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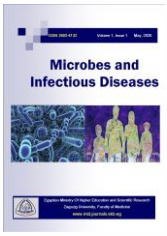

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Original article

Prevalence of multidrug resistant and extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* in the soil of hospital waste dumps

Oluwatayo A. Makinde*

Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, PMB.

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ABSTRACT

Background: Hospitals are important sources of waste, some of which may be contaminated by infectious agents. Thus, the release of improperly decontaminated hospital waste may lead to the introduction of pathogenic microorganisms into the environment. Due to limited resources, solid wastes generated from hospitals in developing countries are sometimes collected in open dump sites on hospital grounds for further processing. The health hazard of this waste management method was evaluated in this study by quantifying the prevalence of multidrug-resistant and Extended β-lactamases (ESBL)-producing *Pseudomonas aeruginosa (P. aeruginosa*) in the soil of selected hospital waste dumps. **Methods:** Bacteria strains isolated from soil samples on cetrimide agar were identified as *P. aeruginosa* based on their cultural morphology, motility and biochemical characteristics. The susceptibility of identified *P. aeruginosa* isolates to selected antibiotics was evaluated by disc diffusion assay. **Result**s: Forty-four of the bacteria isolates cultured from the soil samples were identified as *P. aeruginosa*. The fluoroquinolones antibiotics were most active against the *P. aeruginosa* isolates followed by the cephalosporins antibiotics tested. The phenotypic screening of the isolates for ESBL production showed that 29.55% of the isolates were ESBL producers. **Conclusion**: Soils of clinical dump sites are potential reservoir for multidrug resistant strains of *P. aeruginosa* from where they can be disseminated into the environment and as such constitute an infection exposure risk to humans.

Introduction

Healthcare facilities in addition to non-hazardous wastes, also generate a significant number of hazardous wastes [1, 2]. Such hazardous wastes include wastes colonized by pathogenic microorganisms which can be released into the environment if such wastes are not properly disinfected [3]. Resource-limited hospitals in developing countries lack the resources to properly manage contaminated waste thus resulting in the release of inadequately treated waste into the environment [4].

A common method for the management of solid waste in hospitals in developing countries is the use of a transit waste dump on hospital grounds from where wastes are further processed [4-6]. This procedure can lead to the contamination of soil surrounding the dump site with pathogenic

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* *Corresponding author:* Oluwatayo Ayotunde Makinde
E-mail address: oluwatayo.makinde@aaua.edu.ng
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microorganisms colonizing the solid wastes resulting in the dissemination of pathogenic microorganisms into the groundwater during rainfalls [4, 7]. This study evaluated the public health risk of this method of hospital waste disposal by evaluating the antibiotics susceptibility profile of *P. aeruginosa* isolated from soil collected from waste dumps on the grounds of selected hospitals in the Akoko area of Ondo State, Nigeria.

Pseudomonas aeruginosa is one of the most isolated microorganisms from hospital environments and a common cause of hospital-acquired infection [8, 9]. *Pseudomonas aeruginosa* infection can also be acquired from the community [10-12]. *P. aeruginosa* are versatile pathogen, also known to cause infection in animals, and can persist in diverse environmental niches in soil and water [13-15]. Thus, clinical strains of *P. aeruginosa* released into the environment via improperly managed solid waste can persist in the environment and pose health hazards to humans. *P. aeruginosa* has several virulence factors and is known to cause several acute, severe, and chronic infections in humans [16]. Common *P. aeruginosa* infections include pneumonia, ulcerative keratitis, skin and soft tissue infections, urinary tract infections, bloodstream infections, and surgical site infections [12, 17].

A major concern about the release of clinical pathogens into the environment is the potential for clinical bacterial strains to develop resistance to multiple antibiotics. *P. aeruginosa* is known to have the potential to develop resistance to multiple antibiotics [18]. Common antibiotics resistance mechanisms employed by *P. aeruginosa* include the use of multi-drug efflux pumps, downregulation of outer membrane porin and β-lactamases production [18, 19]. This study aims to evaluate the antibiotics susceptibility profile of *P. aeruginosa* isolated from soil samples collected from hospital solid waste dumps. The ability of the *P. aeruginosa* isolates to produce Extended β-lactamases (ESBL) was also evaluated.

