Isolation and characterisation of antibiotics resistant bacteria from Industrial wastewater in Choba, Port Harcourt

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Abstract:
The widespread and indiscriminate release of inadequately treated industrial wastewater into aquatic environments poses significant threats to public health, as it contributes to antibiotic-resistant bacteria (ARB). The purpose of this study was to identify and characterise ARB from industrial wastewater samples to investigate their presence and assess their potential environmental and health implications. Wastewater samples were collected at the discharge point in Port Harcourt, Rivers State, Nigeria. The generated wastewater comes from cleaning equipment, processing raw materials, and packaging products. Contaminants include starch, oil, grease, organic solids, and cleaning agents like detergents. The physical and chemical parameters of wastewater were analysed. The disk diffusion method was used to determine the antibiotic susceptibility of bacterial pathogen isolates, and bacteriological tests were conducted in the lab using standard protocols for aerobic bacteria. The results revealed a pH of 8.4, temperature of 28.5°C, turbidity of 8.43 NTU, and elevated levels of total dissolved solids (147.67 mg/L) and chemical oxygen demand (74.4 mg/L). Microbial analysis identified nine bacterial genera, including *Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis,* and *Bacillus subtilis*, with the bacterial isolates exhibiting varied resistance to commonly used antibiotics. *Pseudomonas aeruginosa* displayed the highest multiple antibiotic resistance (MAR) index of 0.7. Antibiotic resistance screening revealed resistance to up to seven antibiotics among Gram-negative isolates and up to four antibiotics among Gram-positive isolates. The findings highlight the potential role of industrial wastewater in harbouring antibiotic-resistant bacteria, posing risks to public health and ecosystems. This highlights the need for more effective and action-based regulations on industrial waste management to lessen the spread and prevalence of antimicrobial resistance.

Keywords: **Antimicrobial resistance profile, Food processing effluent, Virulent genes, Wastewater, Industrial waste management.**

1. INTRODUCTION

INDUSTRIAL wastewater management is a growing global concern due to the rapid expansion of industries and their significant contribution to environmental pollution [1][2]. Globally, industrial wastewater constitutes a substantial portion of water pollution, impacting rivers, lakes, and groundwater. Water is an essential component of industrial processes, and its quality and availability pose great significance to people and businesses alike [3]. In the food processing sector, wastewater is defined by its high organic and nutrient content, including carbohydrates, proteins, fats, oils, and salts. Managing this wastewater becomes even more essential as production scales increase to satisfy growing consumer demands. Antibiotic resistance is considered one of the most critical challenges to global health, food security, and sustainable development today. The rapid rise of antibiotic-resistant bacteria (ARB) is driven not only by the overuse and misuse of antibiotics in healthcare and agriculture but also by environmental factors, particularly the role of industrial wastewater. Industrial effluents, such as those generated by food and beverage processing facilities [4][5] chemical manufacturing industries, textile and dye industries [6], pulp and paper industries [7], and petroleum refineries, are increasingly recognised as reservoirs for ARB and vectors for the spread of antibiotic resistance genes (ARGs) in the environment, because of their heavy dependence on water for their industrial processes.

Food factories located in regions such as Nigeria, Indonesia, and India are major contributors to wastewater in the food processing sector. These factories rely heavily on water for processes such as boiling, steaming, frying, and cleaning, all of which generate effluents containing residues of starch, oils, and salts, and potentially trace amounts of antibiotics or antimicrobial agents used for equipment sterilisation or as preservatives. The nutrient-rich effluent creates an ideal breeding ground for bacteria, facilitating the survival and growth of resistant strains. Additionally, the discharge of such untreated or inadequately treated wastewater into the environment can introduce ARB into natural water bodies, soils, and agricultural systems, contributing to the global spread of resistance. In many regions, faulty infrastructure and relaxed environmental regulations are contributory factors of industrial wastewater management, further complicated by non-compliance by industries [8]. As several industries lack the

appropriate infrastructure to manage their wastewater efficiently [9], untreated effluents are discharged into water bodies, which can lead to long-term consequences, including biodiversity loss, groundwater contamination, and increased prevalence of waterborne diseases [10][11]. The increasing prevalence of antibiotic-resistant bacteria (ARB) is a pressing global issue, with significant implications for human health, environmental sustainability, and socio-economic development. Considering the large-scale food production at food factories, the quantity of resulting wastewater is disproportionately high, and this remains largely underexplored. Hence, the current study was conducted to isolate and characterise antibiotic-resistant bacteria (ARB) from industrial wastewater obtained from a food factory. The findings are expected to contribute to the increasing body of knowledge on environmental antimicrobial resistance and provoke action-based interventions to reduce the spread of microbial resistance from industrial discharge.

