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Antibiotic Resistant Pattern of Bacteria Isolated from Effluent Discharge Points of Pharmaceutical Industries in Imo State Nigeria

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Abstract

Background

Antibiotic resistance of pharmaceutical effluent refers to the phenomenon where antibiotic resistant bacteria and antibiotic residues are present in the waste water discharged from pharmaceutical manufacturing facilities. The discharge of pharmaceutical effluent into the environment has significant impact on ecological systems and overall health of the environment.

Aim

The aim of this study is to investigate the antibiotic resistant patterns of bacteria isolated from effluent discharge points of pharmaceutical industries in Imo state, Nigeria.

Methods

Effluent discharges were collected from multiple discharge points of pharmaceutical facilities and bacteria isolates underwent antimicrobial susceptibility test against commonly used antibiotics.

Results

This study reveals a significant prevalence of antibiotic resistant bacteria, including multi-resistant strains in the pharmaceutical effluent. The presence of resistance to beta lactams, fluoroquinolones and tetracyclines suggest potential challenge in treating infectious diseases and raises concerns for public health.

Recommendations

These include improving waste water treatment processes, promoting responsible antibiotics use in the pharmaceutical industry, strengthening regulatory oversight, fostering collaboration among stakeholders.

Urgent measures are needed to mitigate the risks associated with antibiotics resistance, safeguard public health and protect the environment in Imo State.

Keywords: Antibiotic Resistance, Pharmaceutical Industries, Effluent Discharges, Imo State

Introduction

Pharmaceutical industries play a crucial role in society by producing essential drugs for human and animal health. However, these industries are also known to release large quantities of effluents containing various chemicals, including antibiotics, into the environment. This discharge of antibiotics contributes to the emergence and spread of antibiotic-resistant bacteria, posing a significant public health concern. The antibiotic resistance of pharmaceutical effluent is a significant concern for public health and environmental sustainability. Pharmaceutical manufacturing facilities produce a large volume of effluent that contains various chemicals, including active pharmaceutical ingredients (APIs) and antibiotic residues. These effluents are often released into water bodies without adequate treatment, leading to the contamination of aquatic environment [1].

The discharge of antibiotic effluents into the environment can contribute to the development and spread of antibiotic-resistant bacteria. The continuous exposure of bacteria to sub-lethal concentrations of antibiotics in effluent creates a selection pressure for the survival of resistant strains. These resistant bacteria can then be released into the environment, potentially reaching natural water bodies, agricultural fields, and even drinking water sources [2]. The World Health Organization (WHO) has identified antibiotic resistance as one of the biggest threat to global health, food security, and development today. It is estimated that at least 700,00 people die each year due to drug-resistant infections, and this number could rise to 10 million by

2050 if no action is taken. Antibiotic resistance also poses a significant economic burden on healthcare system and society as a whole [4].

Effective strategies to address antibiotic resistance including improving antibiotic use, enhancing infection prevention and control measures, promoting research and development of new antibiotics, and reducing environmental contamination. It is essential to raise awareness and take collective action to tackle this global health threat and preserve the effectiveness of antibiotics for future generations [5]

Imo state, located in Nigeria, houses several pharmaceutical industries, making it an important area to investigate the antibiotic-resistant patterns of bacteria in their effluents. While antibiotic resistance is extensively studied, there is a lack of comprehensive research specifically focusing on the effluents of pharmaceutical industries in Imo State. This study aims to bridge this gap by providing specific data on antibiotic-resistant patterns in the effluents, contributing to the existing body of knowledge on antibiotic resistance in different settings.

Aim

The aim of the study is to investigate the antibiotic-resistant patterns of bacteria isolated from effluent discharge points of pharmaceutical industries in Imo State. The study seeks to understand the prevalence and types of antibiotic-resistant bacteria present in the effluent, as well as their resistance patterns against commonly used antibiotics.

Materials and Methods

Sample collection and analysis

Site for sample collection was effluent discharge points from three pharmaceutical industries namely; Reagan Remedies Ltd at New Owerri, Nichben Pharmaceutical Industries Nig. Ltd at Owerri/Onitsha Road, Owerri and A&J pharmaceutical Nig. Ltd at Egbu, Owerri. The samples were collected aseptically using sterile sample containers and transported in ice-packed containers to the microbiology laboratory for analysis.

Media preparation

Media used for the work were nutrient agar, macconkey agar and muller Hinton agar for the isolation of bacteria and anti-sensitivity test respectively, all media used were prepared according to the manufacturer's instructions.

