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INFLUENCE OF BIOMEDICAL WASTE ON NOSOCOMIAL INFECTIONS

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Abstract: **Biomedical waste and its influence on nosocomial infections was studied. Passive air sample was performed using settle plates, waste water samples from the drippings, soil sediment underlying solid waste and soil adjacent to the dump site were collected from a depth of 0-30cm with UCTH and General and Hospital Calabar, Calabar . The samples were evaluated using standard microbiological techniques. The result of the total microbial count of air samples within the waste dumpsite of the hospital showed that the total viable count (TVC), total coliform count (TCC) and total staphylococcal count (TSC) ranged from 164.1±0.5 to 179.0±0.8cfu/m^3, 21.0±0.2 to 30.0±0.3cfu/m^3, 6.0±0.1 to 9.0+0.2cfu/m^3 respectively, while the air within the reception unit (AR) ranged from 62.0±2.0 to 73.2±2.8cfu/m^3, 6.3±1.0 to 10.4±1.2cfu/m^3, 3.9±0.1 to 6.0±0.3cfu/m^3 respectively. Unlike the control air samples, a lower TVC, TCC and TSC which ranged from 21.4±0.71 to 29.8±0.92cfu.m^3, 2.0±0.01 to 7.4±0.02cfu/m^3, 0 to 3.8±0.01cfu/m^3 respectively were observed. The total microbial count of soil samples underlying solid waste samples from the hospitals had a TVC, TCC and TSC which ranged from 2.4±0.4 x 10^8 to 3.9±0.8 x 10^8cfu/g, 2.0±0.6 x 10^4 2.9 ± 0.9 x 10^4cfu/g, 2.3±0.7 x 10^2 to 3.47±1.0 x 10^2cfu/g respectively, while that of samples from the soil adjacent to the dumpsite ranged from 4.2±0.2 x 10^7 to 4.8±0.2 x 10^8cfu/g, 2.2±0.5 x 10^4 to 2.4± 0.7 x 10^4cfu/g, 2.0. ± 0.1 x 10^2 to 2.81 ± 0.6 x 10^2cfu/g respectively. Similarly, a lower count was observed in the control soil from the two hospitals with the TVC, TCC and TSC ranging from 3.4±0.7 x 10^4 to 3.9±0.8 x10^4cfu/g, 1.8±0.2 x 10^3 to 2.0±0.3 x 10^3cfu/g, 1.7±0.1 x 10^2 1.9± 0.2 x 10^2cfu/g respectively. The microbial count of the dripping samples from the waste dumpsite in UCTH and General hospital Calabar had a TVC, TCC, and TSC that ranged from 7.4±1.8 x 10^7 to 9.2±2.0 x 10^7cfu/ml, 3.4±1.0 x 10^3 to 3.8±1.0 x 10^3cfu/ml and 1.5±0.6 x 10^2cfu/ml to 2.1±0.8 x 10^2cfu/ml respectively. The microorganisms isolated from the various samples and control were identified as *Staphylococcus aureus, Escherichia coli, Klebsiella sp., Bacillus sp., Streptococcus sp., Pseudomonas sp., Corynebacterium sp.,* and *Shigella sp.* However, the high microbial load densities encountered in this study, suggests that the activities of hospital wastes in the environment is a major health and environmental threat as well as a potential source of nosocomial infection. Therefore, there is an urgent need to raise awareness, education and management strategy on biomedical waste issues to ensure public health and environmental safety.**

