ANTIBIOTIC RESISTANCE PATTERN OF BACTERIAL ISOLATES OF LIQUID WASTES AND WASTE DUMP SOILS OF HOSPITALS IN OWERRI, NIGERIA

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Abstract: The present study investigates the prevalence of antibiotic resistance and resistance patterns among bacterial isolates from liquid wastes and waste dump soils of hospitals in Owerri metropolis Nigeria. The bacterial isolates were identified on the basis of standard cultural, morphological and biochemical characteristics. Antibiotic susceptibility of the isolates was tested by disc diffusion method. A total of 115 bacteria species belonging to 11 different genera were isolated from the hospital waste water and waste dump soils. Multidrug resistance was observed in all the waste water and waste dump soil isolates. The waste water and waste dump soil isolates were all resistant to Amoxycillin, Tetracycline, Gentamycin, Erythromycin and Chloramphenicol. However, of the waste water isolates, 4 (66.7%) were sensitive to Zinnacef, 3 (50%) to Nitrofurantoin and Pefloxacin, while 2 (33.3) were sensitive to Ciprofloxacin. While in the waste dump soils, all the isolates except *Bacillus* spp. 4 (80%) were also sensitive to Nitrofurantoin, while all except *Bacillus* spp. and *S. aureus* 3 (60%) were sensitive to Zinnacef . The high prevalence of antibiotic-resistant bacteria with various degrees of resistance to antibiotics observed in this study could represent a potential public health risk. This will contribute to the hazards of poorly managed hospital waste. To help check this growing problem of antibiotics resistance, this study recommends a national policy on health care waste management and formulation of a national drug policy in Nigeria.

Keywords: Antibiotics resistance, environmental bacteria, hospital wastes, public health

Introduction

With the advent of the antibiotics, more drugs were developed to treat serious infections. The indiscriminate and irrational use of antibiotics by health personnel and self medication by patients has increased resistance of microbes to these drugs (Ku¨mmerer, 2004; WHO, 2011). These antibiotics are also used as additives in animal feed which further complicates resistance (European Food Safety Authority, 2008; WHO, 2011)

Improper disposal of untreated hospital waste into rivers, drainages and roads especially in developing nations creates a major problem on public health (Alagoz and Kocasay, 2007; Abah and Ohimain, 2010) and is of major concern. The release of bacteria into the environment, e.g. through hospital wastewater, favours the exchange of genetic materials with previously non-resistant populations thereby increasing the dispersion of resistant capacity in the environment (Davison, 1999). If the

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hospital effluents are not treated, concentrated forms of infectious agents and antibiotic resistant microbes are shed into the environment resulting in the spread of antibiotic resistant genes and of diseases such as cholera, typhoid fever and gastroenteritis.

Trace amounts of antibiotics have been detected in waste water effluents (Kolpin *et al.*, 2002), this has the potentials to selected and develop antibiotic resistance in microbes when they are exposed to it for a long time (Smith *et al.*, 1999). Thus, when such selective pressure contributes to persistence and dissemination of resistant genes, the natural environments become reservoirs of resistant bacteria and resistance genes (Tacão *et al.*, 2012). The transfer of genetic material by conjugation among free-cells could be very restricted in aquatic environments, but it could occur in wastewaters where there is presence of antibiotics, solid flocs and sludge and contact among large numbers of bacteria promoted by their high density (Reinthaler *et al.*, 2003).

The persistence of antibiotic resistant bacteria and the ability to spread its genetic information in environment is largely determined by their capacity to survive under adverse conditions occasioned by biotic and abiotic factors. Although abiotic factors (adverse temperatures, depletion of nutrients, light, salinity and others) may not remove resistant bacteria, they can induce viable but non cultural state in some bacteria (Barcina and Arana, 2009). In this state, the cells retain activity and are able to exchange genetic information and, in some cases recover culturability. The effect of biotic factors is mainly predation by protozoa that effectively eliminate prey bacteria. This may be a crucial process in preventing bacterial and resistant gene spread.

