# Antibiotics susceptibility profile of bacteria isolated from abattoir wastewater in Port Harcourt, River State, Nigeria

## Abstract

Abattoir environment can become a significant reservoir for antibiotics resistance bacteria, particular in abattoir that do not treat their waste before discharge. This study seeks to verify the antibiotics susceptibility profile of bacteria isolated from abattoir wastewater in Port Harcourt, River State, Nigeria. The bacteria were isolated from the wastewater using standard plating technique. The antibiotics sensitivity pattern of all bacterial isolates was determined by Kirby-Bauer disk diffusion method.Isolates with high multiple antibiotic resistance (MAR) index (≥0.5)were screened for genes for pathogenicity (icaC, adhesion gene) and antibiotic resistance (QnrA gene). The abattoir wastewaters were contaminated by bacteria resistant to no less than two of the antibiotics experimented with (MAR index range, 0.2-1.0). The isolates were identified as *Acinetobacter brisouii*, *Bacillus pumilus,Bacillus* sp. and*Priestiaflexa*. Among these isolates, 2(50.0%)harboured the QnrA gene while 3(75.0%) harboured the icaC gene. The findings underscore the importance of abattoir wastewater as environmental flashpoint for antibiotic resistance. Detection of bacteria with multiple antibiotics resistance in abattoir wastewater would inform cautious use of antibiotic, to check the spread of antibiotic resistance emanating from this source.

Keywords: abattoir wastewater, antibiotic resistance, pathogenicity

## 1. Introduction

Abattoirs constantly produce large amount of wastewater, particularly from washing operation. Abattoir wastewatercontains a variety of organic pollutantsincluding microorganisms dispelled from the visceral and skin of slaughtered animal[1].Presence ofmicroorganisms in abattoir wastewater, particularly those with strong propensity to cause infectious diseases, is a serious public health hazard [2].

In third world countries, owing to reasons of poverty and ignorance, abattoir waste gets dumped indiscriminately into the environment. Many people are not aware of how the effluent from abattoirscontributes toenvironmental degradation and affects the health of flora and fauna. If abattoir waste is spilled into the environment, enteric pathogens and excessive nutrients could find their way to surface waters, groundwater, and soil [3].

Abattoir wastewater contains a high concentration of antibiotics, heavy metals and organic pollutants which can create an environment conducive to the development and spread of antibiotics resistant bacteria[4].Several studies have informedon the instances of pathogenic bacteria in abattoir wastewater, includingWHO priority bacteria which are designated opportunistic bacterial pathogens that with widespread antimicrobial resistance, namely *Klebsiella pneumoniae*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, *Enterobacter*sppand*Staphylococcusaureus* [5]. The presence of antibiotics resistant bacteria in abattoir wastewater is a growing public health concern as it increases the risk of transmission of infectious diseases.

Microbiological quality assessment reports onabattoir wastewater have consistently revealed the presence of classical and opportunisticpathogensincluding [6-8].Olawale et al. [9] investigated the antibiotics susceptibility profiles of bacteria isolated from abattoir wastewater in Ilorin, Nigeria. The study isolated various Gram-negative and positive bacteria species, belonging to the families Enterobacteriaceae, Enterococcaceae, Pseudomonadaceae, Staphylococcaceae and Streptococcaceae. The results revealed high rates of resistance to commonly used antibiotics such as ampicillin, sulfamethoxazole andtetracycline,. The study emphasized the need for strict antibiotic stewardship practices to prevent the dissemination of antibiotic-resistant Gram-negative bacteria.Akpan et al. [10] investigated the antibiotics susceptibility patterns of Gram-negative bacteria (*Escherichia coli*, *Enterobacter* sp.,*Klebsiella* sp., *Shigella* sp., *Pseudomonas* sp. and*Salmonella* sp.) isolated from abattoir wastewater in Abeokuta, Nigeria, and reported that a substantial proportion were multidrug resistant.

Though antibioticscan be enhanced by human activities, it can also be driven by environmental factor. Heavy metals in the environment for instance, are known to increase antibiotics resistance in bacteria [11].Zhang et al. [12] isolated a strain of *Bacillus flexus* from oil reservoir that was resistant to ampicillin, erythromycin, fosfomycin, fosmidomycin, gentamicin,teicoplanin, tetracycline and vancomycin. Shivaji [13] reported that a strain of *Bacilluspumilus*isolated from air sample at high altitude, was resistant to ampicillin, ciprofloxacin, lincomycin and novobiocin.