Keywords: **Biomedical, Nosocomial, Infections, Waste, Pollution, Environment.**

1. INTRODUCTION

Many waste are produced as a result of human activities, such waste may be dangerous and therefore need safe disposal (Al-Shenqiti *et al*., 2017). Industrial waste sewage and agricultural waste polluted water, soil and it can also be dangerous to human beings and environment. Solid waste can be classified into different types depending on their source (Anitha and Indira, 2012). It include; household waste, industrial waste, biomedical waste or hospital waste or infectious waste. Hospital waste is considered as hazardous because they contain toxic substances. This waste is generated during the diagnosis, treatment or immunization of human beings or animals or in research activitiesin these fields. Liquid waste can be divided into two components, liquid reagents or chemicals discarded and the cleaning and washing water channelled into the drain (Ekhaise and Omavwoya, 2008). Until recently, medical waste management was not generally considered an issue. In the 1980s and 1990s, concerns about exposure to human immunodeficiency virus (HIV) and hepatitis B virus (HBV) led to questions about potential risks inherent in medical waste. Thus, hospital waste generation has become a

prime concern due to its multidimensional/ ramifications as a risk factor to the health of patients, hospital staff and extending beyond the boundaries of the medical establishment to the general population (Ekeleme *et al*.,2013). Hospital waste refers to all waste, biologic or non -biologic that is discarded and not intended for further use. Medical waste is a subset of hospital waste, it refers to the material generated as a result of diagnosis, treatment or immunization of patients and associated biomedical research. Biomedical waste (MBW) is generated in hospitals, research institutions, healthcare teaching institutes, clinics, laboratories, blood banks, animals houses and veterinary institution (Kalpana *et al*.,2016).

Biochemical waste, also known as infectious waste or medical waste is defined as waste generated during the diagnosis, testing, treatment, research or production of biological produces for humans or animals. Biomedical waste includes syringes, live vaccines, laboratory samples, body parts, bodily fluids and waste, sharp needles, cultures and lancets.

Improper management of waste generated in healthcare facilities causes a direct health impact on the community, the healthcare workers and on the environment. The waste generated in these institutions essentially consists of solids and liquids, which may be hazardous, infectious and non-infectious. It has been estimated that up to 85% to 90% of the waste generated in hospitals is non -infectious (free with any body fluids, which is similar to domestic waste) . It is the remaining 10% to 20% of waste that is of concern because it is hazardous and infectious. In addition, waste that is un-segregated and not treated in the right manner would cause environmental pollution affecting the health of the community. Waste audits done at several hospitals by a few NGOs, arrived at some figures, which can now be used and extrapolated for the whole country. These audits must be conducted only after adequate training on waste segregation is given to healthcare institutions (Uzochukwu *et al*.,2016)

Proper handing, treatment and disposal of biomedical wastes are important elements of healthcare office infection control programme (Al-Shenqiti *et al*., 2017). Correct procedure will help protect healthcare workers, patients and the local community. If properly designed and applied, waste management can be a relatively effective and an efficient compliance-related practice. Proper collection and segregation of biomedical waste are important. At the same time, the quantity of waste generated is equally important (Kalpana *et al*., 2016).

Status of the health of an individual or community is determined by interplay and integration of micro (internal) environment of human beings and macro (external or surrounds) environment. Imbalance in these two may have serious repercussion on the national well-being. Therefore a balance has to be maintained in order to increase living standard and promote healthy society (Ndimela *et al.*, 2015).

"Nosocomial" term is used for any disease acquired by patient under medical care (Krishna, 2010). It is an infection acquired by patient during hospital stay. Recently, a new term "healthcare associated infections" is used for the type of infections caused by prolonged hospital stay and it accounts for a major risk factor for serious health issues leading to death (Apurva *et al*., 2010). About 75% of the burden of these infections is present in developing countries asymptomatic patients may be considered infected if these pathogens are found in the body fluids or at a sterile body site, such as blood or cerebrospinal fluid (Aliyu *et al*., 2006). Infections that are acquired by hospital staff, visitors or other healthcare personnel may be considered as nosocomial.

2. MATERIALS AND METHODS

2.1 Study area

The study was carried out within University of Calabar Teaching Hospital (UCTH) and General Hospital Calabar. The two hospitals are located within Calabar Municipal Local Government Area of Cross River State.

