medical wards, children ward, and medical laboratories at the University of Benin Teaching Hospital and Central Hospital, Benin City. Medical waste specimen was collected into a sterile bacterial transport medium and sent to Inqaba Biotech, South Africa, for analysis.

Microbial DNA extraction

The DNA was extracted from the medical waste using the Zymo BIOMICS DNA Miniprep kit (Zymo Research Corp, Denmark) and following the

manufacturer's instruction. Briefly, 750 µl Zymo BIOMICS lysis solution was poured to a ZR bashing bead lysis tube and firmly sealed. The mixture was placed in a bead beater with a 2 ml tube holder assembly and treated for 5 min at maximum speed. The mixture was spun for 1 min at 10,000xg in a microcentrifuge. Approximately, 400 µl of the supernatant was transferred to a collection tube with a Zymo-Spin III-F filter and centrifuged at 8,000 x g for 1 min. The Zymo-Spin III-F filter was removed from the equation. The filtrate in the collection tube was combined with around 1,200 µl of ZymoBIOMICS DNA binding buffer. In a collecting tube, 800 microliters of the mixture were placed to a Zymo-Spin IICR column and centrifuged at 10,000xg for 1 min. The liquid that flowed through the collection tube was dumped and 400 µl ZymoBIOMICS DNA wash buffer 1 was added to the Zymo-Spin IICR column in a new collection tube, which was centrifuged at 10,000 x g for 1 min while the flow through was discarded. In a collection tube, 700 microliters of ZymoBIOMICS DNA wash buffer 2 were introduced to the Zymo-Spin IICR column and centrifuged at 10,000xg for 1 min. The flow-through was thrown out. MICS DNase/RNase-free water was applied directly to the column matrix, incubated for 1 min, and then centrifuged for 1 min at 10,000xg to elute the DNA. 1.5 ml Zymo-Spin IICR was transferred and added directly to the column matrix in 100 µl ZymoBIOMICS DNase/RNase free water, incubated for 1 min, then centrifuged at 10,000xg for 1 min to elute the DNA. About 600 µl of ZymoBIOMICS HRC prep solution was added to a fresh Zymo-spin III HRC filter and spun at 8,000xg for 3 min. In a clean 1.5 microcentrifuge tube, the eluted DNA was transferred to a prepared Zymo-Spin III HRC filter and centrifuged at exactly 16,000xg for 3 min. For polymerase chain reaction, the filtered DNA was kept in the freezer [10].

Metagenomic sequencing, assembly and taxanomic classification

Full-length 16S PCR was performed using Forward and reverse universal-tail 16S primers (27F and 1492R) covering variable regions v1 to v9. The resulting full-length 16S gene amplicons were sequenced on the Sequel system by PacBio (www.pacb.com).

Raw subreads were processed through the SMRTlink (v10.1) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40).These highly accurate reads were then processed through vsearch (https://github.com/torognes/vsearch) and taxonomic information was determined based on QIMME2. The NCBI NR database was employed in the alignment of the representative sequences of the NR gene catalog for taxonomic annotations[11]. The sequence data for this project have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database with the Accession number PRJNA907864.

Results

Generally, a total of 80 microbial sequence genera were found in medical waste. Sequences that did not match any known microbial organism were the most frequent (57.59%) followed by microbial sequences that correspond to Ruminofilibacter xylanolyticum (19.12%) while the least frequent microbial sequence corresponds to Georgfuchsia toluolica with a prevalence of 0.02% (Figure I).

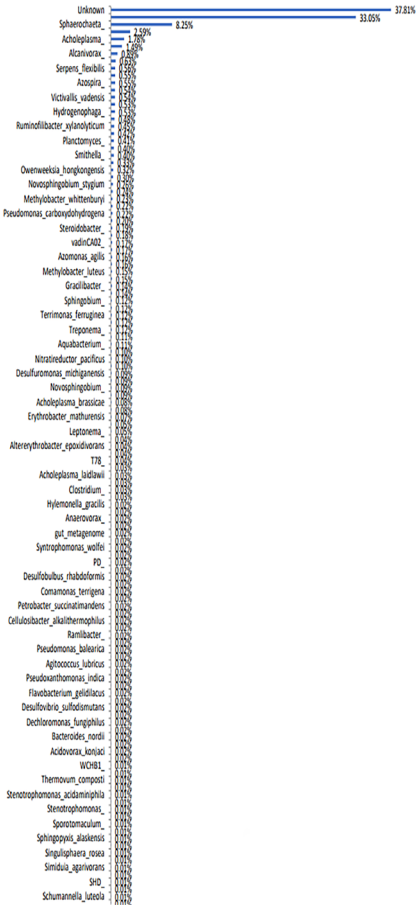
Figure I: Total microorganisms recovered in the study

