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Antibiogram of *Escherichia coli* and *Pseudomonas* Strains Isolated from Wastewater Generated by an Abattoir as It Journeys into a Receiving River

Olutayo I. Falodun, Abimbola O. Adekanmbi*

Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Ibadan, Nigeria
Email: *bimboleen@yahoo.com

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Abstract

Untreated wastewater from abattoir operations contains nutrients and other components that aid the growth of microorganisms especially bacteria. They also serve as a habitat for potentially pathogenic bacteria which might be a source of public health concern. The study was carried out to determine the antibiotics susceptibility profile of Gram-negative bacteria (*Pseudomonas* and *Escherichia coli*) to selected antibiotics. Wastewater samples were collected from ten different sampling points and cultured on Eosin Methylene Blue (EMB) and King's B medium. The bacterial strains obtained from the wastewater samples were subjected to antibiotics susceptibility tests, using the disc diffusion technique. A total of 60 *Pseudomonas* and 100 *Escherichia coli* were isolated out of which none of the *Pseudomonas* strains showed resistance to imipenem, colistin sulphate, meropenem and aztreonam, while 100% resistance was observed to ceftazidime and piperacillin. All the *Escherichia coli* strains were resistant to oxacillin and ceftazidime, while the percentage resistance to aztreonam, ertapenem, cefoxitin and tetracyline was 6%, 11%, 43% and 58% respectively. Eighty-five percent (85%) of the total *Escherichia coli* showed resistance to more than two antibiotics, while 14% showed resistance to ceftazidime and oxacillin, with only one isolate showing resistance to ceftazidime and cefoxitin. There is the need for an effective treatment of wastewater generated from abattoir operations to prevent the potential spread and transmission of antibiotic resistant bacteria to the human population who depends heavily on some of the water bodies, receiving input from abattoir wastes.

Keywords

Abattoir, *Pseudomonas* Sp., *Escherichia coli*, Multidrug Resistance, Antibiotics

*Corresponding author.

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1. Introduction

Abattoir effluents are wastewater generated from the slaughtering activities of animals in the abattoirs and usually consist of intestinal contents like blood and water [1]. Although operations in the abattoirs are of benefits to man in the provision of meat for human consumption, it can be hazardous to public health with respect to the untreated generated wastes which are discharged into the environment [2]. Like many other sewage types, effluents from the abattoir flow into water bodies such as ground water, streams, rivers, lakes and oceans thereby introducing enteric pathogens, excess nutrients and other contaminants into the water sources [3] [4]. The abattoir effluents released without treatment feed natural bodies of water with nutrients and the monitoring of the bacterial status of such effluents are of public health importance [5] [6] especially in Nigeria, a country where abattoir effluents like most other wastewater are untreated before they are discharged [1] [7].

The detection of pathogenic organisms as well as the incidence of proteolytic and lipolytic bacteria in a water body is suggestive of impending health hazards and public health concern [8]. Micronutrients in abattoir wastewater sustain the prevalence of pathogenic and entropic organisms that constitute biohazards in water bodies [8] [9]. The risk becomes high when the enteric pathogens which are present in the water bodies are resistant to antibiotics; this is because, if such bacteria cause infection, they will be very difficult to treat [10]. Furthermore, there could be transmission of antimicrobial resistance to autochthonous bacteria from faecal bacteria through lateral transfer, when the resistance genes are carried by transferable and mobile genetic elements such as plasmids; thus contributing to antimicrobial resistance multiplication and the spread in the environment [11]. Gram-negative infections include those caused by *Klebsiella*, *Acinetobacter*, *Pseudomonas aeruginosa* and *E. coli* as well as many other less common bacteria.

Pseudomonas species are gram-negative aerobic bacilli widely distributed in the natural environment and particularly abundant in soils and water; they are opportunistic and ubiquitous pathogens, probably due to their limited nutritional requirements and tolerance of adverse physical and chemical conditions [12]. *Pseudomonas* species show a wide continuum of resistance to different classes of antimicrobials agents and one of the principal factors linked to the emergence of microbial resistance is the abusive and indiscriminate use of antimicrobial agents [13]. The emergence of *Pseudomonas* strains with variable and growing levels of antimicrobial resistance has generated considerable health concern. *E. coli* is a common inhabitant of human and animal intestinal tract. It is a gram-negative facultative aerobic organism and the most common in the family *Enterobacteriaceae*. *E. coli* is also one of the standard indicator organisms for faecal pollution in environmental water [14]. Animal faeces can also contain pathogens like *E. coli* O157 and *Salmonella* species which can cause infection in humans [15]. This study was designed to determine the presence of gram-negative bacteria (*Pseudomonas* species and *Escherichia coli*) at different points from the flow of abattoir effluents in Bodija market in Ibadan North Local Government Area of Oyo State into a receiving river as well as determine their susceptibility to selected antibiotics.