2.2 Sample collection

2.2.1 Passive air sampling (settle plates)

Passive air sampling was performed using settle plates. Petri-dishes containing a solid media comprising of Nutrient, MacConkey and Mannitol salt agar were left open to air for 30 minutes. Air samples were collected within their biomedical dumpsites, the reception unit and outside the hospitals as control. The plates were covered after 30 minutes and transported to the laboratory of Department of Microbiology for incubation at 37^0C for 24hrs.

2.2.2 Biomedical waste dumpsite soil samples

Biomedical waste soil samples for microbiological analysis were collected from sediment underlying the solid waste dumpsite located in UCTH and General Hospital, Calabar and another control sample was also collected from soil

samples adjacent the dumpsites. All the samples were transferred to polythene bags approved by Nigeria's Federal Environmental Protection Agency (FEPA). These were sealed and transported to the Laboratory of Department of Microbiology, University of Calabar for further analysis.

2.2.3 Hospital waste drippings

Waste water samples were collected from the drippings of the solid waste dumpsite of UCTH and General Hospital Calabar, into sterile universal containers. The samples were then transported to the laboratory for further analysis.

2.3 Materials

2.3.1 Media

Media used in this study were, Nutrient agar, MacConkey agar, Blood agar and Mannitol salt agar. These different media used in isolation were prepared according to the manufacturer's instructions.

2.3.2 Laboratory equipment

Laboratory equipment used for this study include; pressure pots, autoclave, masking tape, micro- wave oven, wire-loop, petri-dishes, conical flasks, stock bottles, universal containers, microscope, glass slides.

2.4 Methods

2.4.1 Isolation and enumeration of microorganisms from the collected samples

The biomedical waste dumpsite and waste water samples were processed as per the method of Apurva *et al.*, (2010). 1g/ml of the soil and waste water samples were separately suspended into 99mls of sterile distilled water and shaken vigorously for 2 minutes. Then the suspension were serially diluted using 10 fold serial dilutions and then 10^{-4} and 10^{-5} fold dilution was streaked onto nutrient, MacConkey and Manittol salt agar contained in petri-dishes and the plates were incubated at 37^0C for 24hours. After which the total viable, total coliform and total staphylococcal counts were recorded.

2.4.2 Purification of isolates

Following enumeration, colonies were picked at random and sub-cultured repeatedly onto Nutrient agar for purification. Purified isolates were stocked in Nutrient agar slants for further studies.

2.4.3 Characterization and identification of isolates

Purified isolates were characterized by gram morphology and biochemical test using the scheme in Bergey's Manual of Determinative Bacteriology.

3. RESULTS

3.1 Total microbial counts of samples collected from UCTH and General Hospital Calabar

Table 1 present the result of total microbial count of air samples within the waste dumpsite and reception unit of UCTH and General Hospital Calabar. It showed that the TVC, TCC and TSC of air within the dump sites of the hospital ranged from 164.1±0.5 to 179.0±0.8cfu/m^3, 21.0±0.2 to 30.0±0.3cfu/m^3, 6.0±0.1 to 9.0+0.2cfu/m^3 respectively, while the air within the reception unit (AR) ranged from 62.0±2.0 to 73.2±2.8cfu/m^3, 6.3±1.0 to 10.4±1.2cfu/m^3, 3.9±0.1 to 6.0±0.3cfu/m^3 respectively. Unlike the control air samples, a lower TVC, TCC and TSC were observed to range from 21.4±0.71 to 29.8±0.92cfu.m^3, 2.0±0.01 to 7.4±0.02cfu/m^3, 0 to 3.8±0.01cfu/m^3 respectively.