Materials and method

Sample collection

Soil samples were collected from the solid waste dumping sites on the grounds of three selected hospitals in the Akoko area of Ondo state Nigeria. Soil samples were collected using a sterile spatula into sterile McCartney bottles from the top layer of the soil. Samples were collected from each site three times on different sampling days. Soil samples were collected in duplicates from 5 different spots of each sampling site. The spatula used was sterilized in between sampling by cleaning with soapy water and subsequently 70% ethanol. Samples were properly labeled and transferred to the laboratory for microbiological analysis.

Physicochemical analysis of soil samples

The moisture content and pH of the collected soil samples were evaluated using standard procedures. To evaluate the moisture content of the soil sample, 5 g of each soil sample was measured into a crucible in triplicates and placed in an oven at 105^0C for 24 hours. Moisture contents were evaluated based on the initial and final weight of the soil sample after total dehydration. Soil pH was evaluated by weighing 20g of the soil sample into 20ml of distilled water in a 100ml beaker. The beaker was shaken vigorously. The pH of the soil suspension was evaluated using a pH meter (Hanah instrument).

Isolation of *P. aeruginosa* from soil sample

Pseudomonas aeruginosa was isolated from soil samples according to the method described by [20] with modification. One gram of each soil sample was weighed into 9 ml of sterile distilled water in well-labeled sterile 20 ml sample bottles in triplicates. The soil suspension was thoroughly shaken with the aid of a vortex to disperse the individual particles. The stock was serially diluted by transferring 0.1 ml of the soil suspension into 0.9 mL of sterile distilled water. This was repeated until the 5th dilution. For each sample, 0.1 ml of the stock, 3rd, and 5th diluents were transferred onto the surface of already prepared Cetrimide agar. The inoculum transferred onto the plates was spread evenly on the plates with the aid of a sterile glass spreader. The inoculated plates were allowed to dry and incubated at 37ºC for 24 hours. After the incubation period, the plates were examined for yellow-green colonies which are characteristic of *P. aeruginosa* growths on cetrimide agar. Non-repetitive presumptive *P. aeruginosa* colonies were sub-cultured on cetrimide agar to obtain pure cultures for further characterization and storage. Presumptive *P. aeruginosa* isolates were identified based on their Gram staining reaction, motility, and biochemical characteristics. Biochemical reactions performed include the catalase test, oxidase test, citrate utilization, indole, and methyl red test.

Evaluation of antibiotics susceptibility profile of isolates

The isolates were grown in nutrient broths at 37ºC for 24 hours. Cells were recovered from the overnight culture by centrifugation and resuspended in PBS. The cells were washed in PBS and finally resuspended in PBS. The optical density of the overnight culture was adjusted in PBS to obtain a final OD similar to the 0.5 McFarland standard. A sterile cotton swab was used to spread the adjusted inoculum of each isolate on freshly prepared Mueller-Hinton agar. The surface of the inoculated medium was allowed to dry for 15 minutes after which antibiotics discs were aseptically placed on the surface of the inoculated plates using sterile forceps, the disk was immediately pressed down lightly with the forceps to ensure complete contact between the disk and the agar surface. The plates were incubated at 37ºC for 24 hours after which the zone of inhibition was measured. The susceptibility of the isolates to each antibiotic was interpreted based on the breaking points recommended by the Clinical and Laboratory Standards Institute [21].

Evaluation of ESBL production by isolates

ESBL productions by isolates were detected phenotypically using the double disk synergy test (DDST) method according to [22]. Amoxicillin-clavulanic acid disk (20/10µg) was aseptically placed at the center of Mueller-Hinton (MH) agar already inoculated with the adjusted broth of the test isolates as earlier explained. Ceftazidime and cefotaxime (30µg) single antibiotic disks were each placed adjacent to the central disk (amoxicillin-clavulanic acid) at a distance of 15 mm from the center of the amoxicillin-clavulanic acid disk. The plates were incubated at 37oC for 24 hrs. Extension of the zone of inhibition towards amoxicillin-clavulanic acid was interpreted as ESBL production.