2. Materials and Methods

2.1. Study Site

The study site was the Bodija abattoir located in Ibadan North Local Government Area of Oyo state, Nigeria. The descriptions of the sampling points and the Geographical Positioning System (GPS) readings have earlier been reported by Adekanmbi and Falodun [16].

2.2. Sample Collection

Wastewater samples from the Bodija abattoir were collected at different points as it flows via a drainage channel into a nearby receiving river. The samples were transported to the laboratory for microbiological analysis.

2.3. Isolation and Characterization of *Pseudomonas* Sp.

Serial dilutions were carried out on the wastewater samples and 1 ml of the appropriate dilution was plated out on King's B medium, using the standard pour plate technique of Harrigan and MacCance [17]. The plates were incubated at 35˚C for five-seven days for growth to occur. Colonies showing visible greenish-yellow colouration (due to pigment production) were selected and further sub-cultured to obtain pure cultures.

2.4. Isolation of *Escherichia coli*

One ml aliquot of the appropriate dilutions was plated out on Eosin Methylene Blue (EMB) agar using the pour plate technique. The plates were incubated at 35˚C - 37˚C for 24 hours and growth observed. Colonies showing the green metallic sheen typical of *Escherichia coli* were selected and sub-cultured to obtain pure cultures.

2.5. Characterization of the Bacterial Isolates

The bacterial isolates (*Pseudomonas* sp. and *Escherichia coli*) were characterized using morphological, biochemical and sugar fermentation tests and compared with the scheme of Sneath [18].

2.6. Antibiotic Susceptibility Test

Antibiotic susceptibility test for the Gram negative isolates was carried out using the disc diffusion technique. The antibiotics were selected based on the CLSI [19] standards and they included: imipenem (10 µg), aztreonam (10 µg and 30 µg), ceftazidime (30 µg), colistin sulphate (10 µg), piperacillin (30 µg), meropenem (10 µg), tetracycline (30 µg), cefoxitin (30 µg), and oxacillin (1 µg). All the antibiotic discs were purchased from Oxoid, UK. The bacterial were seeded onto Mueller Hinton agar plates by picking an overnight 18 - 24 hour culture of the isolates with an inoculating loop and suspended in a tube containing 0.85% saline. The turbidity was adjusted to 0.5 McFarland standards. The suspension was spread uniformly over the already prepared Muller Hinton agar plate using sterile swab stick, and antibiotics were placed on the plates with the aid of sterile forceps. The plates were inverted and incubated at 35˚C - 37˚C for 18 - 24 hours. Zones of inhibition were measured after the incubation period and recorded. The values were compared with the CLSI [19] standards.

3. Results

A total of sixty (60) *Pseudomonas* species and one hundred (100) *Escherichia coli* were isolated from the waste water samples over the sampling duration. The percentage resistance of the isolated Gram negative strains is shown in **Figure 1** and **Figure 2**. There was 100% resistance to ceftazidime and piperacillin by the *Pseudomonas* strains, while there was no observed resistance to imipenem, meropenem, colistin sulphate and aztreonam. On the other hand, all (100%) the *Escherichia coli* were resistant to oxacillin and ceftazidime, while the resistance to aztreonam, ertapenem, tetracycline and cefoxitin was 6%, 11%, 58% and 43% respectively.

The phenotypic pattern of resistance of the isolates is shown in **Table 1** and **Table 2**. All the *Pseudomonas* strains showed the same pattern of resistance to the antibiotics (CAZ, PRL); while the *Escherichia coli* strains had different patterns of resistance to the six antibiotics tested against them. The highest frequency was 28 strains, showing resistance to CAZ, TE, OX; while 24 strains showed resistance to CAZ, TE, FOX, OX. In all, 38% of the *Escherichia coli* showed resistance to more than three antibiotics.