Unknown	57.59%
Ruminofilibacter_xylanolyticum	19.12%
Sphaerochaeta_	5.48%
Clostridium_stercorarium	1.75%
Clostridium_	1.66%
Clostridium_thermosuccinogenes	1.24%
RFN20_	1.12%
Ignatzschineria_	1.11%
Wohlfahrtiimonas_	0.99%
Comamonas_terrigena	0.74%
Clostridium_hungatei	0.66%
Parabacteroides_	0.64%
wall_spirochete	0.57%
Bacteroides_	0.53%
Serpens_flexibilis	0.52%
Bacteroides_coprosuis	0.42%
Bacteroides_nordii	0.39%
Victivallis_vadensis	0.34%
Rhodospirillum_rubrum	0.25%
Sporobacter_termitidis	0.23%
Comamonas_	0.23%
gut_metagenome	0.22%
Parabacteroides_gordonii	0.22%
Acetonema_longum	0.18%
Gracilibacter_	0.17%
Dysgonomonas_gadei	0.16%
Dysgonomonas_	0.16%
Pelotomaculum_	0.15%-
Tetrathiobacter_kashmirensis	0.14%
Olivibacter_sitiensis	0.12%
Coprococcus_	0.11%
Anaerofilum_	0.11%
Paralcaligenes_ureilyticus	0.11%
Stenotrophomonas_	0.11%
Papillibacter_cinnamivorans	0.10%
Sedimentibacter_	0.10%
BF311_	0.10%
Kerstersia_gyiorum	0.10%
Desulfotomaculum_aeronauticum	0.09%
Atopobium_rimae	0.09%
Clostridium_methylpentosum	0.09%
Clostridium_hathewayi	0.08%
Prevotella_	0.07%
Stenotrophomonas_retroflexus	0.07%
Prevotella_ruminicola	0.07%
Cytophaga_xylanolytica	0.07%
Thermincola_ferriacetica	0.07%
Paenochrobactrum_glaciei	0.05%
Acholeplasma_brassicae	0.05%
Salmonella_	0.05%
Luteimonas_mephitis	0.05%
Cellvibrio_	0.05%
Oligella_	0.05%
Luteimonas_	0.05%
Acholeplasma_morum	0.05%
Peptococcus_	0.04%
Jonquetella_anthropi	0.04%
vadinCA02_	0.04%
Pseudoxanthomonas_indica	0.04%
Bacteroides_uniformis	0.04%
Caloramator_	0.04%

Figure II: Microbes recovered from a tertiary hospital in Benin City

Paenibacillus_	87.40%
Unknown_	6.08%
B_	1.83%
Luteimonas_	0.88%
Unknown	0.81%
Serpens_flexibilis	0.38%
Pseudomonas_	0.24%
Acholeplasma_	0.21%
Hydrogenophaga_	0.21%
Ruminofilibacter_xylanolyticum	0.17%
Arcobacter_cryaerophilus	0.16%
RFN20_	0.15%
Paenibacillus_edaphicus	0.12%
Bacillus_firmus	0.12%
Planctomyces_	0.11%
Aequorivita_	0.08%
Demequina_	0.08%
Parapedobacter_	0.07%
Thauera_	0.06%
Clostridium_thermosuccinogenes	0.05%
Cellvibrio_	0.04%
Sphingomonas_wittichii	0.04%
Sedimentibacter_	0.04%
Arenibacter_	0.04%
Steroidobacter_	0.04%
Fluviicola_	0.04%
Actinomyces_	0.04%
Plesiocystis_	0.03%
Caldicoprobacter_	0.03%
Arthrobacter_	0.03%
Comamonas_	0.03%
Clostridium_	0.03%
Persicitalea_jodogahamensis	0.03%
Devosia_	0.03%
PSB_	0.03%
Coprococcus_	0.03%
Gemmatimonas_	0.03%
Lactobacillus	0.03%

Figure III: Microbes recovered from a secondary hospital in Benin City



The sequences of microbes recovered from medical waste generated from the tertiary hospital corresponded to Paenibacillus species predominated with a prevalence of 87.40% while sequences corresponding to Paenibacillus mucilaginosus, Phaeospirilum fulvum, Novosphingobium stygium, Clostridium stercorarium, Brevibacillus thermoruber, Bacillus koreensis, Dysgonomonasspecies, Sphaerochaeta species were the least frequent with prevalence of 0.01% each (Figure II).

Figure II: Microbes recovered from a tertiary hospital in Benin City

Microbial sequences that did not match any known microbial organism were the most prevalent (37.81 %) in medical waste from the secondary hospital used in this study while sequences corresponding to Novosphingobium acidiphilum are among the 58 microbial sequences with the least prevalence – 0.01% each. A total of 79 microbial sequences were recovered from the medical waste obtained from the tertiary hospital (Figure III).

Discussion

Clinical waste is not receiving the adequate attention it deserves in poor resource nations, particularly in Africa owing to the scarcity of resources [12, 13]. Poor management of waste has been reported to be a product of epidemics [14, 15]. Healthcare workers have been reported to suffer from a wide range of infections emanating from poor waste management [15, 16].