Serial dilution

Serial dilution was adopted by Cappuccino [6]. Ten-fold serial dilution was carried out using sterile distilled water as the diluent. 10 test tubes containing nine milliliter per volume (9.0 ml/v) of sterile distilled water were used for each of the sample. They were labeled and arranged appropriately in a test tube rack. 1ml of the sample was serially diluted in test-tube containing 9ml of distilled water using a sterile syringe, each transfer was followed by a gentle agitation in order to mix the contents uniformly. The procedure was repeated for all the diluents in the same manner. The serial dilution was performed aseptically beside a lit Bunsen burner to prevent contamination.

Identification of the Bacteria Isolates

Identification of pure colonies using morphological characteristics were based on morphological differences, colonies were isolated from their axenic culture. Petri-dishes were divided into quadrants and sub culturing carried out by streaking. Colony morphology observations formed a major identifying criteria for bacteria.

The characteristics observed include (circular, irregular, spreading) elevation (flat, slightly raised or markedly raised), pigmentation (red, white, pink, colorless), size (pinpoint, small, medium, large) and texture.

This test is to identify members of *vibranaceae* and most members of the *enterobacteriaceae* which are also motile. The mobility medium was inoculated using a needle to make 5 stabs of the test organism to the depth of l-2cm of the bottom of the tube. The tube was incubated at 37°c for 24hrs. The line of incubation was examined for cloudiness showing the organisms is motile [7].

Antibiotic culture sensitivity

In third step a 100 ml of muller hinton was prepared under sterile condition and poured into sterile test plates. A sterile cotton swab was dipped into a sample from well-mixed colonies in distilled water and applied onto nutrient agar plate. Sensitivity was checked by using 6 different commercially available anti- microbial discs (wafers) and were placed on the plate by means of multi-disc dispenser and pressed firmly onto agar plate with sterile forceps by using the agar- disc diffusion. The inoculated plates containing the antibiotics were incubated at 37°C for 24 hours after which the diameter of zone of inhibition around each antibiotic dise were then measured to the nearest millimeter (mm). Zones of inhibition of growth were examined around the disc; susceptible: Zone of inhibition of>l 5mm. Resistant: Zone of inhibition of <15mm. The discs were dispensed onto agar surface aseptically by forceps and firm contact was ensured. A distance of 24mm was maintained between the discs. After overnight incubation at 37°C the plates were examined for the zone of inhibition. Zone diameters were measured by calipers and strains were reported sensitive or resistant according to the chart supplied.

Results

Total viable count of isolates from pharmaceutical effluents

Table (no) shows the total viable count of isolates from pharmaceutical effluents. The total heterotrophic count of pharmaceutical effluents ranged from 2.0 x 10^3 to 3.6 x 10^3 while the coliform count ranged from 1.9 x 10^3 to 2.5 x 10^3.

Table 3.1: Total viable count of isolates from pharmaceutical effluents

Sample	THC(CFU/g)	TCC(CFU/g)
A	3.6 xl0^3	2.5 xl0^3
B	3.5 xl0^3	2.4 xl0^3
C	2.0 xl0^3	1.9 xl0^3

Keys

Sample A; Reagan Remedies Ltd
Sample B: Nichben Pharmaceutical Industries
Sample C: A & J Pharmaceutical
THC: Total heterotrophic count
TCC: Total coliform count
CFU/g: Colony forming unit per gram

Biochemical test of the bacterial isolate from pharmaceutical effluents

Table (no) shows the biochemical properties of the isolated bacteria from pharmaceutical effluents and their reactions to the various test the biochemical results shows that the bacteria isolated were *Escherichia coli, Pseudomonas spp. Serratia spp, Staphylococcus spp, Staphylococcus spp* and *Proteus spp*.

Table 3.2: Morphological and Biochemical Identification of Bacteria isolated from UTls of Subjects

S/N	Morphology Suspected	Gram staining	Catalase	Citrate	Indole	Methyl red	Voges Proskaeur	Oxidase	Motility	Organism
1.	Yellow, Glassy, Round, Cocci in cluster									wrinkled translucent colony
2.	Cream, smooth, short rod in single									
t.	Greenish, Opaque, Flat iiinl odourcolony									
4.	Reddish, Opaque, Raised, Glittery Colony									
5.	Irregular									

Staphylococcus spp
Escherichia coll
+ Pseudomonas spp
Serratia spp
+ Proteus spp

Resistance of bacterial isolates against individual antibiotic

Table (no) below shows the resistance of bacterial isolates against individual antibiotic. However, certain degrees of sensitivity were obtained, for instance all *Staphylococcus* strains were sensitive to Gentamicin and Cotrimoxazole, all strains Proteus were sensitive to Gentamicin, Nalidixic acid and Ofloxacin, while all strains of Pseudomonas were sensitive to Gentamicin and Nalidixic acid. All the Sains of *Escherichia* and *Serratia* showed various forms of resistance to all the antibiotics.