II. Materials And Methods

Sample collection

Samples were taken from the food industry wastewater discharge point in Port Harcourt, using plastic containers sanitised with 70% alcohol and standard procedures for the analysis of water and wastewater outlined by [12]. The company makes Pasta, Baking flour, Power oil, Indomie, and Semolina. The sample containers were repeatedly rinsed with industrial effluent before being filled with the sample. Well-labelled sterile bottles were used to collect 100 ml of industrial effluents from two sampling points and immediately transferred to the University of Port Harcourt's Microbiology laboratory for additional examinations. All samples were kept in cooler boxes. Every sample was kept at 4°C and examined within 24 hours of being collected.

Physicochemical properties of wastewater samples

On-site, a digital pH meter was used to measure the pH of wastewater samples. Standard laboratory procedures were used to test temperature, heavy metals, phosphate, sulphate, nitrate, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and total suspended solids [13]. Every time wastewater was sampled, information on the wastewater treatment plant's operational parameters, including flow rate, residence time, and desludging rate, was gathered.

Isolation and identification of bacterial isolates

Standard methods for aerobic bacteria were used in the laboratory to conduct bacteriological analysis. A portion of the wastewater sample was initially enhanced in Selenite F Broth, which aids in inhibiting the development of bacteria other than Shigella and Salmonella, in order to identify Salmonella species. A loop-shaped portion of the sample was streaked onto Salmonella-Shigella (SS) agar following enrichment, and it was then incubated for 24 hours at 36–37°C. The preliminary *Salmonella* spp. identification was based on its colony's appearance and biochemical properties, including shape, size, texture, colour, elevation, motility, Gram stain reaction, and tests for urease, indole, oxidase, lactase, citrate and catalase. The identification of *Pseudomonas* spp., *E. coli*, and *Streptococcus* spp. in the sample was done after being cultured on MacConkey agar and incubating at 36–37°C for 24 hours. Individual colonies were then isolated and purified for further analysis. These pure cultures were studied for morphological and biochemical features to confirm their identity.

Quantification of *Pseudomonas* spp., *E. coli*, *Streptococcus* spp., *Salmonella* spp. and other bacteria was carried out using the pour plate method. Serial dilutions (10^{-2} to 10^{-8}) were prepared, and 1.0 ml from each dilution was mixed with molten agar and poured into Petri dishes. After the medium solidified, the plates were incubated at 37°C for 48 hours. The resulting colonies were counted and multiplied by the dilution factor to estimate the bacterial concentration in the original sample.

Characterisation of the isolated bacteria

The conventional biochemical tests carried out include Voges-Proskauer test, Indole test, triple sugar iron agar test, Methyl-red test, and Citrate utilisation test. Motility test using nutrient broth, nutrient agar, EMB agar and SS agar accordingly, and Gram staining were carried out [14]. Using established protocols, gram-positives were detected by their various physiological tests, including coagulase, hemolysis, and catalase [15].

Antimicrobial Susceptibility Testing

The antibiotic resistance of bacterial pathogens was assessed using the disk diffusion method, following the guidelines described in reference [15. Bacteria were cultured in nutrient broth for 24 hours, and the turbidity was adjusted using a 0.5 McFarland standard. Fresh Mueller-Hinton agar was then inoculated with the standardised bacterial suspension using sterile cotton swabs. After inoculation, the agar plates were left to dry before being incubated aerobically at 36–37°C for 24 hours. Commercially available antibiotic discs, pre-soaked with specific drugs, were placed on the surface of the agar. The plates were again incubated at 37°C for 24 hours.