To our knowledge, this is the first metagenomic analysis of medical waste in this study area. A total of 80 sequence genera were recovered in this study. The majority of the sequence (57.59%) did not match any of the earlier submissions in GenBank. Sequences that correspond to Ruminofilibacter xylanolyticum (19.12%) were the most prevalent while the least frequent microbial sequence corresponds to Georgfuchsia toluolica with a prevalence of 0.02%. The reason for this finding may be due to the fact that these unknown sequences may not have been previously reported and/ or submitted to Genbank. Tian et al. [17] observed the presence of Ruminofillibacter in a composting process as it was reported to have enhanced the degrading of macromolecules like cellulose, agar, and chitin. It is imperative to observe that most of the organisms recovered from medical wastes in this study were environmental organisms with little or no medical importance in humans[18]. However, Sphaerochaeta was one of the most frequently recovered organisms in this study. Sphaerochaeta are symbiotic bacteria that belong to the phylum Spirochaetes that are commonly distributed in nature and are phylogenetically ancient and distinct groups of microorganisms [19].

The propensity of spirochetes to inhabit greatly anatomical and ecological habitats is remarkable and indicates a high diversity of the bacterial members of this phylum [19]. Treponema was reported from the medical wastes obtained in this study. In this genus, T. pallidum is known to cause syphilis and if untreated on time, it may spread to the joints, heart, and nervous system [1]. Syphilis is a disease of public health importance that, if not treated promptly, can have a devastating effect on one's health. Cardiovascular and neurological illness, as well as unfavorable pregnancy outcomes such as stillbirth and congenital syphilis, are all indications of syphilis [20]. However, T. pallidum was not recovered in this study. This study underscores the risk of improper waste management which could pose a serious health implication to the category of health workers where they may become a conduit of transmitting these infections to the community should they become infected through improper waste management.

Clostridium stercorarium, C. thermosuccinogenes, C. hungatei, C. methylpentosum, C. hathewayi, and Clostridium were recovered in this study. Clostridia are anaerobic organisms with at least 209 species and five subspecies [21]. Generally, the ability of colonization of the intestine differs between species and strains of Clostridium as its adhesion contributes tremendously to the colonization and predominance of this organism in the colon [22]. The five species of Clostridium (Clostridium stercorarium, C. thermosuccinogenes, C. hungatei, C. methylpentosum, and C. hathewayi) observed in this study have been reported as known thermophiles where the C. thermosuccinogenes naturally produces succinic acid as one of their main fermentation products [23]. The species of Clostridium recovered in this study have not been implicated in any disease pattern. It is recommended that health workers are encouraged to receive the requisite vaccination that will confer specific immunity to some infections while those who have received the vaccine be encouraged to take a booster shot for prevention.

Bacteroides nordii was reported as one of the bacteria observed in this study. This bacterium has been reported by Song et al. [24] from the clinical specimen of human intestine. The phylum Bacteroidetes have been implicated as a potential pathogen in colorectal cancer [25]and reported to have a significant effect on diseases with localization outside of the gut such as depression, anxiety, chronic fatigue syndrome, autism cirrhosis, and aging [26]. Wohlfahrtiimonas chitiniclastica was also recovered (0.99%) in medical waste. This organism can cause both local skin/soft tissue infection and sepsis [27]. The presence of this bacterium has been associated with fly larvae infestation in open wounds. However, an infection with this organism has been reported to be rare in humans [28].

Sphaerochaeta was the most prevalent bacteria recovered from the secondary health institution whereas Paeniabacillus was the most recovered bacteria in waste from the tertiary health institution in this study. P. phoenicis and P. lautus are the common isolates of humans as they are capable of either being a pathogen or contaminant. It can cause a true infection, particularly in cases of abscesses, wound exudates, ocular infections, and diverse fluids [29].Pseudomonas was also isolated among sequences obtained from the waste retrieved from the secondary health institution in our study. Pseudomonas can be found in both the soil and the water. Pseudomonas aeruginosa is one of the many different forms of Pseudomonas that cause illnesses in people. Following surgery, this species has been known to cause infections in the blood, lungs (pneumonia), and other regions of the body [30].

The species of Pseudomonas reported in this study were P. carboxydohydrogena, P. balearica, P. thermotolerans, and P. citronellolis. P. balearica has been previously reported by Salva Serra et al. [31] from an oil-polluted environment. This observation makes P. balearica a species of interest for bioremediation of areas polluted with recalcitrant aromatic hydrocarbons, some of which are among the most prevalent and persistent pollutants in the oil-polluted environment. This may be the reason for the observation of P. balearica as our study area, Edo State is classified as an oil-producing area. The species of Pseudomonas recovered in this study have not been implicated to have clinical importance in humans.

Conclusions

A total of 80 microbial sequence genera were recovered in the medical waste in this study thus, provided a database of microbial diversity for future studies. Sequences of microbial genera recovered were Ruminofilibacter xylanolyticum Georgfuchsia toluolica, Paeniabacillus, Phaeospirilum fulvum, Novosphingobium stygium, Clostridium stercorarium, C. thermosuccinogenes, C. hungatei, C. methylpentosum, and C. hathewayi, P. carboxydohydrogena, P. balearica, P. thermotolerans, and P. citronellolis, Brevibacillus thermoruber, Bacillus koreensis, Dysgonomosa species, and Sphaerochaeta species. P. balearica has been implicated as an organism that is found in oil-polluted environments like our study area. Measures to reduce infections from medical waste among healthcare workers are advocated.

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