Results

This study evaluated the susceptibility of *P. aeruginosa* isolated from soil samples collected from a hospital solid waste dump. To understand the impact of environmental conditions on the persistence of *P. aeruginosa* in the soil sampled, the moisture and pH of the soil samples were measured. As shown in **table (1),** the moisture content of the soil samples ranges from 6.75 to 21.50 %. Likewise, the pH of the soil samples ranges from 6.68 to 7.06.

A total of 44 isolates were identified as *P. aeruginosa* based on their Gram staining reaction and biochemical characteristics. **Figure 1** shows the number of *P. aeruginosa* isolated from the three sampled sites. Site B had the highest number of *P. aeruginosa* positive samples while site A had the lowest number of *P. aeruginosa* positive samples. The susceptibility of the *P. aeruginosa* isolates to selected antibiotics is shown in **table (2).** Ofloxacin showed the best activity against the isolates followed by ciprofloxacin with ceftazidime showing the weakest activity against the *P. aeruginosa* isolates. The phenotypic ESBL production screening of the *P. aeruginosa* isolates showed that 29.55% of the total isolates screen were positive for ESBL production **(Figure 2).**

Table 1. Physicochemical characteristics of soil samples.

Samples	Percentage moisture	pH
A1	21.50±1.2	6.95±0.8
A2	11.25±1.5	6.98±1.1
A3	14.75±0.8	6.68±0.5
B1	6.75±1.3	6.87±1.2
B2	6.75±1.5	6.92±0.9
B3	8.25±1.4	6.93±0.8
C1	9.00±1.3	7.06±1.5
C2	8.10±1.0	7.04±1.2
C3	10.23±1.7	6.82±0.7

Table 2. Antibiotic susceptibility profile of *P. aeruginosa* isolated from soil samples (n = 44).

Antibiotics	**S (%)**	**I (%)**	**R (%)**
Cefepime	40.90	13.64	45.45
Ceftazidime	27.27	9.09	63.66
Gentamicin	36.36	9.09	54.55
Levofloxacin	54.55	13.64	31.82
Ciprofloxacin	59.09	13.64	27.27
Ofloxacin	72.72	0	27.27

Cefepime (CFM), Ceftazidime (CAZ), Gentamicin (GEN), Levofloxacin (LVX), Ciprofloxacin (CPR) and Ofloxacin (OFL)

Figure 1. Number of *P. aeruginosa* strains isolated from each sampling site.

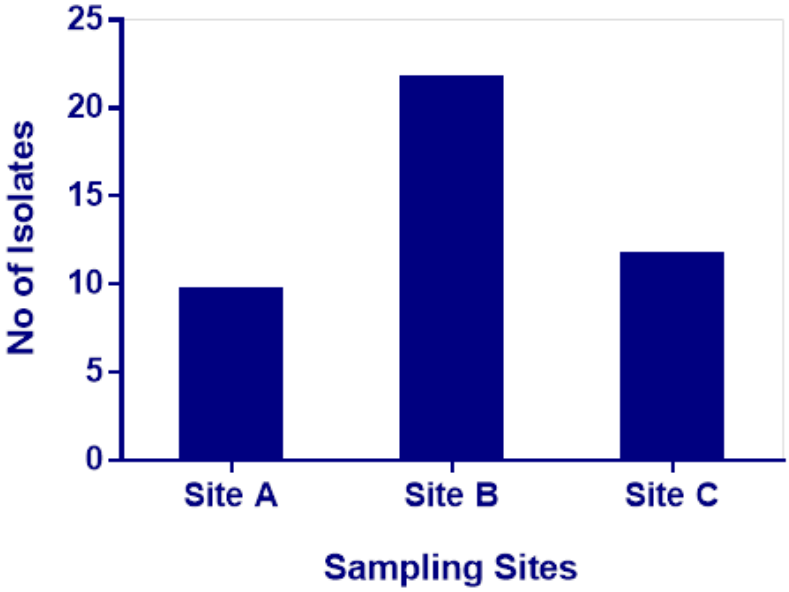
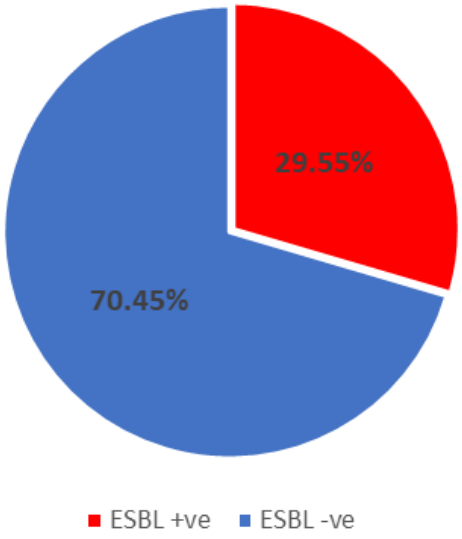