The bacterial isolates were tested against 10 antibiotics from six different drug classes, including Penicillin (10 µg), Nalidixic acid (30 µg), Quinolones: (Ciprofloxacin (10 µg), Ofloxacin (10 µg)), Sulfonamides (Co-trimoxazole or sulfamethoxazole-trimethoprim (10 µg)), Beta-lactams (Augmentin; a combination of amoxicillin and clavulanic acid, 30 µg), Pefloxacin (10 µg), Aminoglycosides (Gentamicin (10 µg)), Cephalosporins: Cephalexin and Cephorex (30 µg each) and Streptomycin (30 µg), as described by [17]. Antibiotic doses that inhibited microbial growth were used to identify inhibition zones, which were quantified to the closest millilitre. The isolates were classified as susceptible (S), intermediate (I), or resistant (R) based on their level of susceptibility. Each isolate's multiple antibiotic resistance (MAR) index was independently determined using the methodology outlined by [18].

Quality control

Type culture (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922) was employed as quality control for the susceptibility testing of antibiotics as recommended by Clinical and Laboratory Standards Institute [19].

DNA Extraction

Cultures of bacteria kept in Mueller-Hinton broth storage were extracted and subsequently used to identify virulent and antibiotic-resistant genes (ARGs). For the extraction procedure, 1 ml of the suspension was put into a test tube with 1 ml of sterile deionised water (ddH_2O). The suspension was then heat-shocked for ten minutes at 95°C in a water bath. In order to evaluate potential contamination during the DNA extraction procedure, a negative control of ddH_2O was introduced and evaluated concurrently. After that, the mixture was centrifuged at 14,000 × g for five minutes. For further molecular analysis, the bacterial DNA-containing supernatant was moved to a fresh 2 ml tube and kept at -20°C.

Quantification of DNA

A drop (about 2 µl) was placed in the sample area, and the NanoDrop spectrophotometer program was used to analyse the DNA in order to determine its concentration and purity.

Detection of Virulence Genes using PCR

Following the extraction of the DNA of the bacterial isolates, detection of the virulent genes of the isolates by PCR was done as described by other studies [20][21]. Table 1 contains the list of the PCR primers that were utilised to amplify the pathogenic genes. To obtain bacterial extracts, overnight cultures of bacteria were suspended in 1 ml of distilled water and boiled for 10 minutes at 95 ^{0}Celsius. The cell extracts were immediately employed as a DNA template for the PCR reaction after being boiled and allowed to cool on ice. The Phusion™ flash high-fidelity PCR master mix (Finnzymes instrument, Finland) was used for the PCR. A final volume of 10 µl was used for the reactions, which included 2 µl of PCR water, 5 µl of Phusion flash PCR master mix, and 0.5 µM of primer sets in 1 µl volume of each. The reaction conditions were as follows: initial denaturation at 95ºC for 30 seconds, followed by 35 cycles of annealing process at 95ºC for one second, 58ºC for 5 seconds and 72ºC for 15 seconds. The final extension was applied at 72°C for one minute. The presence of a particular DNA band on a 1.5% agarose gel, stained with ethidium bromide, and examined under a UV transilluminator was used to identify the detection. The sizes of the PCR products were estimated based on the migration pattern of a 100-bp ladder.

PCR-based detection of antibiotic-resistant genes (ARGs)

The DNA extraction technique was validated using polymerase chain reaction (PCR) directed against the bacterial 16S rRNA gene (Table 2). To ascertain whether each gene was present in each of the bacterial isolates, PCR amplification was performed against 10 ARGs.

The set of primers used for each gene is shown in Table 2.

At a final volume of 25 µl, the PCR mixture for every reaction included 12.5 µl of 2 × Taq PCR Master Mix (0.1 µl of Taq polymerase/µl, 0.5 mM dNTP, and 3 MgCl2), 0.5 µl of each primer (1 µM), 1 µl of template DNA, and ddH2O. Every PCR reaction was conducted without the use of template controls. One microliter of 105 copies of each ARG's DNA standards, as measured by gel electrophoresis, made up the positive controls. Using gel electrophoresis and the predicted amplicon length (measured with a DNA ladder), the presence or absence of a certain target gene was evaluated.

