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ISOLATION AND ANTIBIOGRAM OF ESCHERICHIA COLI ISOLATED FROM HOSPITAL REFUSE DUMP SITES

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ABSTRACT: Hospital waste is a potential health hazard to health care workers. Wastes in dump sites with no proper waste handling method are a source of pathogens to the soil, which in turn contribute to the emergence of community acquired infections. Soil samples were collected from two different hospitals in Awka, Anambra State. This study isolated, identified and determined the antibiogram of genetic bacteria isolated from waste dump sites in some selected hospitals in Awka metropolis, Nigeria. The bacterial isolates were identified on the basis of standard cultural, morphological and

biochemical characteristics. Antibiotic susceptibility of the isolates was evaluated using the Kirby Bauer disc diffusion method. Different bacterial species were isolated from the hospital waste dump soil. The bacterial isolates included Escherichia coli, Staphylococcus aureus, Salmonella sp, and Psudeomonas aeruginosa. The isolates were resistant to these antibiotics like Amoxicillin, Ciprofloxacin and Levofloxacan. The presence of resistant enteric pathogenic species at the various dump sites indicates poor management of antibiotic disposal in hospitals. Effective waste management practice should be put in place so as to control the wide spread of these antibiotic-resistant bacteria in the environment resulting to public health challenges.

Keywords: *Escherichia Coli, Antibiotic Resistance, Antibiogram, Hospital, Dumpsite*

Introduction

Waste generated within healthcare facilities represents a significant environmental and public health concern on a global scale. Such waste streams are produced during medical activities including diagnosis, treatment, immunization, and research involving humans and animals. Healthcare waste encompasses a wide spectrum of materials, ranging from relatively harmless refuse to highly hazardous substances. Of particular concern are infectious components which, when poorly managed, pose serious threats to human health and ecological systems (Awari et al., 2023). Inadequate waste handling practices frequently result in the uncontrolled release of hospital waste into surrounding soils and environments, thereby contributing to environmental degradation within and around medical facilities.

Hospital-generated waste is characteristically diverse, consisting of discarded biological materials and chemical substances that are no longer suitable for use. These include blood and body fluids, microbial cultures, sharps such as syringes and scalpels, expired pharmaceuticals, chemical reagents, radioactive materials, contaminated dressings, and other clinical disposables. Many of these materials harbor pathogenic microorganisms, including drug-resistant strains, making improper disposal a serious hazard to both environmental safety and public health (Ranjita et

al., 2012; Anitha et al., 2012; Awari et al., 2024). When mismanaged, such wastes become reservoirs for disease transmission and environmental contamination.

The uncontrolled discharge of untreated hospital waste introduces a complex mixture of pollutants into natural ecosystems. These contaminants include toxic chemicals, radionuclides, and antibiotic-resistant bacteria, all of which can disrupt ecological balance and microbial community dynamics (Andy et al., 2018; Awari et al., 2023). Particularly alarming is the environmental dissemination of multidrug-resistant (MDR) organisms, which are commonly associated with hospital settings and are increasingly recovered from soils surrounding healthcare waste dumpsites (Obasi et al., 2024). These resistant bacteria often originate from clinical environments and are subsequently released into the environment through indiscriminate disposal practices (Sunday et al., 2012).

The challenge of hospital waste management is especially pronounced in low- and middle-income countries such as Nigeria, where waste treatment facilities and regulatory enforcement are often inadequate. The release of untreated hospital effluents allows high concentrations of infectious agents and antibiotic-resistant organisms to enter surrounding environments, facilitating the horizontal spread of antibiotic resistance genes (ARGs) among environmental microorganisms (Tacão et al., 2012). Empirical studies conducted in Nigeria have demonstrated the persistence, adaptability, and public health significance of pathogenic bacteria in contaminated soils and waste sites (Adepeju et al., 2023; Agu et al., 2013).