The input of resistant bacteria as well as of antibiotics to the environment seems to be an important source of resistance in the environment; it thus constitutes a major threat to public health. Therefore, the development of antibiotic resistance in bacteria and their spread in the environment is a serious public health concern because an individual patient can develop an antibiotic resistant infection by contacting a resistant organism and can spread it in the communities.

Hospitals are meant to safeguard the health of the community. They however, produce wastes that carry high potential for infection and injury. The waste produced by the hospitals if disposed of improperly, can pose an even greater threat than the original diseases. Inadequate and improper handling and treatment may have serious public health consequences and a significant impact on the environment, affecting patients, staff and the community (Sharma *et al.*, 2010; Ndidi *et al.*, 2009; Abah and Ohimain, 2011; Ogbonna, 2011). In Nigeria, majority of the hospitals do not practice safe waste treatment and disposal (Ogbonna *et al.*, 2012; Abah and Ohimain, 2010). Thus, the aim of this study was to assay the antibiotics resistance pattern of bacterial isolates of liquid wastes and waste dump soils of hospitals in Owerri metropolis. This study is imperative to explore the antibiotic pressure on the environment as a result of the hospital waste management and disposal. There has also not been any study on the impact of poor waste management and disposal on the prevalence and resistance pattern of antibiotic resistant bacteria in environmental samples.

Materials and methods

Nine hospitals in Owerri metropolis were selected as representative of health care institutions in the area. The hospitals are the Federal Medical Centre, Federal University of Technology Owerri Medical Centre, Imo State Specialist Hospital, Umuguma, St. David Hospital, Amanda Hospital, Umezurike Hospital, Holy Rosary Hospital Emekuku, Christiana Hospital Egbu, Federal Polytechnic Nekede Medical Centre.

Collection of samples

Hospitals' liquid waste

Hospital effluent water specimens (1500 ml) were collected for each of the nine hospitals studied from the outermost chambers before discharging into the public drainage system, following standard procedures. Samples were collected in clean sterile containers. The collected samples were taken to the laboratory in an ice box within an hour of collection for analysis.

Hospitals' waste dump site soil

The soil samples (100 g) were collected from waste dump site of the hospitals. Surface soils (0 – 10 cm) were randomly collected using a sterile trowel. It was then bulked to form a composite sample and then transported immediately to laboratory for analysis within two hours of collection.
Three samples each were collected from the respective sampling station in each hospital.

Isolation, characterization and identification of bacterial isolates

A 10 fold serial dilution of the water and soil samples was done and aliquots (0.1 ml) of appropriate dilutions of samples' suspensions streaked on freshly prepared nutrient agar plates supplemented with fulcin (500 mg/l) an antifungal agent, and incubated aerobically at 37°C for 24 h.

Bacterial colonies differing in size, shape and colour in different plates were selected and further sub-cultured on nutrient agar by the streak plate technique and then incubated at 37°C for 24 h after which, they were transferred to and maintained on agar slants in a refrigerator till further characterization and identification.

The bacterial isolates were characterized based on colonial and cell morphology, growth on differential selective media and biochemical tests which include Gram's reaction, indole tests, methyl red, Voges-Proskauer, citrate utilization, motility, endospore formation, utilization of carbohydrates such as glucose, sucrose, mannitol, lactose and fructose, oxidase, catalase, coagulase and starch hydrolysis test (Oyeleke and Manga, 2008). The bacterial isolates were identified by comparing their characteristics with those of known taxonomy using the schemes of Cowan and Steel (1993).

Antibiotic resistance test

The resistance of the bacterial isolates of water and soil samples to antibiotics was assayed according to the Kirby–Bauer disc diffusion method (Bauer *et al.*, 1996).