III. Results

Table 1: Physical and chemical features of industrial wastewater

Entities	Value	Recommended limit (FMENV)
pH	8.4	6.0 – 9.0
Temperature (^{0}C)	28.5	40
Turbidity (NTU)	8.43	5
Electrical conductivity (mho/cm)	351.39	400
Total Dissolved Solids (mg/L)	147.67	500
Total Soluble Solids (mg/L)	17.37	50
Biological Oxygen Demand (mg/L)	33.21	10
Chemical Oxygen Demand (mg/L)	74.4	50
Nitrate (mg/L)	18.26	10
Phosphate (mg/L)	12.38	5
Sulphate (mg/L)	189.15	50
Chloride (mg/L)	54.03	250

Key: FMENV=Federal Ministry of Environment Nigeria; mg/l=milligram per litre; µs/cm=micro siemens per centimeter; NTU= Nephleometric Turbidity Units

Table 2: Total viable counts of bacteria from industrial wastewater

Culture medium	Bacterial count (Mean ± SE) (cfu/g×10^5)
THB	7.70±0.36
MacConkey agar	4.25±0.33
Turbidity (NTU)	4.25±0.26
Salmonella Shigella agar	0.64±0.22
Centrimide agar	0.06±0.00
Mannitol salt agar	0.1±0.05

Counts represent means of triplicate samples ± standard error
Key: cfu/g= Colony forming units per gramme;S.E=Standard error

Table 3: Morphological characteristics of bacterial isolates

ID	Color	Shape	Margin	Surface	Elevation	Opacity	Size (mm)	Samples
THB								
WA	White	Circular	Entire	S&S	Flat	Opaque	0.3	IF1
WA	Light blue	Circular	Entire	S&S	Flat	Translucent	0.2	IF2
MAC								
WA	Pink	Circular	Entire	S&S	Raised	Opaque	0.4	IF3
WA	Cream	Circular	Entire	S&S	Raised	Opaque	0.5	IF4
SSA								
WA	Pink	Circular	Entire	S&S	Flat	Opaque	0.4	IF5
CA								
WA	Cream	Circular	Entire	S&S	Flat	Opaque	0.4	IF6
EMB								
WA	MSG	Circular	Entire	S&S	Flat	Opaque	0.4	IF7
WA	MSG	Circular	Entire	S&S	Flat	Opaque	0.5	IF8
WA	Pink	Circular	Entire	S&S	Flat	Opaque	0.3	IF9

Key: THB=; WA=; EMB=Eosin Methylene Blue agar; MSG=Metallic sheen green; MAC=MacConkey agar;

Table 4: Biochemical characteristics of bacterial isolates

Isolate ID	IF1	IF2	IF3	IF4	IF5	IF6	IF7	IF8	IF9
Catalase	+	+	-	-	+	+	+	+	+
Citrate	+	-	-	+	+	-	+	+	+
Glucose	+	-	+	+	+	-	+	+	+
Lactose	-	-	+	+	+	-	+	+	+
Indole	+	-	-	-	+	-	+	+	-
MR	-	-	-	+	+	-	+	+	-
VP	+	-	+	-	-	-	-	-	+
Motility	+	+	-	-	+	+	+	+	+
Starch	-	+	-	+	-	+	-	-	-
TSI	BB-	AA-	AA-	AB-	AB-	AA-	AB+	AA+	AB-
H2S	+	-	-	+	-	-	-	-	-

'+'=Positive; '-'=Negative

Table 5: Rate of occurrence of bacterial isolates

Bacterial isolate	Number of isolates	Occurrence (%)
Bacillus subtilis	5	13.16
Pseudomonas aeruginosa	9	23.68
Enterococcus faecalis	5	13.16
Streptococcus spp.	3	7.89
Escherichia coli	13	34.21
Enterobacter aerogenes	3	7.89
Total	38	100

Table 6: Antimicrobial susceptibility test for the Gram-positive bacterial isolates