Although hospitals are designed to protect and restore health, paradoxically, the waste they generate may pose risks exceeding those of the diseases they treat if not properly managed. Exposure to improperly disposed medical waste has been associated with healthcare-associated infections, occupational hazards, and environmental contamination (Medubi et al., 2006; Awah et al., 2016). Consequently, effective surveillance, regulation, and management of hospital waste are critical to minimizing environmental contamination and preventing pathogen dissemination.

Among the bacterial species frequently encountered in hospital waste environments, *Escherichia coli* is of particular importance. This Gram-negative member of the family *Enterobacteriaceae* is a well-known etiological agent of a wide range of gastrointestinal illnesses, including traveler's diarrhea and dysentery. The species encompasses numerous strains with pathogenic potential, capable of causing diseases that range from mild infections to severe, life-threatening conditions such as septicemia and renal failure. Its clinical relevance is amplified by its remarkable ability to acquire and disseminate antimicrobial resistance determinants (Awari et al., 2024; Obasi et al., 2024).

Originally identified in 1885 by Theodor Escherich as *Bacterium coli commune*, *E. coli* was first regarded as a harmless intestinal commensal. Subsequent investigations, particularly following outbreaks of infantile gastroenteritis in the early twentieth century, revealed the pathogenic nature of specific strains. Since then, extensive serological, genetic, molecular, and antimicrobial resistance studies have established *E. coli* as one of the most extensively studied bacterial species.

Due to its ease of cultivation and rapid growth under laboratory conditions, *E. coli* has become a cornerstone model organism in microbiology and biotechnology. It is a chemoheterotroph requiring external carbon and energy sources and is capable of rapid replication, with generation times as short as 20 minutes under optimal conditions (Tortora, 2010; Redorbit, 2013). Beyond its commensal role, distinct *E. coli* pathotypes are responsible for intestinal and extraintestinal infections, including urinary tract infections and neonatal meningitis (WHO, 2015).

The virulence of pathogenic *E. coli* strains is mediated by a variety of factors that disrupt host cellular functions such as signal transduction, protein synthesis, ion transport, cytoskeletal integrity, and mitochondrial activity. These virulence traits are frequently encoded on mobile genetic elements, including plasmids, bacteriophages, transposons, and pathogenicity islands, facilitating horizontal gene transfer and the emergence of highly virulent, drug-resistant strains (WHO, 2015; Umeoduagu et al., 2023; Awari et al., 2024; Obasi et al., 2024).

Despite the significant public health implications, data on the antibiotic resistance characteristics of *E. coli* isolated from hospital dumpsites in Nigeria remain limited. This lack of information constrains effective environmental risk assessment and evidence-based policy formulation. Therefore, the present study aimed to isolate *Escherichia coli* from hospital waste dumpsites and to evaluate the antimicrobial susceptibility patterns of the isolates, thereby contributing to improved hospital waste management and antimicrobial resistance mitigation strategies.

MATERIALS AND METHODS

Study Area

The investigation was conducted in Awka, Anambra State, Nigeria, focusing on two major healthcare facilities: Amaku Teaching Hospital and Eldorado Hospital. Both institutions are located within densely populated urban areas and generate substantial quantities of medical waste due to high patient turnover.

Sample Collection

Soil samples were obtained from waste disposal sites within the selected hospitals. Four soil samples, each weighing approximately 5 g, were collected from different points around the dumpsites at a depth of 5–6 cm using sterile sampling containers. Samples were appropriately labeled and immediately transported to the microbiology laboratory for analysis.

Microbiological Analysis

Sample Dilution and Culturing

One gram of each soil sample was suspended in 9 mL of sterile distilled water to obtain the primary stock suspension. Serial tenfold dilutions were prepared up to 10^{-6}. Aliquots of 1 mL from selected dilutions (10^{-2} and 10^{-3}) were aseptically inoculated onto sterile Petri dishes using the spread plate technique. Inoculated plates were incubated at 37°C for 18–24 hours.