According to Abia et al. [4] wastewater is a significant environmental reservoir for antibiotics resistanceand that the problem is much of an issue in Africa, becauseof indiscriminate use of antibiotics, derisory state of sanitation and the prevalent practice of not treating wastewater before discharge into the environment.Abattoir wastewater ought to be treatedbefore discharge into the environment to avert adverse alteringin microbiological properties.When the wastewater is laden with pathogenic and antibiotic resistant microorganisms, the spread to disease and antibiotics resistance is heightened.This study seeks to assess the antibiotics susceptibility profile of bacteria isolated from wastewater in abattoir located in Port Harcourt Rivers State.

## 2. Material and Methods

### 2.1 Wastewater Collection from Abattoirs

Wastewater was garnered from discharge points of Chokochoabattoir,Port Harcourt, Rivers State, Nigeria, in sterile 2.0litre sample vials. The samples were collected in the morning when slaughtering activity is at its peak. The samples were quickly shipped in ice chest to Microbiology Laboratory, University of Port Harcourt, for isolation of bacteria.

### 2.2 Isolation and Characterisationof Bacterial Isolates

The following bacteriological media were used for the isolation of culturable bacteria present in the wastewater: nutrient agar, MacConkey agar salmonella-shigella agar, thiosulphate citrate bile salt agar, eosin methylene blue agar and Cetrimide agar, by spread plate technique.

Distinct colonies on agar plates were sub-cultured in nutrient agar to plate out pure isolates. Isolates were characterized through Gram staining and a battery biochemical test, in accordancewithAneje[14].

### 2.3 Bacterial Antibiotics Susceptibility Testing

The antibiotics sensitivity pattern of all bacterial isolates was determined by Kirby-Bauer disk diffusion method described by Anele et al. [15] on Mueller Hinton (MH)agar.

The antibiotics used are Amoxycillin/Clavulinate(AU)30µg, Amoxicillin (AML) 30 µg,Ampiclox (APX) 20 µg, Ampicillin (APX) 10 µg, Cefalexin (CEP) 10 µg,Chloramphenicol (CH) 30 µg, Ciprofloxacin (CPX) 5 µg,Cotrimoxazole (SXT) 30 µg,Erthromycin (E) 15µg, Gentamicin (CN)10µg,Levofloxacin (LEV) 20 µg, Nalidixic Acid (NA) 30 µg, Norfloxacin (NB) 10 µg,Ofloxacin (OFX) 5 µg, Reflacine(PEF) 10 µg,Rifampicin (RD) 20µgand Streptomycin (S) 30µg.

Sterile MH agar was dispensed into sterile petri dishes and allowed to solidify. Afterwards, 0.1ml of the bacteria isolates agar plates using a sterilised hockey stick. Then multiple antibiotics disc wasplaced firmly on the agar plates using a sterilized forceps. Plates were incubated at 35±2$^{0}$C for 24hrs.

Zone of growth impedanceobserved was measured to the nearest millimetre, recordedand interpreted as sensitive, intermediate or resistant for each of the assayed antimicrobial agent, based on EUCAST2019 categories [16].

Multiple antibiotic resistance (MAR) index(the ratio of antibiotics an organism was resistant to in comparison to all antibiotics was exposed to) was calculated for each isolate [9].

### 2.4 Structural and Functional Genes Amplification and Sequencing

Isolates were ascertained on the basis of their 16S rRNA sequences following genomic DNA amplification(ABI 9700 Applied Biosystems thermal cycler) using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers.

Amplification oficaC genes from the selected isolates was done using primersicaCF: 5’-TAACTTTAGGCGCATATGTTTT -3’ and icaCR: 5’- TTCCAGTTAGGCTGGTATTG -3’ primers.

The QnrA gene from isolates was amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers.

Table 1 shows the PCR conditions to amplify 16S Rrna, icaCR and QnrA genes

| Cycle | Step | Temperature | Duration |
|---|---|---|---|
| 1 | Initial denaturation | 95ºC | 5 minutes |
| 35 | Denaturation | 95ºC | 30 seconds |
|  | Annealing | 58ºC | 30 seconds |
|  | Extension | 72ºC | 30 seconds |
| 1 | final extension | 72ºC | 5 minutes |

DNA sequencing was carried out using an Applied Biosystems (Foster City, CA, USA) automatic DNA sequencer (ABI PRISM 3130 x 1 Genetic Analyzer) and an Applied Biosystem Big Dye (ver. 3.1) kit.

## 3. Results

### Antibiotics susceptibility profile of bacteria

Table2 shows antibiotics sensitivity pattern of Gram-positive bacterial isolates obtained from Wastewater samples. All isolates showed resistance to rifampicin and levofloxacin, and are resistant to two or more of the antibiotics. MAR index range was 0.2-0.5.