Code	IF1	IF3	IF4
Bacterial isolate	*Bacillus subtilis*	*Enterococcus faecalis*	*Streptococcus* spp.
LEV	S	S	S
E	S	S	S
PEF	S	S	S
CN	S	20	20
APX	18	19	20
Z	16	20	23
AM	S	19	18
R	S	S	S
CPX	S	S	S
AZ	S	S	S

PEF=pefloxacin; CN=gentamicin; APX=ampiclox; Z=zinnacef; AM=Amoxicillin; R= Rocephin; CPX=Ciprofloxacin; AZ=Azithromycin; LEV=levoflocacin; E=Erythromycin; S=sensitive; R=Resistant, Zone of inhibition≤15mm (sensitive); Zone of inhibition≥15mm (Resistant)

Table 7: Antimicrobial susceptibility test for Gram-negative bacterial isolates

Code	IF2	IF5	IF6	IF7	IF8	IF9
Bacterial isolate	*P. aeruginosa*	*E. coli*	*P. aeruginosa*	*E. coli*	*E. coli*	*E. aerogenes*
CPX	S	S	S	S	S	S
AM	15	R	R	16	15	10
AN	R	R	R	RS	R	R
CN	18	15	19	S	20	S
PEF	S	S	S	S	S	S
OFX	S	S	S	S	S	S
S	S	S	20	S	S	S
RXT	S	22	18	S	S	S
CH	S	R	15	14	15	16
SP	S	15	18	S	S	S

CPX=Ciprofloxacin; PEF=pefloxacin; AZ=Azithromycin; APX=ampiclox; Z=zinnacef; AM=Amoxicillin; R= Rocephin; CN=gentamicin; LEV=levoflocacin; E=Erythromycin; S=sensitive; R=Resistant, Zone of inhibition≤15mm (sensitive); Zone of inhibition≥15mm (Resistant)

Table 8: Multiple antibiotic resistance index of the bacterial isolates

Code	Bacterial isolate	Resistance pattern	Number of antibiotics	MAR Index
IF1	*Bacillus subtilis*	APX, Z	2	0.2
IF3	*E. faecalis*	CN, APX, Z, AM	4	0.4
IF4	*Streptococcus* spp.	CN, APX, Z, AM	4	0.4
IF2	*Pseudomonas aeruginosa*	AM, AU, CN	3	0.3
IF5	*Escherichia coli*	AU, AM, SXT, SP	4	0.4
IF6	*Pseudomonas aeruginosa*	CN, AU, S, AM, SXT, CH, SP	7	0.7
IF7	*Escherichia coli*	AM, AU	2	0.2
IF8	*Escherichia coli*	AU, CN	2	0.2
IF9	*Enterobacter aerogenes*	AU, CH	2	0.2

CPX=Ciprofloxacin; PEF=pefloxacin; AZ=Azithromycin; APX=ampiclox; Z=zinnacef; AM=Amoxicillin; R= Rocephin; CN=gentamicin; LEV=levoflocacin; E=Erythromycin; S=sensitive; R=Resistant, Zone of inhibition≤15mm (sensitive); Zone of inhibition≥15mm (Resistant)

Physicochemical Properties

The result of the physicochemical properties of the wastewater in Table 1 showed the following Parameters pH (8.4), Temperature (28.5°C), electrical conductivity (351.39 µS/cm),

Chloride 54 mg/l, Total soluble solids (17.37 mg/l), Total dissolved solids 147.67 mg/l were within permissible FMENV limits. However, the following parameters: chemical oxygen demand (COD, 74.4 mg/L), turbidity (8.43 NTU), Biological oxygen demand 33.31 mg/l Nitrate 18.26 mg/l, phosphate 12.5 mg/l and Sulphate 189.15 mg/l exceeded the FMENV permissible limit.

Microbial load and prevalence of Bacterial pathogens from wastewater.

The microbial load presented in Table 2 showed a total heterotrophic bacterial count of 7.7×10^5 CFU/g, MacConkey agar (4.25×10^5 CFU/g), Salmonella Shigella agar (4.25×10^5 CFU/g), and Centrimide agar 0.006×10^5 CFU/g) and Mannitol agar (0.1×10^5 CFU/g.)