Figure 2. Phenotypic evaluation of ESBL production by *P. aeruginosa* isolates.



Discussion

The discovery of antibiotics is one of the remarkable milestones in healthcare. Their use in healthcare provided the opportunity to treat previously deadly infectious diseases [23]. However, the emergence of antibiotics resistant pathogenic bacteria strains poses a significant threat to the fight against infectious diseases as it can

severely limit the therapeutic options available to treat hitherto easily treated infections. The hospital environment is a known hot bed for the emergence of antibiotics resistant bacteria strains which may be released into the environment through poorly decontaminated wastes [24, 25]. This study evaluated the antibiotics susceptibility profile of *P. aeruginosa* isolated from soil of solid waste dumps on hospital grounds.

P. aeruginosa represent an ideal organism that can be used to evaluate the potential for poorly managed contaminated hospital wastes to disseminate antibiotics resistant pathogens into the environment owing to its ability to colonize wide range of hosts and survive in divers' environmental conditions as well as its potential to develop antimicrobial resistance [26, 27]. Multidrug resistant *P. aeruginosa* isolated from soil sample in this study might have been released into the soil from contaminated clinical samples dumped on the transit waste site sampled. The multidrug resistant *P. aeruginosa* isolates can be disseminated into the environment through runoffs where humans can become exposed thus constituting a public health hazard.

The moisture content observed in this study was relatively high and consistent at the locations which could be responsible for the high number and persistency of *P. aeruginosa* in the site. Previous studies have also implicated high humidity and moisture content for the colonization and survival of *P. aeruginosa* in the environment [28, 29].

In this study, ofloxacin followed by ciprofloxacin and levofloxacin showed the best activity against *P. aeruginosa* isolates. Fluoroquinolones are known to be effective against aerobic Gram-negative bacilli like *P. aeruginosa* and are commonly used in the treatment of *Pseudomonas* infections [30].

The cephalosporin used in this study; cefepime and ceftazidime only showed moderate activity against the *P. aeruginosa* isolates. Various studies have described the isolation of *P. aeruginosa* strains resistant to cephalosporin of various generations from the environment [31, 32]. Recently, increased resistance has been observed against third generation cephalosporins for Gram-negative bacilli, especially *P. aeruginosa* as observed in this study [33, 34].

P. aeruginosa isolates are known to frequently harbor the genes responsible for ESBL production which confers resistance to cephalosporin class of antibiotics [35]. In this study, *P. aeruginosa* isolates were screened for ESBL production. Thirteen (29.55%) of the isolates were positive for ESBL production. Other studies evaluating ESBL production in *P. aeruginosa* strains isolated from soil samples have also reported ESBL production in some isolated strains [20, 36]. ESBL production confers the ability to resist second and third generation cephalosporins on bacteria cells thus limiting treatment options. The detection of ESBL production in some of the *P. aeruginosa* isolates in this study indicate the danger pose by improper management of hospital waste.

Conclusion

Pseudomonas aeruginosa is part of the group of pathogenic bacteria refer to as the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) which are common causes of healthcare associated infections. In this study antibiotics resistant strains of *P. aeruginosa* were isolated from soil samples collected from hospital waste dumps indicating the potential for poorly managed contaminated hospital wastes to disseminate pathogenic microorganisms in the environment. It is therefore imperative that hospital waste should be properly disinfected before discharge into the environment.

Competing interest

The author declares that he has no competing interest

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