All the plates were incubated for 20 minutes to allow excess moisture dry before inoculation and placement of antibiotic discs. A single loop of each isolate was inoculated into sterile normal saline and standardized. The standardized suspension was aseptically inoculated on the surface of Mueller–Hinton agar plates and antibiotic

sensitivity discs were aseptically laid on the surface of plates to test for the resistance pattern of bacteria isolates. The antibiotics are Zinnacef (10 µg), Nitrofurantoin (10 µg), Tetracycline (30 µg), Ciprofloxacin (20 µg), Amoxycillin (20 µg), Erythromycin (10 µg), Chloramphenicol (30 µg), Pefloxacin (20 µg) and Gentamycin (30 µg).

The plates were incubated at 37°C for 24 hours. After incubation, zone of growth inhibition around each disc was measured and used to classify the organisms as sensitive or resistant to the antibiotic following the interpretive standard of the Clinical and Laboratory Standards Institute (2012). Antibiotic sensitivity test was carried out on all isolates.

Results

Prevalence of aerobic bacteria in hospitals' waste effluent.

The result of the prevalent bacteria species of the waste water samples from the various hospitals examined is shown in Table 1.

Table 1: Prevalence of aerobic bacteria in hospitals' waste effluent (%)

Hospitals	No of Isolates	*Klebsiella* spp.	*Pseudo.* spp.	*E. coli*	*S. aureus*	*Staph.* spp.	*Strept.* spp.	*Others
H1	9	1	2	3	1	1	-	1
H2	4	-	-	1	1	-	1	1
H3	5	-	1	2	-	2	-	-
H4	9	1	2	3	1	1	1	-
H5	5	-	1	2	-	-	2	-
H6	8	2	1	3	-	2	-	-
H7	9	-	2	2	3	1	1	-
H8	7	1	-	2	1	-	2	1
H9	6	-	-	3	1	-	1	1
	62	5 (8.06%)	9 (14.5%)	21 (33.9%)	8 (12.9%)	7 (11.3%)	8 (12.9%)	4(6.45%)

Table 1 Legend: H1-H9, 9 hospitals studied;*, *Salmonella* spp., *Proteus* spp., *Enterobacter* spp.; *Staph.* spp., *Coagulase negative Staphylococcus* spp.; *Strept.* spp., *Streptococcus* spp; *Pseudo.* spp, *Pseudomonas* spp.

From the result, *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *S. aureus*, *Coagulase negative Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., *Proteus* spp. and *Enterobacter* spp. were the bacterial species isolated. *E. coli* had the highest occurrence in the hospital waste water samples (33.9%) while *Salmonella* spp. *and Enterobacter* spp. had the least occurrence of 1.61% each (Table 1).

Prevalence of aerobic bacteria in hospitals' waste dump soil.

The prevalent aerobic bacteria species isolated from the hospital waste dump site soils samples in the various hospitals examined are shown in Table 2.

Table 2: Prevalence of aerobic bacteria in hospitals' waste dump soil

Hospitals	No of Isolates	*Klebsiella* spp.	*Pseudomonas* spp.	*E. coli*	*S. aureus*	*Bacillus* spp.	*Others
H1	9	-	2	3	2	2	-
H2	4	1	1	1	-	1	-
H3	7	1	-	2	1	2	1
H4	8	-	1	3	1	2	1
H5	6	1	2	2	1	-	-
H6	3	-	-	1	1	1	-
H7	6	-	2	2	1	1	-
H8	4	-	1	2	-	1	-
H9	6	-	1	2	1	2	-
	53 (100%)	3 (5.7%)	10 (18.9%)	18 (33.9%)	8 (15.1%)	12 (22.6%)	2 (3.8%)

Table 2 Legend: H1-H9, 9 hospitals studied;, Enterobacter spp. and Citrobacter spp.*

From the result *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, *S. aureus*, *Bacillus* spp., *Enterobacter* spp. and *Citrobacter* spp. were the species isolated. *E. coli* had the highest occurrence in the hospitals' waste dump soil 18 (33.9%) while *Enterobacter* spp. and *Citrobacter* spp. had the least occurrence of 1 (1.89%) each (Table 2).