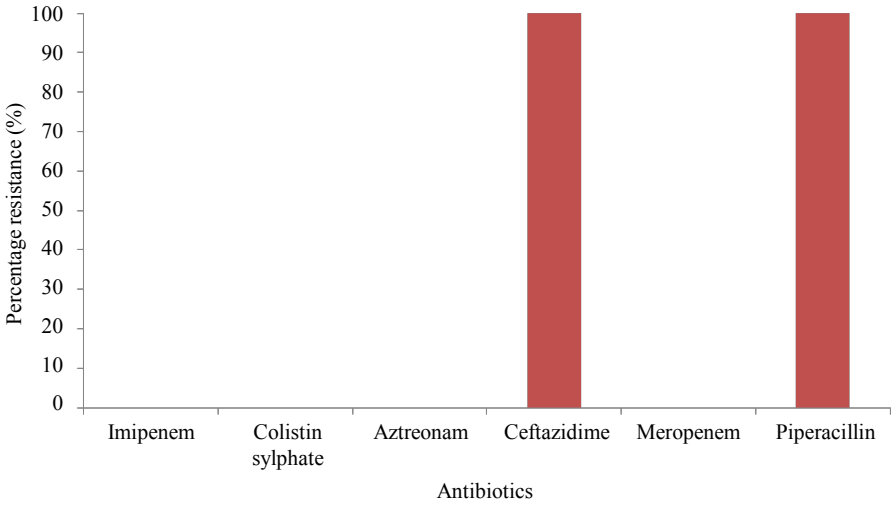

Figure 1. Percentage resistance of the *Pseudomonas* strains to the tested antibiotics.

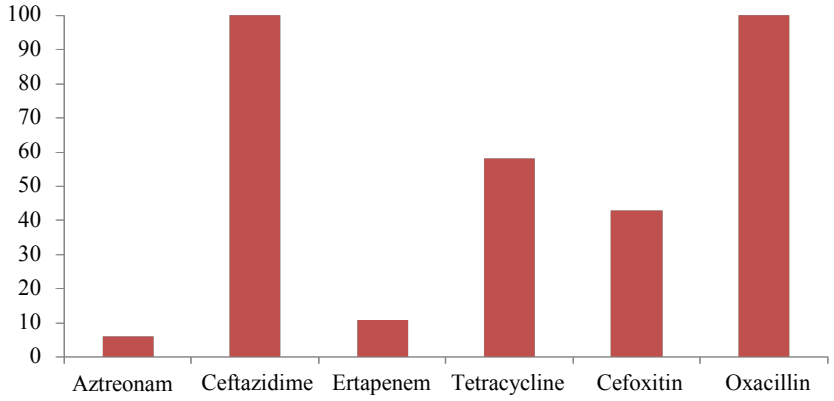

Figure 2. Percentage resistance of the *Escherichia coli* to the tested antibiotics.

4. Discussion

The isolation of *Escherichia coli* and *Pseudomonas* species from the Bodija, Ibadan abattoir further substantiates the fact that these Gram negative bacteria are associated with abattoir effluents as they have been previously isolated from abattoir wastewater in Nigeria [7] [20]-[22] and elsewhere [12] The observed resistance patterns of *Pseudomonas* strains in this study differs from the observation of Igbinosa *et al*. [20] regarding resistance to imipenem, aztreonam and meropenem. All the isolates in this study showed no resistance to the antibiotics whereas Igbinosa and his colleagues reported varying level of resistance to the drugs. However, resistance to ceftazidime and piperacillin (100%) as observed in this study was higher than the 80% and 71.4% resistance reported by the same authors to the two antibiotics respectively. The noticed disparity may be due to the nature of the wastewater studied. Igbinosa and colleagues collected their samples prior to discharge into the receiving water body as against the samples from this study that were already discharged before sampling.

Moreover, the observation from this study was similar to a study carried out in a swine slaughterhouse in Brazil [12]. As observed in this study, all the *Pseudomonas* strains were completely resistant to imipenem and aztreonam. However, only 2.9% of the total strains in their study were reported to be resistant to meropenem while there was no resistance by the *Pseudomonas* strains in this study to the same antibiotic. Furthermore, all the isolates in this present study exhibited complete resistance to piperacillin and ceftazidime while Kelly *et al*. [12] reported that 100% and 91.2% of their *Pseudomonas* strains were resistant to the respective drugs. Similarly, both studies observed that the *Pseudomonas* isolates did not exhibit multi-resistance to the antimicrobials tested. The resistance obtained for imipenem (0%), ceftazidime (100%) and piperacillin (100%) against the *Pseudomonas* strains is not in agreement with the 18% (imipenem), 34% (piperacillin) and 36% (ceftazidime) reported from another study in India [23]. In addition, the result of the resistance pattern of the *Pseudomonas* sp. to colistin sulphate and piperacillin were similar to a previous report on wastewater and wastewater impacted marine coastal zone where the *Pseudomonas* isolates from the study were completely susceptible to colistin sulphate and only one of the 146 isolates was resistant to piperacillin [24]. In a study carried out on clinical isolates in Jamaica, the reported *Pseudomonas* species resistance was 17.6% (piperacillin), 19.6% (ceftazidime) and 9.8% (imipenem and meropenem) [25]. This was not in agreement with the result of this present study that showed the isolates being completely resistant to imipenem and meropenem while a higher resistance was observed for ceftazidime and piperacillin.