Enumeration and Isolation of Pure Cultures

After incubation, visible colonies were counted and bacterial load was calculated using the formula:

CFU/mL = (Number of colonies × Dilution factor) / Volume plated

Distinct colonies were purified through repeated subculturing using the streak plate method on nutrient agar. Cultures yielding uniform colony morphology were considered pure.

Identification and Characterization of Isolates

Bacterial identification was achieved through evaluation of colony morphology, Gram staining, and a series of biochemical assays following standard protocols (Cheesbrough, 1984; 2002). Test results were interpreted using Bergey's Manual of Determinative Bacteriology (9th Edition, 1994).

Gram Staining

Bacterial smears were prepared on clean glass slides using sterile saline, heat-fixed, and sequentially stained with crystal violet, iodine, ethanol, and safranin. Slides were examined under oil immersion microscopy (×100 objective) to determine Gram reaction and cellular morphology.

Biochemical Tests

Standard biochemical tests including catalase, citrate utilization, methyl red, indole, sugar fermentation, motility, and Voges–Proskauer assays were conducted according to established procedures.

Antibiotic Susceptibility Testing

Isolates were standardized to 0.5 McFarland turbidity and inoculated onto Mueller–Hinton agar plates. Antibiotic discs including ciprofloxacin, ofloxacin, streptomycin, amoxicillin, levofloxacin, penicillin, rifampicin, and nalidixic acid were aseptically placed on the agar surface. Plates were incubated at 37°C for 24 hours, after which zones of inhibition were measured in millimeters.

RESULTS

Microbiological analysis revealed the presence of three bacterial species: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Total bacterial counts

ranged from 3.0 × 10^5 to 4.5 × 10^5 CFU/mL. Morphological and biochemical characteristics confirmed the identity of the isolates. Antibiotic susceptibility testing of presumptive *Escherichia coli* isolates demonstrated variable resistance and sensitivity patterns across the tested antibiotics.

Table 4.1: The bacterial count of the isolates from hospital refuse dump site

Hospital Soil Sample	**Bacterial Count (×10^5 cfu/ml)**
Sample 1	30
Sample 2	45
Sample 3	37
Sample 4	32

Table 4.2 Morphology Characterization of Bacterial isolates

Isolate	**Elevation**	**Margin**	**Colour**	**Texture**	**Shape**	**Probable Isolate**
1	Convex	Entire	Dark metallic sheen	Smooth	Circular	*Escherichia coli*
2	Raised	Entire	Yellowish	Smooth	Circular	*Staphylococcus aureus*
3	Convex	Entire	Dark metallic sheen	Smooth	Circular	*Escherichia coli*
4	Raised	Entire	Cream	Smooth	Irregular	*Pseudomonas aeruginosa*

Table 4.3 Biochemical Characterization of bacterial isolate from soil sample

Isolate	**Shape**	**Gram Stain Reaction**	**CAT**	**IT**	**CUT**	**MR**	**MT**	**Organism Identified**
Soil 1	Rod	–	+	+	+	+	+	*Escherichia coli*
Soil 2	Rod	+	–	–	–	–	–	Not probable *Escherichia coli*
Soil 3	Rod	–	+	+	+	+	+	*Escherichia coli*
Soil 4	Rod	+	–	–	–	–	–	Not probable *Escherichia coli*

Keys: + = Positive & - = Negative

CAT = Catalase test, MR = Methyl Red test, IT = Indole test, MT = Motility test, CUT = Citrate utilization test

Table 4.4 Sugar Fermentation of Bacterial Isolates

Isolate	Glucose	Fructose	Lactose	Sucrose
1	AG+	AG+	AG−	AG+
2	AG−	AG−	AG−	A−
3	AG−	AG−	AG−	A−
4	AG+	AG+	A+	AG+

Keys: + Positive - Negative

A+ Positive and Acid produced

AG+ positive and produces both Acid and Gas

Table 4.5 Antibiogram of Bacterial Isolates to selected Antibiotic (zone of inhibition measured in mm)