Resistance to antibiotics among bacterial isolates of hospitals' waste effluent.

From the result, the isolates were all resistant in various degrees to the antibiotics Amoxycillin, Tetracycline, Gentamycin, Erythromycin and Chloramphenicol (Table 3).

Table 3: Percentage resistance to antibiotics among bacterial isolates of hospitals' waste effluent

Antibiotics	*Klebsiella* spp.	*Pseudomonas* spp.	*E. coli*	*S. aureus*	*Staph.* spp.	*Strept.* spp.
Pefloxacin	0.0	33.3	32.0	24.3	0.0	0.0
Amoxycillin	83.6	89.8	80.5	35.1	11.3	17.4
Tetracycline	54.0	87.3	78.5	67.7	33.0	66.5
Nitrofurantoin	0.0	56.6	10.0	14.2	0.0	0.0
Zinnacef	0.0	0.0	0.0	17.5	0.0	12.0
Ciprofloxacin	67.0	33.3	6.3	17.4	0.0	0.0
Gentamycin	81.4	87.8	76.0	41.3	23.5	11.4
Erythromycin	18.4	74.3	77.0	69.2	10.3	23.5
Chloramphenicol	80.0	83.3	93.0	68.9	21.0	44.0

Table 3 Legend: Staph. spp., Coagulase negative Staphylococcus spp.; Strept. spp., Streptococcus spp.

S. aureus and *Streptococcus* spp. were the only isolates resistant to Zinnacef, 17.5% and 12.0% respectively. *Pseudomonas* spp., *E. coli* and *S. aureus* were resistant to Pefloxacin and Nitrofurantoin, while *Klebsiella* spp., *Pseudomonas* spp., *E. coli* and *S. aureus* were resistant to Ciprofloxacin (Table 3).

Resistance to antibiotics among bacterial isolates of hospitals' waste dump soil.

From the result, all the isolates were resistant in various degrees, to Amoxycillin, Tetracycline, Gentamycin, Erythromycin and Chloramphenicol.

Table 4: Percentage resistance to antibiotics among bacterial isolates of hospitals' waste dump soil

Antibiotics	*Klebsiella* spp.	*Pseudomonas* spp.	*E. coli*	*S. aureus*	*Bacillus* spp.
Pefloxacin	0.0	22.0	14.0	0.0	10.0
Amoxycillin	80.0	78.0	67.0	44.0	54.0
Tetracycline	44.0	83.3	56.4	70.5	77.3
Nitrofurantoin	0.0	0.0	0.0	0.0	66.0
Zinnacef	0.0	0.0	0.0	10.0	10.0
Ciprofloxacin	34.0	22.0	10.0	11.0	0.0
Gentamycin	78.0	54.9	56.0	29.5	74.0
Erythromycin	10.0	77.5	46.0	23.8	67.3
Chloramphenicol	57.0	67.7	63.0	30.0	78.0

All the isolates except Bacillus spp. were sensitive to Nitrofurantoin. All the isolates, except Bacillus spp. and S. aureus were also sensitive to Zinnacef (Table 4).

Hospital waste water bacterial isolates and antibiotics to which they are sensitive.

The result of the hospital waste water bacterial isolates and antibiotics they are sensitive to is shown in Table 5.

Table 5: Hospital waste water bacterial isolates and antibiotics to which they are sensitive

Isolates	Antibiotics to which they are sensitive
Klebsiella spp.	Pefloxacin, Nitrofurantoin, Zinnacef
Pseudomonas spp.	Zinnacef
E. coli	Zinnacef
Staphylococcus aureus	Nil
Staphylococcus spp.	Pefloxacin, Nitrofurantoin, Zinnacef, Ciprofloxacin
Streptococcus spp.	Pefloxacin, Nitrofurantoin, Ciprofloxacin

From the result, *S. aureus* was the most resistant species isolated, being sensitive to none of the antibiotics tested. The coagulase negative *Staphylococcus* was the most susceptible bacterial isolate and is susceptible to Pefloxacin, Nitrofurantoin, Zinnacef, Ciprofloxacin. *Pseudomonas* spp. and *E. coli* were susceptible to only Zinnacef (Table 5).