The result in Table 5 showed six bacterial isolates identified as *Bacillus subtilis, Pseudomonas aeruginosa, Enterococcus faecalis, Streptococcus sp, Escherichia coli, and Enterobacter aerogene*s. The percentage frequency presented in Table 5 revealed that *Escherichia coli* was the most prevalent, with the highest prevalence of 34.21%, *Pseudomonas aeruginosa* 23.68%, *Bacillus subtilis* and *Enterococcus faecalis* had a percentage frequency of 13.16%, *Enterobacter aerogenes* and *Streptococcus* sp had the least percentage frequency of 7.89%.

Patterns of Antibiotic Resistance and the Index of Multiple Antibiotic Resistance (MAR).

In Table 6, the antibacterial susceptibility test for the gram-positive isolates: *Bacillus subtilis, Enterococcus faecalis, and Streptococcus* sp demonstrated that they were all resistant to zinnacef and Ampiclox, while *Enterococcus faecalis* and *Streptococcus* sp were resistant to gentamycin and amoxicillin. Similarly, in Table 7, all gram-negative bacterial isolates, *Pseudomonas aeruginosa, Enterobacter aerogene*, and *Escherichia coli* showed resistance to Augmentin and gentamicin. Different strains of *Escherichia coli* and Pseudomonas aeruginosa showed resistance to Amoxicillin, while *Enterobacter aerogenes* showed no resistance to Amoxicillin. Some strains of *Pseudomonas aeruginosa* and E. *coli* were resistant to gentamicin, while other strains of *Pseudomonas* were resistant to Streptomycin. The MAR Index for the bacterial isolates presented in Table 8 ranged from 0.2 to 0.7 when tested against ten (10) antibiotics, with *Pseudomonas aeruginosa* having a MAR index of 0.7 and having resistance to Augmentin, gentamicin, Streptomycin, sparfloxacin, Amoxicillin, Chloramphenicol and Septrin. Different strains of *E. coli* were isolated as seen by their resistance profile to antibiotics. *E. coli* strain (IF5) with MAR index of 0.4 was resistant to Augmentin, Amoxicillin, Septrin and Sparfloxacin. While other strains of *E. coli* have a MAR index of 0.2, *E. coli* code IF7 showed resistance to Augmentin and Amoxicillin, and *E. coli* (IF8) showed resistance to Augmentin and gentamicin. Generally, the different strains of *E. coli* showed resistance to Augmentin. *Enterobacter aerogenes* showed resistance to Augmentin and chloramphenicol. Generally, all strains of the isolated gram-negative bacteria exhibited resistance to Augmentin in this study. *Streptococcus* sp and *Enterobacter faecalis* have a MAR index of 0.4, both showing resistance to the same antibiotics: Amoxicillin, gentamicin, zinnacef and ampiclox. *Bacillus subtilis* has a MAR index of 2, showing resistance to Ampiclox and Zinnacef. Generally, the gram-positive bacterial isolate showed a similar resistance pattern to Ampiclox and Zinnacef.

AMPLIFICATION OF ANTIBIOTIC RESISTANCE GENES

The PCR amplification of quinolone resistance-conferring gene (*qnr*A) genes in antibiotic-resistant bacteria isolated from industrial wastewater is presented in Figure 1. Five antibiotic-resistant bacteria were screened for *qnr*A genes. The gel electrophoresis revealed that *qnr*A genes were present in all of the isolates with an amplicon size of 430 bp.

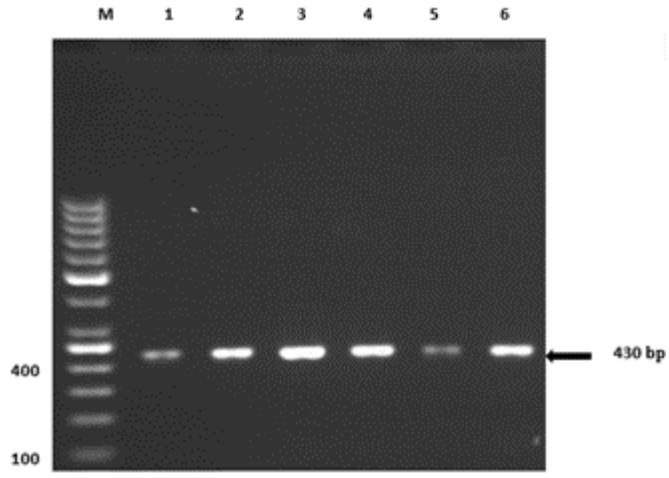

Figure 1: Gel electrophoresis amplification of *qnr*A genes in antibiotic-resistant bacteria isolated from industrial wastewater