Sample	APX	OFX	RD	PN	AMX	LEV	CEP	S	CPX	NA
Sample 1	–	12	–	13	–	–	12	17	–	14
Sample 2	16	–	22	–	18	22	–	–	15	–
Sample 3	–	15	–	17	–	–	13	28	–	12
Sample 4	15	–	20	–	13	16	–	–	21	–

KEY:

CPX= Ciprofloxacin, OFX= Ofloxacin, CFP: Cefoperazon S= Streptomycin

APX= Ampiclox AMX= Amoxicillin LEV= Levofloxacan PN= Penicillin

NA= Naficillin RD= Rifampicin

DISCUSSION

Findings from this investigation reveal a pronounced level of microbial pollution in soils obtained from hospital refuse disposal sites, as evidenced by total bacterial counts between 3.0×10^5 and 4.5×10^5 CFU/mL. Such elevated microbial burdens reflect sustained input of organic-rich medical waste, which provides nutrients and favorable microenvironments that support bacterial persistence and multiplication. Comparable microbial loads have been documented in environments influenced by healthcare waste and are generally interpreted as indicators of suboptimal waste

segregation, treatment, and disposal practices, particularly within resource-limited healthcare systems.

The isolation of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* from the dumpsite soils raises considerable public health concerns. These bacteria are widely recognized for their roles in opportunistic and hospital-acquired infections, and their detection in the environment suggests direct contamination from improperly discarded clinical materials, including soiled dressings, laboratory residues, and human biological waste. The frequent recovery of *E. coli* is especially suggestive of fecal contamination, underscoring deficiencies in the containment and treatment of human excreta within hospital waste streams.

Colony morphology and biochemical testing produced results consistent with established descriptions of the identified organisms. Isolates presumptively identified as *E. coli* exhibited characteristic growth patterns and biochemical reactions, including Gram-negative staining, catalase and indole positivity, methyl red reactivity, motility, and the ability to ferment specific sugars with acid and gas production. These combined phenotypic traits provided a reliable basis for differentiation from other non-*E. coli* isolates, reinforcing the validity of the identification methods used.

Antimicrobial susceptibility profiling revealed diverse resistance patterns among the isolates, with *E. coli* demonstrating reduced susceptibility to several widely used antibiotics, notably β-lactam agents such as penicillin, amoxicillin, and ampiclox. In contrast, moderate sensitivity was observed against selected fluoroquinolones and other agents, including streptomycin and rifampicin. This variability likely reflects selective pressure arising from extensive antibiotic use in hospital settings, which favors the emergence and persistence of resistant strains. Of particular concern is the presence of resistant *E. coli* in the environment, as such organisms can function as reservoirs of resistance determinants capable of horizontal transfer to other bacteria.

Resistance to β-lactam antibiotics observed in this study is consistent with reports from similar hospital-associated environments, where resistance is commonly linked to mobile genetic elements encoding β-lactamase enzymes. The comparatively

higher susceptibility to fluoroquinolones may indicate lower selective pressure or slower resistance development to these drugs locally; however, the potential for future resistance emergence remains significant.

Collectively, these results highlight hospital refuse dumpsites as important ecological niches for pathogenic and antibiotic-resistant bacteria. The recovery of clinically relevant species exhibiting varied resistance profiles emphasizes the urgent need for strengthened biomedical waste management systems, including proper segregation, treatment, and disposal of hospital waste. In the absence of such measures, contaminated soils may continue to serve as sources for the dissemination of pathogenic organisms and antimicrobial resistance, posing ongoing risks to environmental and public health.

CONCLUSION

This study demonstrates that hospital refuse dumpsites harbor significant populations of pathogenic bacteria, notably *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, many of which exhibit resistance to commonly used antibiotics. The findings underscore the environmental and health hazards associated with poor hospital waste management and reinforce the need for effective waste treatment, regulatory enforcement, and routine environmental monitoring to curb the spread of antibiotic-resistant pathogens.