Table 2 present the result of the total microbial count of soil samples underlying solid waste and soil adjacent to the dump site of UCTH and General Hospital Calabar. It showed that the TVC, TCC, and TSC of the underlying solid waste samples from the two hospitals ranged from 2.4±0.4 x 10^8 to 3.9±0.8 x 10^8cfu/g, 2.0±0.6 x 10^4 2.9 ± 0.9 x 10^4cfu/g, 2.3±0.7 x 10^2 to 3.47±1.0 x 10^2cfu/g respectively, while that of samples from the soil adjacent to the dumpsite ranged from 4.2±0.2 x 10^7 to 4.8±0.2 x 10^8cfu/g, 2.2±0.5 x 10^4 to 2.4± 0.7 x 10^4cfu/g, 2.0. ± 0.1 x 10^2 to 2.81 ± 0.6 x 10^2cfu/g respectively. Similarly, a lower count was observed in the control soil from the two hospitals with the TVC, TCC and TSC ranging from 3.4±0.7 x 10^4 to 3.9±0.8 x10^4cfu/g, 1.8±0.2 x 10^3 to 2.0±0.3 x 10^3cfu/g, 1.7±0.1 x 10^2 1.9± 0.2 x 10^2cfu/g respectively. The microbial count of the dripping samples from the waste dumpsite in UCTH and General hospital Calabar had a TVC, TCC, and TSC that ranged from 7.4±1.8 x 10^7 to 9.2±2.0 x 10^7cfu/ml, 3.4±1.0 x 10^3 to 3.8±1.0 x 10^3cfu/ml and 1.5±0.6 x 10^2cfu/ml to 2.1±0.8 x 10^2cfu/ml respectively.

Table 3, present the result of microbial count of dripping samples from the waste dumpsite in UTCH and General hospital Calabar. It showed that the TVC, TCC and TSC of the dripping samples collected from the two hospital ranged from 7.4±1.8 x 10^7 to 9.2 ± 2.0 x 10^7cfu/ml. 3.4±1.0 x 10^3 to 3.8±1.0 x 10^3cfu/ml and 1.5±0.6 x 10^2cfu/ml to 2.1 ± 0.8x 10^2cfu/ml respectively.

3.2 Biochemical characterization and identification of isolates

Table 4 presents the result of biochemical characterization and identification of isolates from the biomedical waste samples. It showed that bacterial species isolated from the samples were identified as *Staphylococcus aureus*, *Strepotococcus sp.*, *Escherichia coli*, *Bacillus sp.*, *Klebsiella sp.*, *Corynebacterium sp.*, *Shigella sp.*, and *Pseudomonas sp.*

TABLE 1: Total microbial count of air samples within the waste dumpsite and reception unit of UCTH and General Hospital Calabar

Sample	UCTH Counts (cfu/m^3)			GHC Counts (cfu/m^3)		
	VTC	TCC	TSC	TVC	TCC	TSC
AW	164.1±0.5	21.0±0.2	6.0±0.1	179.0±0.8	30.0±0.3	9.0±0.2
AR	62.0±2.0	6.3±1.0	3.9±0.1	73.2±2.8	10.4±1.2	6.0±0.3
EA	21.4±0.71	2.0±0.01	-	29.8±0.92	7.4±0.02	3.8±0.01

Key: AW = Air with waste dumpsite, AR= Air within Reception unit, EA = Control air, TVC = Total viable count, TCC = Total coliform count, TSC = Total Staphylococcal count

TABLE 2: Total microbial count of soil samples underlying solid waste and soil adjacent to the dumpsite of UCTH and General Hospital Calabar

Sample	UCTH Counts (cfu/m^3)			GHC Counts (cfu/m^3)		
	VTC	TCC	TSC	TVC	TCC	TSC
SW	2.4±0.4x10^8	2.0±0.6 x10^4	2.3±0.7x 10^2	3.9±0.8x10^8	2.9±0.9x10^4	3.47±0.1x10^2
SD	2.4±0.2x10^7	2.2±0.5x10^4	2.0±0.1x10^2	4.8±0.2x10^8	2.4±0.7x10^4	2.81±0.6x10^2
ES	3.4±0.7x10^4	1.8±0.2x10^3	1.7±0.1x10^2	3.9±0.8x10^4	2.0±0.3x10^3	1.9±0.2x10^2

Key: SW= Soil underlying solid waste, SD = Soil adjacent dumpsite, ES= Control soil, TVC = Total viable count, TCC = Total coliform count, TSC = Total Staphylococcal count