Hospital waste dump soil bacterial isolates and antibiotics to which they are sensitive.

The result of the hospital waste dump soil bacterial isolates and antibiotics they are sensitive to is shown in Table 6.

Table 6: Hospital waste dump soil bacterial isolates and antibiotics to which they are sensitive

Isolates	Antibiotics to which they are sensitive
Klebsiella spp.	Pefloxacin, Nitrofurantoin, Zinnacef
Pseudomonas spp.	Zinnacef, Nitrofurantoin
E. coli	Zinnacef, Nitrofurantoin
Staphylococcus aureus	Pefloxacin, Nitrofurantoin, Zinnacef
Bacillus spp.	Nitrofurantoin, Zinnacef, Ciprofloxacin

The result shows that *Klebsiella* spp. and *S. aureus* were sensitive to Pefloxacin, Nitrofurantoin, Zinnacef, while *Bacillus* spp. was sensitive to Nitrofurantoin, Zinnacef, Ciprofloxacin. *Pseudomonas* spp. and *E. coli* were susceptible to only Zinnacef and Nitrofurantoin (Table 6).

Discussion

The bacterial species isolated in the hospitals' waste effluents studied were identified as *Klebsiella* spp., *Pseudomonas* spp., *E. coli, S. aureus,* Coagulase negative *Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., *Proteus* spp. and *Enterobacter* spp. The sources of liquid waste in hospitals include indoor and outdoor departments, operational theatres and laboratories. It is of major concern that these waste waters are contaminated with blood and other infectious materials. *Klebsiella* spp., *Pseudomonas* spp., *E. coli, S. aureus, Bacillus* spp., *Enterobacter* spp. and *Citrobacter* spp., were also isolated from the hospitals' waste dump soils. The *E. coli, Streptococcus* spp., *Salmonella* spp. and *Enterobacter* spp. are pathogens which may have entered the waste water and waste dump soil from contaminating infectious materials and/or faecal matter. *S. aureus, Pseudomonas* spp. and Coagulase negative *Staphylococcus* spp. are opportunistic pathogens whose discharge with the waste water and solid waste may lead to serious public health issues. *Klebsiella* spp., *Pseudomonas* spp., *E. coli*, Staphylococci, *Proteus* spp. *and Salmonella* spp. have been reported by previous studies as pathogens commonly isolated from hospital wastes (Mizra, 1996; Akande, 1999). Omojasola *et al.* (2009) also observed the presence of *Streptococcus pyogenes, Pseudomonas* spp., *E. coli* and *Actinomyces israelii* in hospital waste and concluded that they portend a potential health hazard to the environment. The high prevalence of *E. coli* in the waste water and soil (33.9% each) may indicate feacal contamination. Presence of diapers in the solid waste was a common sight in most of the waste dumps.

The results of the antibiotics resistance pattern of the isolates from the waste water and waste dump soil indicated that the bacterial strains isolated have developed resistance to most of the antibiotics tested. All the isolates from the waste water and waste dump soil showed multi-drug resistance to Amoxycillin, Tetracycline, Gentamycin, Erythromycin and Chloramphenicol. Thus these antibiotic agents might be ineffective as therapeutic agents of choice in the treatment of infections caused by these organisms. Bacteria resistant to antibiotics and disinfectants have been detected in environmental compartments such as waste water,

surface water, ground water, sediments and soils (Ku¨mmerer, 2004). The emergence and spread of resistance in pathogenic bacteria that consequently cannot be treated by previously successful regimens, is the greatest threat to the use of antibiotics. Once in the environment, these antibiotic resistant species can transfer such resistance to pathogenic bacteria that will cause infections and spread the resistance in the communities.