The occurrence of *Enterobacteria* especially *E. coli* in wastewater has been extensively reported by several authors. Danishta *et al.* [26], Amaya *et al.* [27] and Galvin *et al.* [28] all reported the occurrence of *E. coli* in wastewater obtained from environmental, hospital and other sources in various parts of the globe. The occurrence of *E. coli* in this study confirmed their reports that *Escherichia coli* and other bacterial strains are frequently encountered in wastewater samples.

The occurrence of high level of resistance to antibiotics by *E. coli* has also been well documented; Alhaj *et al.* [29] reported that anthropogenic activities have contributed significantly to the spread of antibiotic resistance in the environment. They were of the view that antibiotic resistant *E. coli* can enter the environment via sewage and every other form of faecal contamination. This cannot be ruled out, however, because of the widespread use of antibiotics in animal husbandry; which has led to increased resistance in most environmental isolates.

Table 1. Phenotype of resistance of the *Pseudomonas* strains to the selected antibiotics.

Phenotype of resistance	Number of isolates showing trait	Percentage number of isolates (%)
[1]CAZ, [2]PRL	60	100

Footnote: [1]ceftazidime, [2]piperacillin.

Table 2. Phenotype of resistance of the *Escherichia coli* strains to the selected antibiotics.

Phenotype of resistance	Number of isolates showing resistance	Percentage number of isolates (%)
[2]CAZ, FOX	1	1
[2]CAZ, [5]OX	14	14
[1]ATM, [2]CAZ, [4]FOX	1	1
[1]ATM, [2]CAZ, [5]OX	2	2
[2]CAZ, [4]FOX, [5]OX	14	14
[2]CAZ, [6]TE, [5]OX	30	30
[2]CAZ, [3]ETP, [5]OX	2	2
[1]ATM, [2]CAZ, [3]ETP, FOX	1	1
[1]ATM, [2]CAZ, [4]FOX, [5]OX	1	1
[1]ATM, [2]CAZ, [3]ETP, [5]OX	2	2
[1]ATM, [2]CAZ, [3]ETP, [6]TE	1	1
[2]CAZ, [3]ETP, [4]FOX, [5]OX	1	1
[2]CAZ, [3]ETP, [6]TE, [5]OX	2	2
[2]CAZ, [6]TE, [4]FOX, [5]OX	24	24
[1]ATM, [2]CAZ, [3]ETP, [6]TE, [5]OX	2	2
[1]ATM, [2]CAZ, [3]ETP, [4]FOX, [5]OX	1	1
[1]ATM, [2]CAZ, [6]TE, [4]FOX, [5]OX	1	1
Total	**100**	**100**

Footnote: [1]aztreonam; [2]ceftazidime; [3]ertapenem; [4]cefoxitin; [5]oxacillin; [6]tetracycline.

From the study of Galvin *et al*. [28], there was a percentage resistance of 14% to ceftazidime of the 254 *E. coli* isolated in their study and this is not in agreement with the present study where 100% resistance was observed to the same antibiotic. Similarly, there was no agreement in the resistance of the *E. coli* from both studies to cefoxitin, as 15% of the total isolates from the former showed resistance as against 43% observed in the present study.

The result of this study showed that 58% of the *E. coli* isolates were resistant to tetracycline and this value is higher than the 20% reported in a similar study conducted in Lagos a neighboring state by Akano *et al.* [1]. Furthermore, 18% resistance to tetracycline was also reported in a recent study by Farmer *et al.* [30] which is lower compared to the value observed in this present study. The disparity in the values might be attributed to the differences in the nature of the samples, while this present study was carried out on abattoir effluents, the other study was on fresh water samples. The observation from the present study revealed that 59.7% of the *E. coli* strains were resistant to ceftazidime which is also in agreement with the over 50% resistance recently reported for *E. coli* to the same antibiotic in a study carried out in a municipal dumpsite in Tanzania [31].

5. Conclusion

The findings of this study showed that the *Pseudomonas* strains did not exhibit multi-resistance to the tested antibiotics which was an indication that the risk of spreading *Pseudomonas* strains that could be multi-resistant seems very low. However, the multi-resistance exhibited by the *E. coli* strains underscores the need to closely monitor multidrug resistant pathogens in the effluents before discharge into the environment and the need to en-

force treatment of the effluents before disposal.

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