TABLE 3: Total microbial count of air dripping samples from the waste dump site in UCTH and General Hospital Calabar

Sample	UCTH Counts (cfu/m^3)			GHC Counts (cfu/m^3)		
	VTC	TCC	TSC	TVC	TCC	TSC
D	7.4±1.8x10^7	3.4±1.0 x10^3	1.5±0.6x 10^2	9.2±0.2x10^7	3.8±1.0x10^3	2.1±0.8x10^2

Key: D = Dripping samples, TVC = Total viable count, TCC = Total coliform count, TSC = Total Staphylococcal count

TABLE 4: Biochemical characterization and identification of isolates from the biomedical waste samples

Isolate code	Gram –staining	Motility	Shape	Catalase	Coagulase	Oxidase	Urease	H_2S production	Indole production	Methyl –Red	Voges proskaur	Glucose	Mannitol	Lactose	Sucrose	Citrate	Probable organism
A_1	+	Non-motile	Cocci in cluster	+	+	-	-	-	-	+	-	+	+	-	-	-	*Staphylococcus aureus*
A_2	+	Non-motile	Cocci in chain	-	+	-	-	-	-	+	-	+	-	-	-	-	*Streptococcus sp.*
A_3	-	Motile	Rod	+	-	-	-	-	+	+	-	+	+	+	-	-	*Escherichia coli*
A_4	+	Motile	Rod	+	-	+	-	-	+	-	+	+	-	-	-	+	*Bacillus sp.*
A_5	-	Motile	Rod	+	-	-	-	-	-	-	+	+	-	+	+	-	*Klebsiella sp.*
A_6	+	Non-motile	Rod	+	-	-	-	-	+	+	-	+	-	-	-	-	*Corynebacterium sp.*
A_7	-	Non-motile	Rod	-	-	+	-	-	+	-	+	+	-	-	-	-	*Shigella sp.*

Key: + = Positive, - = Negative

3.3 Occurrence and distribution of the microbial isolates

Table 5 present the result of occurrence and distribution of the organisms identified samples collected with UCTH and General Hospital Calabar. It showed that *Staphylococcus aureus* and *Bacillus sp.*, were present in all the samples collected within the hospitals, while the other bacterial isolates had a varied distribution in the collected samples.

Table 5: Identified occurrence and distribution of the organisms from samples collected within UCTH and general hospital, Calabar

Organisms	AW	AR	SW	D
Staphylococcus aureus	+	+	+	+
Escherichia coli	-	-	+	-
Klebsiella sp.	+	-	+	+
Bacillus sp.	+	+	+	+
Streptococcus sp.	-	+	+	-
Pseudomonas sp.	-	-	+	+
Corynebacterium sp	-	-	+	-
Shigella sp.	+	-	+	+

Key: + = Present, -= Absent, AW = Air within waste dumpsite, AR = Air within reception unit, SW = Soil underlying solid waste, D = Dripping sample