Table 3.3: Resistance of bacterial isolates against individual antibiotic Bacteria Isolates (Antibiotics *Escherichia spp staph, Spp Proteus spp Pseudomonas spp Serratia spp*)

Aug	5(100)	3(60)	ND	3(60)	5(100)
Amx	5(100)	5(100)	1(20)	5(100)	4(80)
Oil	4(80)	3(60)	ND	5(100)	1(20)
Tet	2(40)	2(40)	2(40)	2(40)	5(100)
Nal	3(60)	4(80)	ND	4(80)	4(80)
Gen	1(20)	XD	ND	ND	2(40)
Cot	2(40)	ND	5(100)	4(80)	5(100)
Nit	4(80)	5(100)	5(100)	ND	5(100)

Keys: Gen- Gentamicin Tet- **Tetracycline** Ofl- Ofloxacin Nal- Nalidixic acid cot- Cotrimoxazole Aug- Augmentin Nit- Nitrofurantoin, Amo- Amoxycillin

Discussion, Conclusion and Recommendation

Discussion

The effluent in this study contained large numbers of bacteria. The value obtained in this study is similar to those earlier reported for microbial populations in some polluted rivers in Nigeria that are exposed to human, agricultural and industrial wastes [8]. The index of the microbial load (105) is high and it indicates dense population of bacteria in the effluent. It also contained coliform bacilli with high MPN (1800) as set by coliferm index [9]. The isolation of E. coli in all the tubes used for the coliform test is an indication of faecal contamination of the effluent.

Strains of *Staphylococcus spp, Escherichia coli, Proteus spp, Serratia spp* and *Pseudomonas spp* were isolated from the effluent. All these organisms are potential pathogens of man capable of causing a variety of diseases. *Staphylococcus spp* causes infections of the skin, deeper tissues and organs, pneumonia, enteritis and pseudo membraneous enterocolitis, and food poisoning; Proteus may infect urinary tract and wounds; *E. coli* causes diarrhoea, urinary tract and kidney infections, and peritonitis septicaemia; Serratia causes bacteriuria; and Pseudomonas causes infections of wounds, burns, eyes, and ears [10]. The isolation of these pathogens from the effluent is worrisome because the effluents were collected prior to contact with the external environment. In such a case, it is not impossible to assume that these pathogens were introduced into the production process by human healthy carriers through handling. The continuous contamination of the process may be enhanced through the processing equipment [11].

The antibiotic sensitivity test and assay for the production of b-lactamase were conducted on five strains of each bacterium to ascertain the level of resistance. The results of the antibiotic sensitivity test were interpreted and are presented as the resistance of bacterial isolates to the antibiotics, and the antibiotic resistance pattern among the bacterial isolates. High levels of resistance were obtained among the bacterial isolates (20-100%). None of the antibiotics tested had 100% activity against all the bacterial isolates. However, certain degrees of sensitivity were obtained, for instance all *Staphylococcus* strains were sensitive to Gentamicin and Cotrimoxazole, all strains of Proteus were sensitive to Gentamicin, Nalidixic acid and Ofloxacin, while all strains of *Pseudomonas* were sensitive to Gentamicin and Nalidixic acid. All the strains of *E. coli* and *Serratia* showed various forms of resistance to all the antibiotics.

Conclusion

Pharmaceutical waste contains different microorganisms. Furthermore, with this study, it is concluded that several drug-resistant organisms are found in Pharmaceutical waste effluents. Which spreads the MDR bacterial diseases. Hence, Pharmaceutical waste should be treated and purified before sending outside. Every pharmaceutical industry necessary to discharge drug free waste in the final treated effluent. So that microbial strain becomes less resistant against antibiotics.

Recommendations

Pharmaceutical industries should invest in advanced wastewater treatment technologies specifically designed to remove antibiotic residues and resistant bacteria. Employing techniques such as advanced oxidation, membrane filtration, and activated carbon adsorption can effectively reduce the release of resistant bacteria into the environment.

There should be enhanced regulatory oversight and enforcement to ensure compliance with effluent discharge standards and guidelines. Regular monitoring and

inspections should be conducted to assess effluent quality and ensure that pharmaceutical industries are implementing appropriate treatment measures.

There should be enhanced awareness among stakeholders, including pharmaceutical industry personnel, healthcare professionals, and the general public about the implications of antibiotic resistance and the importance of proper effluent management. Education programs can help promote responsible practices and encourage behavior change.

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