REFERENCE

1. Adindu, C. S., Odili, L. C., Nwagu, E. E., Agu, K. C., Awah, N. S., Okeke, B. C., & Orjionwe, R. N. (2016). Phytochemical and antimicrobial screening of *Cola gigantea* leaves, stem and bark. *Universal Journal of Microbiology Research, 4*(2), 49–54.

2. Adepeju, D. M., Uwanta, L. I., Agu, K. C., Udenweze, E. C., Mbah, C. A., Awari, V. G., & Umeoduagu, N. D. (2023). Mosquito larvae biocontrol potential of *Bacillus thuringiensis* isolated from soil samples. *International Journal of Research Publication and Reviews, 4*(8), 1899–1906.

3. Andy, I. E., & Okpo, E. A. (2018). Prevalence of potential nosocomial bacterial pathogens in liquid waste and waste dump soil of three major hospitals in Calabar Metropolis, South-South, Nigeria. *World Journal of Pharmaceutical Research, 7*(17), 134–147.

4. Anazodo, C. A., Abana, C. C., Agu, K. C., Victor-Aduloju, A. T., Okoli, F. A., Ifediegwu, M. C., Awari, V. G., & Chidozie, C. P. (2024). *In vitro* antifungal efficacy of *Allium cepa* and *Allium sativum*: A comparative study with commercial drugs.

5. Anazodo, C. A., Adepeju, D. M., Okoli, F. A., Obasi, C. J., Abana, C. C., Agu, K. C., Ezenwelu, C. O., Awari, V. G., & Umeoduagu, N. D. (2024). Investigating the susceptibility of otomycosis-causing microorganisms to different ear drops. *International Research Journal of Modernization in Engineering Technology and Science, 6*(6), 1958–1965.

6. Anitha, J. (2012). Isolation and identification of bacteria from biomedical waste. *International Journal of Pharmacy and Pharmaceutical Sciences*, 78–84.

7. Awah, N. S., Agu, K. C., Ikedinma, J. C., Uzoechi, A. N., Eneite, H. C., Victor-Aduloju, A. T., Umeoduagu, N. D., Onwuatuegwu, J. T. C., & Ilikannu, S. O. (2017). Antibacterial activities of aqueous and ethanolic extracts of male and female *Carica papaya* leaves on some pathogenic bacteria. *Bioengineering and Bioscience, 5*(2), 25–29. https://doi.org/10.13189/bb.2017.050201

8. Awah, N. S., Agu, K. C., Okorie, C. C., Okeke, C. B., Iloanusi, C. A., Irondi, C. R., Udemezue, O. I., Kyrian-Ogbonna, A. E., Anaukwu, C. G., Eneite, H. C., Ifediegwu, M. C., Umeoduagu, N. D., Abah, N. H., & Ekong, U. S. (2016). *In vitro* assessment of the antibacterial quality of some commonly used herbal and non-herbal toothpastes on *Streptococcus mutans*. *Open Journal of Dentistry and Oral Medicine, 4*(2), 21–25. https://doi.org/10.13189/ojdom.2016.040201

9. Awari, V. G., Umeoduagu, N. D., Agu, K. C., Okonkwo, N. N., Ozuah, C. L., & Victor-Aduloju, A. T. (2023). The ubiquity, importance and harmful effects of microorganisms: An environmental and public health perspective. *International*

Journal of Progressive Research in Engineering Management and Science, 3(12), 1–10.

10. Awari, V. G., Umeoduagu, N. D., Agu, K. C., Obasi, C. J., Okonkwo, N. N., & Chidozie, C. P. (2024). Antibiogram of pathogenic *Pseudomonas aeruginosa* isolated from hospital environment. *International Refereed Journal of Engineering and Science, 13*(2), 1–10.

11. Cassels, F. J., & Wolf, M. K. (2013). Colonization factors of diarrheagenic *Escherichia coli* and their intestinal receptors. *Journal of Industrial Microbiology, 15*, 214–226.