4. DISCUSSION

The study to evaluate biomedical waste and nosocomial infection was investigated. Result obtained revealed that the air microbial quality of the waste dumpsite (AW) within UCTH and General Hospital, Calabar had a total viable count (cfu/m^3) that ranged from 164.1±0.5 to 179.0±0.8cfu/m^3, total coliform count that ranged from 21.0±0.2 to 30.0±0.3cfu/m^3 and total Staphylococcal count that ranged from 6.0±0.1 to 9.0+0.2cfu/m^3. The air microbial quality within the reception units recorded a total viable count that ranged from 62.0±2.0 to 73.2±2.8cfu/m^3, total coliform count that ranged from 6.3±1.0 to 10.4±1.2cfu/m^3, and total staphylococcal count that ranged from 3.9±0.1 to 6.0±0.3cfu/m^3 respectively. However, the microbial load of the air around the hospitals waste dumpsite and the air within the hospital reception showed high microbial load when compared with the control sample (EA) from the two hospitals with a total viable count that ranged from 21.4±0.71 to 29.0±0.92cfu/m^3, total coliform count of 2.0±0.01 to 7.4±0.2cfu/m^3, and total staphylococcal count that ranged from 0 to 3.8±0.06cfu/m^3. This observation was not surprising as similar study by Ndimela *et al.*, (2015) who reported high level of air microbial contamination in some teaching hospitals biomedical waste dump site in South-Eastern Nigeria. Similar study by Uzochukwu *et al.*, (2016) also reported high level of microbial contamination in air around hospital waste dumpsites in Imo state University Teaching Hospital, Orlu. This observation also agrees with that of Apurva *et al.*, (2010) who reported high bacterial load in the extra mural environment. This observation in this study could be attributed to the biomedical waste dumpsite in the hospitals as they encourage the growth and proliferation of microorganisms which are easily carried by air. Also the dumpsite environment influenced the count obtained from the reception area directly or indirectly and this could have a direct effect on humans in the hospital as well as an increased in disease condition or nosocomial infection (Ndimele *et al.*, 2015). Total microbial count of soil samples underlying the solid waste (SW) and soil adjacent to the dumpsite (SD) in the samples from UCTH and General Hospital, Calabar ranged from 164.1±0.5 to 179.0±0.8cfu/g (TVC), 2.0 ± 0.6 x 10^4g to 2.9±0.9 x 10^4cfu/g (TCC), 2.3±0.7 x 10^2 to 3.47±1.0 x 10^2cfu/g (TSC) and 4.2±0.2 x 10^7 to 4.8±0.2 x 10^8cfu/g (TVC), 2.2±0.5 x 10^4 to 2.4±0.7 x 10^4cfu/g (TCC), 2.0±0.1 x 10^2 to 2.8±0.6 x 10^6cfu/g (TSC) respectively. In the control samples, a lower TVC (3.4±0.7 x 10^4 to 3.9±0.8x 10^4 cfu/g), TSC (1.8 ±0.2 x 10^3 to 2.0±0.3 x 10^3cfu/g), TSC (1.7±0.1 x 10^2 to 1.9±0.2 x10^2cfu/g) was observed as compared to the samples from the soil underlying solid wastes and soil adjacent to the dumpsite. This observation was in line with that of Oyeleke and Istifanus (2009) and Ndimele *et al.*, (2014) who reported a higher total viable and coliform counts in soil samples from biomedical waste dumpsites as compared to that in control soil samples collected from Abia. The high counts of microbial load in the study sites is a reflection of the level of pollution in the environment (Aliyu *et al.*, 2006), and this is an indication of the amount of organic matter present. The high microbial counts observed in this study is of public health as these organisms can easily be carried into the hospitals environment by foot or other means which could increase the risk of infection.

Variations in the microbial site in UCTH and General Hospital, Calabar was observed. The TVC ranged from 7.4±18 x 10^7 to 9.2±2.010^{7}cfu/ml, the TCC ranged from 3.4±1.0 x 10^3 to 3.8±1.0 x 10^3cfu/ml, while the TSC ranged from 1.5±0.6 x 10^2cfu/ml. This water that drips from the hospital waste dumpsites are contaminated with potential microorganisms, in which *Klebsiella sp* are responsible for 3-7 percent of hospital acquired bacterial infections and stands as the eight significant pathogen in healthcare settings (Hassan *et al.,* 2015). It usually colonizes gastrointestinal tract, pharynx and skin and it yets involved in disease such as neonatal, septicemia, pneumonia and wound infections. *Pseudomonas aeruginosa* contributes to 11% of all nosocomial infections and it is a cause of surgical and wound infections, UTI, pneumonia, cystic fibrosis and bacteremia (Hassan *et al.,* 2015).

5. CONCLUSION

The high microbial load densities, the type as well as the occurrence and distribution of microbes encountered in this study, suggests that the activities of hospital biomedical wastes in the environment is a major health and environmental threat and also a potential source of nosocomial infections. Therefore, there is an urgent need to raise awareness, education and management strategies on medical waste issues to ensure public health and environmental safety.

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