The resistance observed here may have been as a result of long exposure of the isolated species to the antibiotics in the hospitals which must have allowed the development of mechanisms for evading antibiotic bactericidal activities by the strains. This may be from the use, misuse and under-use of antibiotics and from the release into the environment of resistant species from patients with untreated or poorly treated hospital wastes. This assertion is supported by the report of Kolpin *et al.* (2002) that trace level concentrations of antibiotics have been discovered in waste water treatment plant effluents and surface waters. Smith *et al.* (1999) also reported that long term exposure of microorganisms to low concentrations of antibiotics in wastewater has the potential for the development of antibiotic resistance in the organisms. Lateef (2004) also reported that in developing countries, drugs are available to the public and thus people may practice self–administration of antibiotics and further increase the prevalence of drug resistant strains.

The result of the hospital waste water bacterial isolates and antibiotics they are sensitive to showed that the isolates are most sensitive to Zinnacef, followed by Pefloxacin and Nitrofurantoin, and then Ciprofloxacin, while the isolates of the dump soil are most sensitive to Zinnacef and Nitrofurantoin, followed by Pefloxacin and then Ciprofloxacin. The high sensitivity recorded with these drugs could be because they are relatively new and not commonly used compared to the others tested. This suggests a relationship between antibiotic usage and the level of drug resistance. This shows that most antibiotic resistant infections can still be treated, however, where infections are resistant to the antibiotics of choice, the length of hospital stay may increase, patient recovery may also be slower, and costs to health services and taxpayers will increase dramatically. Drug resistance to commonly used antibiotics, may force hospital doctors to turn to more toxic antibiotics which can cause serious side effects.

The resistance to antibiotics used in this study by the bacterial isolates is very alarming, and highlights the global problem of antibiotics resistance to public health. Antibiotics resistant pathogens contribute to the hazards of poorly managed waste. They can enter the human body through wounds present on the skin (Omojasola *et al.*, 2009). This problem may continue to be fuelled by the dumping of untreated liquid and solid wastes from hospitals into municipal drain and dump sites. Since resistance to antibiotics has no ecological, sectoral or geographical borders, its appearance in one sector or country affects resistance in other sectors and countries. Thus national authorities, veterinarians, physicians, patients and farmers all have key roles in preserving the power of antibiotics. This requires addressing all risk factors for the development and spread of antibiotic resistance across the full spectrum of conditions, sectors, settings

from health care to use in food-animal production (WHO, 2011).

This study calls for a national policy on health care waste management and formulation of a national drug policy in Nigeria that will ensure proper health care waste management and rational drug use respectively. None of these is in existence in Nigeria at the moment.

References

Abah, S. O. and Ohimain, E. I. (2011). Healthcare waste management in Nigeria: A case study. *Journal of Public Health and Epidemiology* **33**: 99-110.

Abah, S. O. and Ohimain, E. I. (2010). Assessment of Dumpsite Rehabilitation Potential using the Integrated Risk Based Approach: A case study of Eneka, Nigeria. *World Applied Sciences Journal* **4:** 436 - 442.

Akande, T. M. (1999). Hospital waste discharge and health risk. *Medical Digest* 8-13.

Alagoz, B. A. Z. and Kocasoy, G. (2007). Treatment and disposal alternatives for healthcare wastes in developing countries; A case study in Isanbul, Turkey. *Waste Management and Research* **25:** 83-89

Barcina, I. and Arana, I. (2009). The viable bit nonculturable crossroads in the life-cycle of non-differentiating bacteria. *Reviews in Environmental Science and Biotechnology* **8:** 245-255

Bauer, A. W., Kirby, W. M., Sheries, J. C. and Turck, M. (1996). Antibiotic, Susceptibility testing by a Standardized single disc method. *American Journal of Clinical Pathology* **45:** 49-58.