Keys: Lane M=100 bp DNA maker, 1=Enterococcus faecalis, 2 = Escherichia coli (IF5), 3=Pseudomonas aeruginosa (IF6), 4=Escherichia coli (IF8), 5=Pseudomonas aeruginosa (IF2) 6=control

IV. DISCUSSION

The result of the physicochemical properties of the wastewater showed the following Parameters pH (8.4), Temperature (28.5°C) electrical conductivity (351.39 µS/cm), Chloride 54mg/l, Total soluble solid (17.37mg/l) Total dissolved solids 147.67mg/l were within permissible Federal Ministry of Environment (FMENV) and WHO limits this agrees with the report of [22][23]. Temperature influences biological processes and gas solubility [24][25]. EC and TDS indicate that salt and dissolved solids concentration were within limits, the high pH value, which is the degree of acidity or alkalinity, was within the ideal range is 6.5–8.5. The presence of inorganic solids, such as chlorides, affects water conductivity; this could account for the low EC because of low chloride. However, the following parameters: turbidity (8.43 NTU), chemical oxygen demand (COD, 74.4 mg/L), Biological oxygen demand 33.31mg/l, Nitrate 18.26mg/l, phosphate 12.5mg/l and Sulphate 189.15mg/l exceeded the FMENV permissible limit. Though the TSS, which measures suspended particles affecting water clarity, was low but Turbidity was high, which could be a result of chemical pollutants or organic matter. The increase in Turbidity, BOD, and DO showed that effluent contributes to pollution, and this is similar to the findings of [23], who investigated the physicochemical parameters of refinery waste water effluent discharged into Ekerema Creek. The increase in

Biological oxygen demand, Chemical Oxygen Demand, presence of high organic and particulate matter loads can disrupt aquatic ecosystems and reduce dissolved oxygen levels critical for aquatic life [25]. The Nitrates, Phosphate and sulphate levels exceeded the WHO limits, and improper disposal of untreated wastewater into the environment has become a major source of nutrients for algae bloom, causing eutrophication of surface water bodies [26][27]. The high levels of Nitrogen and phosphorus in wastewater promote microbial growth but can cause eutrophication in aquatic ecosystems [28]. In Nigeria, eutrophication is becoming more and more of a concern, especially in the Niger Delta [29]. As a result, there is a fish population decline, poisonous cyanobacterial blooms, oxygen levels drop, and an increase in BOD; thus, the biological integrity of surface waters is jeopardised [30]. Oil and grease from the processing plant can form films which can reduce oxygen exchange. Heavy Metals and Toxins: Common in industrial wastewater, toxic to health and the environment [31].

Counts ranged between 1 x 10^4 - 77x 10^4, the presence of bacteria in the effluent indicates bacterial pollution, the counts were higher compared to the findings of [32] and lower than the counts obtained by [22]. The microbial load of industrial effluents varied among study facilities.

Six bacterial isolates were identified in this study: *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterococcus faecalis*, *Streptococcus* sp, *Escherichia coli* and *Enterobacter aerogenes*. Some of the isolates identified in this study were similar to the reports of [33][34][32]. Out of the six bacteria identified, *Escherichia coli* was the most prevalent, with a prevalence of 34%, while *Streptococcus* sp and *Enterobacter aerogenes* were the least prevalent (7.89%). The findings agree with the study of [32]. The presence of *Escherichia coli* indicates the presence of faecal coliforms, human carriers could introduce these human pathogens into the effluent. Five antibiotics resistant bacteria were screened for *qnr*A genes, the gel electrophoresis revealed that *qnr*A genes were present in all of the isolates with an amplicon size of 430 bp: *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, streptococcus sp and Enterobacter aerogenes* this agrees with the report [35] and [36] that sewage often contains Antibiotic Resistant Bacteria, Antibiotic Resistant Genes, *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp were the common pathogens cited as major drivers of Antimicrobial resistance. [35][36]. The *qnrA* gene is a plasmid-mediated quinolone resistance gene that confers low-level resistance to fluoroquinolones in bacteria like *Escherichia coli*. It's a protein within the pentapeptide repeat family, protecting DNA gyrase and topoisomerase IV from quinolone inhibition. *qnrA* is often found on the same plasmids as extended-spectrum beta-lactamase (ESBL) genes, contributing to multidrug resistance.