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Table 2: Antibiotics sensitivity pattern of Gram-positive bacteria

| | **Antibiotic/ Diameter of zone of inhibition (mm)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | CPX | NB | CN | AML | S | E | RD | CH | APX | LEV | MAR index |
| *Staphylococcus* sp. | 15(S) | 9(I) | 18(S) | 11(I) | 16(S) | 16(S) | 2(R) | 19(S) | 15(S) | 4(R) | 0.2 |
| *Bacillus* sp. | 12(I) | 4(R) | 17(S) | 8(R) | 3(R) | 18(S) | 1(R) | 18(S) | 14(I) | 2(R) | 0.5 |
| *Priestia*sp. | 3(R) | 15(S) | 2(R) | 5(R) | 17(S) | 12(I) | 6(R) | 4(R) | 10(I) | 1(R) | 0.6 |
| *Bacillus* sp. | 10(I) | 1(R) | 18(S) | 8 (R) | 17(S) | 9(R) | 3 R) | 0 (R) | 5 (R) | 1(R) | 0.7 |

Keys: R=Resistance, I= Intermediate, S= Sensitive (EUCAST, 2019)

CPX= Ciprofloxacin, NB= Norfloxacin, CN=Gentamycin, AML=Amoxicillin, S= Streptomycin, E= Erythromycin, RD= Rifampicin, CH= Chloramphenicol, APX= Ampiclox, LEV=Levofloxacin

Table 3 shows antibiotics sensitivity pattern of Gram-negative bacterial isolates obtained from Wastewater. All the isolates were resistant to ampicillin. All the isolates were resistant to at least two antibiotics (MAR index range 0.2-1.0). *Acinetobacter* sp. was resistant to all antibiotics experimented with

Table 3: Antibiotics sensitivity pattern of Gram-negative bacterial

| | **Antibiotic/ Diameter of zone of inhibition (mm)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria | OFX | PEF | CPX | AU | CN | S | CEP | NA | SXT | PN | AMR index |
| *E. coli* | 17(S) | 15(S) | 15(S) | 18 S) | 18(S) | 17(S) | 0(R) | 0(R) | 4(R) | 0(R) | 0.4 |
| *Shigella* sp. | 15(S) | 18(S) | 15(S) | 14(R) | 15(S) | 17(S) | 15(S) | 19(S) | 6 (R) | 5(R) | 0.3 |
| *Acinetobacter* sp. | 6(R) | 8(R) | 10(R) | 9(R) | 7(R) | 6(R) | 11(R) | 6(R) | 5(R) | 3(R) | 1.0 |
| *Enterobacter* sp. | 4(R) | 6(R) | 11(I) | 14(I) | 16(S) | 18(S) | 15(S) | 8(R) | 15(S) | 2(R) | 0.4 |
| *Salmonella* sp. | 15(S) | 5(R) | 10(I) | 17(S) | 15(S) | 6(R) | 4(R) | 15(S) | 18(S) | 5(R) | 0.4 |
| *Pseudomonas* sp. | 11(I) | 14(I) | 10(I) | 7(R) | 15(S) | 14(I) | 10(I) | 15(S) | 13(I) | 6(R) | 0.2 |

Keys: R=Resistance, I= Intermediate, S= Sensitive (EUCAST, 2019)

OFX= = Ofloxacin 5 µg, PEF= Reflacine, CPX=Ciprofloxacin, AU= Amoxycillin/Clavulinate, CN=Gentamycin, S= Streptomycin, CEP= Cefalexin, NA= Nalidixic Acid, SXT= Cotrimoxazole, PN= Ampicillin

### 3.2 Molecular Identification

Analysis of 16s rRNA gene sequence placed the isolates within the *Bacillus*, *Priestia*and *Acinetobacter* sp., and revealed a closely relatednessto, *Acinetobacter brisouii*strain AB859735 (99.8%), *Bacillus pumilus* strain MK123487 (99.9%), *Bacillus* sp. strain MN704100 (99.7%) and*Priestiaflexa*strain OQ726277 (99.8%).

### 3.3 Resistance and virulence genes

The distribution of resistance and virulence genes among the bacterial isolates revealed that 4 bacteria 2(50.0%) harboured the resistance gene QnrA while 3(75.0%) had the icaC gene for adhesion.

The bacterial isolates harbouring the resistance gene QnrAare *Acinetobacter brisouii*and *Bacillus pumilus*while *Priestiaflexa*, *Acinetobacter brisouii*and *Bacillus* sp.harbouredicaC gene for adhesion.