12. Centers for Disease Control and Prevention. (2012). *Pathotypes of human Escherichia coli*. National Center for Emerging and Zoonotic Infectious Diseases.

13. Chartier, Y., Emmanuel, J., Pieper, U., Prüss, A., Rushbrook, P., Stringer, R., Townend, W., Wilburn, S., & Zghondi, R. (Eds.). (2014). *Safe management of wastes from health-care activities* (2nd ed.). World Health Organization Press.

14. Medubi, S. A., Akande, T. M., & Osagbemi, G. K. (2006). Awareness and pattern of needle stick injuries among health workers at University of Ilorin Teaching Hospital, Ilorin, Nigeria. *African Journal of Clinical and Experimental Microbiology, 7*(3), 183–187.

15. Nataro, J. P., & Kaper, J. B. (2017). Diarrheagenic *Escherichia coli*: A comprehensive review of pathogenesis, epidemiology, diagnosis and clinical aspects. *Clinical Microbiology Reviews, 11*, 142–201.

16. Obasi, C. J., Agu, K. C., Anazodo, C. A., Aniekwu, C. J., Okeke, C. B., Adepeju, D. M., Okoli, F. A., & Umeoduagu, N. D. (2024). *In vitro* activity of β-lactams and other antimicrobials against multidrug-resistant *Pseudomonas aeruginosa*. *International Journal of Scientific Research & Engineering Trends, 10*(3), 426–432.

17. Ogbo, F. C., & Agu, K. C. (2015). Evaluation of a new method for testing the pathogenicity of molds to yam tubers. *Edorium Journal of Microbiology, 1*, 9–17.

18. Panda, R. (2012). The effect of hospital solid wastes on the environment. *International Quarterly Journal of Environmental Science, 6*(1–2), 37–40.

19. Prüss, A., Giroult, E., & Rushbrook, P. (2013). *Safe management of wastes from health-care activities*. World Health Organization.

20. Rasheed, S., Iqbal, S., Baig, L. A., & Mufti, K. (2005). Hospital waste management in the teaching hospitals of Karachi. *Journal of Pakistan Medical Association, 55*, 192.

21. Russo, T. A., & Johnson, J. R. (2015). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. *Journal of Infectious Diseases, 181*, 1753–1754.

22. Sears, C. L., & Kaper, J. B. (2016). Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. *Microbiology Reviews, 60*, 167–215.

23. Sunday, M., & Agbaji, E. B. (2012). Influence of hospital waste dumps and incinerator ash on the receiving environment. *Advances in Applied Sciences Research, 3*(5), 2884–2889.

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

25. Tortora, G. J. (2010). *Microbiology: An introduction*. Benjamin Cummings.

26. Ubaoji, K. I., Nwosu, O. K., Agu, K. C., Nwozor, K. O., Ifedilichukwu, N. H., & Okaka, A. N. C. (2020). Gas chromatographic analysis of phytoconstituents and antimicrobial properties of leaf extracts of Nigeria-grown *Ginkgo biloba*. *Journal of Scientific Research in Medical and Biological Sciences, 1*(2), 45–56.

27. Umeoduagu, N. D., Chidozie, C. P., Ifemeje, E. M., Anazodo, C. A., Okoli, F. A., Okonkwo, N. N., Uwanta, L. I., Agu, K. C., & Azuka, G. E. (2023). Halitosis: Antimicrobial susceptibility pattern of some commercially sold toothpastes on

selected isolates. *International Journal of Scientific Research & Engineering Trends, 9*(5), 1309–1315.

28. Vivan, J. B., & Wright, G. D. (2010). Antibiotic resistance in the environment: A link to the clinic. *Current Opinion in Microbiology, 13*, 589–594.

29. Waste Care Corporation. (2012). *Trash compactors and waste disposal* (Reference Article C9-l-CS-020311).

30. World Health Organization. (2014). *World health statistics*. World Health Organization.