Clinical and Laboratory Standards Institute (2012). *Performance standard for antimicrobial susceptibility testing*, Twenty-Second Informational Supplement. CLSI document M-100 S22 CLSI, Wayne Pennsylvania USA.

Cowan, S. T. and Steel, K. J. (1993). *Manual for the Identification of Medical Bacteria*. 2nd Ed. London: Cambridge University Press, pp. 205-209.

Davison, J. (1999). Genetic exchange between bacteria in the environment. *Plasmid* **42**: 73-91.

European Food Safety Authority (2008). Foodborne antimicrobial resistance as a biological hazard, Scientific Opinion of the Panel on Biological Hazards, Adopted on 9 July 2008, http://www.efsa.europa.eu/de/scdocs/doc/765.pdf

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Turman, E. M., Zaugg, S. D., Barber, L. B., Buxton, H. T. (2002). Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. *Environmental Science and Technology* **36:** 1202-1211.

Ku¨mmerer, K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy* **54:** 311-320.

Lateef, A. (2004). The Microbiology of a Pharmaceutical Effluents and Its Public Health Implications. *World Journal of Microbiology and Biotechnology* **22:** 167-171. http://dx.doi.org/10.1023/B:WIBI.0000021752.29468.4e

Mizra, N. (1996). Hospital waste disposal in Nairobi. *Africa Health* **18:** 12-13.

Ndidi, N., Ochekpe, N., Odumosu, P. and John, S. A. (2009). Waste management in healthcare establishments within Jos Metropolis, Nigeria. *African Journal of Environmental Science and Technology* **3:** 459-465.

Ogbonna, D. N. (2011). Characteristics and waste management practices of medical wastes in healthcare institutions in Port Harcourt, *Nigerian Journal of Soil Science and Environmental Management* **2 (5):** 132-141.

Ogbonna, D. N., Chindah, A. and Ubani, N. (2012). Waste management options for health care wastes in Nigeria: A case study of Port Harcourt hospitals, *Journal of Public Health and Epidemiology* **4:** 156-169. DOI: 10.5897/JPHE12.012

Omojasola, P. F., Adetitun, D. O., Oshin, O. O. and Omojasola, T. P. (2009). Environmental Health Hazard Assessment of Hospital Wastes in Ilorin Metropolis. *Nigerian Journal of Microbiology* **23:** 1882 – 1889.

Oyeleke, S. B. and Manga, B. S. (2008). *Essentials of Laboratory Practical in Microbiology*, 1st Ed. Tobest Publishers, Minna, Nigeria. Pp. 28 – 62.

Reinthaler, F. F., Posch, J., Feierl, G., Wüst, G., Haas, D., Ruckenbauer, G., Mascher, F. and Marth, E. (2003). Antibiotic resistance of E. coli in sewage and sludge. *Water Research* **37:** 1685-1690. doi:10.1016/S0043-1354(02)00569-9.

Sharma, D. R., Pradhan, B. and Mishra, S. K. (2010) Multiple drug resistance in bacterial isolates from liquid wastes generated in central hospitals of Nepal. *Kathmandu University Medical Journal* **8**: 40 – 44.

Smith, K. E., Besser, J. M., Hedberg, C. W., Leano, F.T., Bender, J. B., Wicklund, J. H., Johnson, B. P., Moore, K. A. and Osterholm, M. T. (1999). Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. *The New England Journal of Medicine* **340:** 1525-1532. Doi: 10.1056/NEJM199905203402001

Tacão, M., Correia, A. and Henriques, I. (2012). Resistance to broad-spectrum antibiotics in aquatic systems: anthropogenic activities modulate the sissemination of blaCTX-M-like genes. *Applied and Environmental Microbiology*. **78:** 4134–4140.

World Health Organization (2011). *Tackling antibiotic resistance from a food safety perspective in Europe.* World Health Organization http://www.euro.who.int/_data/assets/pdf_file/0005/136454/e94889.pdf.