The antibiotic resistance screening demonstrated multidrug resistance across bacterial isolates, with Pseudomonas aeruginosa showing the highest resistance index (0.7). *Pseudomonas aeruginosa* has a MAR index of 0.7 and has resistance to seven out of 10 antibiotics tested: Augmentin, gentamicin, Streptomycin, Sparfloxacin, Amoxicillin, Chloramphenicol and Septrin. *Pseudomonas aeruginosa* showed the highest resistance index (0.7), which agrees with the finding of [37] where *Pseudomonas* was the predominant isolate from pharmaceutical effluent and showed the highest multiple drug resistance to 11 antibiotics. In this study, *Pseudomonas aeruginosa* showed high sensitivity to ciprofloxacin, and this agrees with the finding of [37]. According to the study, *Pseudomonas aeruginosa* transmits disease to humans through the environment and serves as a reservoir for genes that cause antibiotic resistance. *Pseudomonas* species and other microorganisms derived from pharmaceutical effluents are a major public health concern due to their high levels of antibiotic resistance [37]. *Pseudomonas aeruginosa's* resistance profile raises concerns due to its association with healthcare-acquired infections and resilience to treatment. Gram-positive isolates like *Enterococcus faecalis* and *Streptococcus* spp were resistant to multiple antibiotics, indicating the potential transfer of resistance genes. This underscores industrial wastewater's role as a hotspot for antimicrobial resistance dissemination, as previously noted by [38].

Different strains of *Escherichia coli* were isolated as seen by their resistance profile to antibiotics. *Escherichia coli* strain (IF5) with MAR index of 0.4 was resistant to Augmentin, Amoxicillin, Septrin and Sparfloxacin, while *Escherichia coli* (IF7) was resistant to Amoxicillin and Augmentin, and Escherichia coli (IF8) showed resistance to Gentamycin and Augmentin. This agrees with the work of [32], who revealed that *Escherichia coli* was resistant to amoxicillin, zinnacef, streptomycin, perfloxacin, septrin and ampiclox. The presence of faecal coliforms in the wastewater agrees with the report of [31] that wastewater often carries pathogens from fecal matter and industrial sources. Industrial and healthcare wastewater may contain antibiotics, promoting the growth of antibiotic-resistant bacteria [38]. The findings align with global studies, such as [39], which highlighted the prevalence of resistant bacteria in industrial effluents. Antibiotic-Resistant Bacteria (ARB) present in industrial effluents pose health risks [38]. The presence of antibiotic-resistant bacteria in industrial wastewater is a significant environmental and public health threat. These bacteria can transfer resistance genes to native microbial communities in water bodies used for agricultural or domestic purposes, increasing the risk of untreatable infections in humans and animals.

IV. CONCLUSION

The assessment of the physicochemical, microbial and antibiogram of the wastewater from the food industry sampled revealed that the water is polluted, and the is a pressing need to further treat the wastewater to reduce the high microbial load before discharge into the streams. Six pathogenic microorganisms were isolated, and all had *qnrA* genes, with *Pseudomonas aeruginosa* having the highest MAR. Different strains of *Escherichia coli* were isolated as well, and the isolated pathogens can pose a serious health risk to those who use stream water around the community where the factory is located. The various resistance patterns exhibited by the isolates to antibiotics pose a serious health concern. This study calls for monitoring of wastewater quality and further treatment before

discharge, and the need to improve sanitation around the factory.

DISCLOSURE OF CONFLICT OF INTEREST

No conflict of interest to be disclosed.

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