Table 4: Distribution of virulence and resistance genes among the bacterial isolates

| Organism | Accession Number | QnrA | icaC |
|---|---|---|---|
| *Acinetobacter brisouii* | OR462205 | + | + |
| *Priestiaflexa* | OR462206 | - | + |
| *Bacillus* sp. | OR462207 | - | + |
| *Bacillus pumilus* | OR462208 | + | - |
| | *Total* | *2(50.0%)* | *3(75.0%)* |

### Discussion

The bacteria isolated from wastewater in this study are opportunistic pathogens.Results for antibiotics sensitivity pattern of Gram-positive bacterial isolates obtained from wastewater samples shows that all isolates showed resistance to rifampicin and levofloxacin, and are resistant to two or more of the antibiotics. Results for antibiotics sensitivity pattern of Gram-negative bacterial isolates obtained from Wastewater shows that all the isolates were resistant to ampicillin. All isolates were resistant to at least two of the antibiotics tested.

In the present study *Bacillus* sp. were resistant to norfloxacin,amoxicillin, erythromycin, rifampicin, chloramphenicol, ampiclox, levofloxacin, rifampicin and levofloxacin. Antibiotics resistance in *Bacillus* sp. from clinical and environmental samples has been severally reported.Sundaramanickam [17] reported resistance of *B.pumilus*isolated from shrimp to 14 antibiotics including norfloxacin,amoxicillin, erythromycin,rifampicin, chloramphenicol, ampiclox, levofloxacin and levofloxacin.György [18] reported that*B. stratosphericus*SALKÖ, isolated from commercially available spice were resistant to amoxiclav, azithromycin, oxacillin, penicillin G and rifampicin.Zhang et al. [12] isolated a strain of *Bacillus flexus* from oil reservoir that showed resistance to ampicillin, erythromycin, fosfomycin, fosmidomycin, gentamicin,teicoplanin, tetracycline and vancomycin.

*Acinetobacterbrisouii*isolated from abattoir wastewater in the present study was resistant toreflacine, ciprofloxacin, amoxycillin/clavulinate, streptomycin, cefalexin, nalidixic acid, ofloxacin, gentamycin,nalidixic acid, cotrimoxazole, streptomycinandampicillin.*Acinetobacter baumannii* is the well-known pathogenic *Acinetobacter* species, but it is not the only member of that species that are resistant to commonly used antibiotics against Gram-negative bacteria. Antibiotics resistance has been reported in non-*Acinetobacter* species such as *A.brisouii* [19,20].

The isolate *Priestiaflexa*was resistant to ciprofloxacin, gentamycin, amoxicillin, streptomycin, rifampicin, chloramphenicol and levofloxacin. Deswal [21] reported a strain of *P.flexa* isolated from human faecesthat was resistant to cefixime, clavulanic acid/ceftazidime, nafallin, methicillin, trimethoprim, kanamycin and nalidixic.

The presence of multi-drug resistant strains of bacterial isolates in abattoir wastewater is a pointer it could act as a reservoir and conduit for the propagation antibiotic resistance in the environment. Similar view was expressed in the study by Ogbonna andAzuonwu[2] in which multi-drug resistant strains of bacteria were isolated from abattoir effluents. This is a source for concern going by the growing trend of antibiotics resistance globally.

## Resistance and Virulence Genes

Bacterial isolates in wastewater samples were identified on the basis of their 16S rRNA genes to be closely related to *Bacillus* sp., *B.pumilus*, *P.flexa*and *A.brisouii*. These isolates were also analysed to ascertain if they are carriers of resistance and virulence genes. The bacterial isolates harbouring the resistance gene QnrAare *A.brisouii*and *B.pumilus*while *P.flexa*, *A.brisouii*and *Bacillus sp.*harbouredicaC gene for adhesion. Sokolov [22] detected icaC in Staphylococcus species but not in Bacillus. TheicaC gene is part of icaABCD cluster under the control ica operon, known for the synthesis of polysaccharide intercellular adhesin (PIA), a major component of the extracellular matrixnecessary for biofilm formation by bacteria [23,24]. Biofilm contribute to microbial pathogenicity as adhesion to surfaces is an important pathogenic factor and also enhances antibiotics resistance by their impermeable nature [23,25]

*Bacillus pumilus*and *A.brisouii*harboured QnrA gene.The presence of QnrA gene has been associated with decreased susceptibility to quinolones inbacterial species.*Acinetobacterbrisouii*was resistant to levofloxacin, ofloxacin, ciprofloxacin and nalidixic acid, while *B.pumilus* was resistant to levofloxacin and norfloxacin. The QnrA gene is a plasmid-mediated quinolone resistance gene that encodes for a protein that protects the DNA gyrase and topoisomerase IV, from the effect of quinolone antibiotics [26].

Treatment of wastewater can reduce microbial load and improve on the physiochemical properties of abattoir wastewater [27]. This would help in checking the spread of antibiotics resistance.

## Conclusion

The findings underscore the importance of abattoir wastewater as environmental flashpoint for antibiotic resistance. Detection ofbacteria with multiple antibiotics resistancein abattoir wastewaterwouldinformcautious use of antibiotic,tocheck the spread of antibiotic resistance emanating from this source.

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