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Antibiogram of Bacteria Isolated in the Air of Some Public Toilets in Port Harcourt Metropolis, Rivers State, Nigeria

Williams, Janet Olufunmilayo[1*] and Lumati, Jessica[1]

[1]Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author WJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author LJ managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Antimicrobial resistance is one of the major challenges facing the health sector. This study was aimed at investigating the antimicrobial susceptibility pattern of bacterial isolates from indoor air of public toilets in motor parks with a view of developing an antibiogram. The study sites included the public toilets in Mile 3 Motor Park, Rivers Transport Company (RTC) park and a General Motor Park in Waterlines, Port Harcourt, Nigeria. The indoor air was sampled using the plate exposure and disc diffusion techniques in determining the antimicrobial susceptibility pattern of the bacterial isolates. The bacterial isolates in the genera, *Staphylococcus, Bacillus, Providencia, Pseudomonas, Escherichia, Enterobacter* and *Klebsiella* species were identified. The antibiogram of the bacteria isolated showed that the bacterial isolates exhibited multi-drug resistant species as the isolates were resistant to more than two antibiotics. Ciprofloxacin and Tarivid are the drug of choice and recommended for treatment of infections from these study sites.

**Corresponding author: Email: janet.williams@ust.edu.ng;*

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1. INTRODUCTION

An antibiogram is a table that displays the antibiotic susceptibility pattern of specific microorganisms to different types of antimicrobial drugs [1]. According to the [2], Antimicrobial resistance (AMR) has surfaced as a global problem facing public health and as a result of this, there is prolonged illness and a higher risk of death since diseases arising from these resistant microorganisms do not respond to the antimicrobial drugs. Furthermore, the diseases caused by multidrug-resistant microorganisms which are virtually non-treatable are the most alarming. The development of antibiotic resistance in many bacteria constitute serious problems in the control of infectious diseases [2]. The indiscriminate use of antibiotics has been found to be the most crucial factor in the resistance of bacteria to antibiotics [3]. Resistance to antibiotics takes place when an antimicrobial drug is unable to completely inhibit the growth of bacteria and the resistant bacteria continue to multiply in the presence of therapeutic levels of the antibiotics [4].

A building containing one or more toilets that is easily accessible for use (for urination, defecation or both) to everyone or the public is referred to as public and they are mainly found in different establishments, schools, airports, cinemas, motor parks, etc. The toilet is a room or small building housing one or more toilets and is made available for public usage [5]. The public toilets (away from home toilet) comprise of traditional 'street toilets' and toilets that are no longer used which is accessed by the public. In some public toilets, people only access them after paying while some are accessed without payment of an amount of money. A toilet assessed by different persons could be a reservoir for infections especially if the toilets are not properly managed. In a study of public toilets in Lagos by [6], the public toilet was referred to as "paying to get infections". Infection via the use of public toilets is increasing and there is a need to identify the microorganisms that may be responsible for these infections so as to speed up treatment. The rate of drug resistance is also on the increase and it would be of immense help if bacterial isolates in public toilets are screened for their susceptibility levels to antimicrobial drugs. Thus, this study was aimed at providing an antibiogram of bacterial isolates in the indoor air of public toilets.

2. MATERIALS AND METHODS

2.1 Study Area/ Duration of study

The public toilets in the Mile 3 Motor Park, Rivers Transport Company (RTC) Motor Park and a general Motor Park in Waterlines were the areas of study. The GPS coordinates for the study area are 4.8031^{o}N and 6.9909^{o}E, 4.8167^{o}N and 7.0068^{o}E, 4.8156^{o}N and 7.0078^{o}E respectively. The study was conducted between March and May 2018.

2.2 Sampling of Indoor Air

The indoor air was sampled by exposing plates containing growth media in the different toilets for fifteen (15) minutes as described by previous studies [7].

2.3 Isolation, Purification and Identification of Bacterial Isolates

The indoor air bacterial isolates were sampled by exposing prepared sterile plates of nutrient and Mac Conkey agar in duplicates in the respective public toilet rooms. Plates were further taken to the Microbiology laboratory of the Rivers State University and incubated at 37 ^{O}C for 24 hours. After incubation, discrete colonies on plates were picked and streaked on freshly prepared sterile nutrient agar plates. This was done until pure bacterial isolates were obtained. Pure bacterial isolates were stored frozen in bijou bottles containing 10% v/v glycerol suspension [8 which was used for further identification. Bacterial isolates were identified using cultural methods as described by [9].

2.4 Collection of Antibiotics Discs

Commercially prepared antibiotics discs (Maxi discs) of Gram-positive and Gram-negative discs were bought from the medical store in Hospital road, borikiri, Port Harcourt, Rivers State. Antibiotics discs were stored in the refrigerator. The Maxi disc concentrations were as follows: Pefloxacin (PEF)10 µg, Gentamycin (CN)10 µg, Ampiclox (APX)30 µg, Zinnacef (Z) 20 µg, Amoxicillin (AM)30µg, Rocephin (R) 25 µg, Ciprofloxacin 10 µg, Streptomycin (S)30 µg, Septrin (SXT)30 µg, Erythromycin(E)10 µg, Augmentin (30 µg), Tarivid (10 µg), Chloramphenicol (30 µg), Sparfloxacin (10µg).

2.5 Antimicrobial Susceptibility of Bacterial Isolates

The disc diffusion method as described by previous studies [10] was used. Pure colonies of bacterial isolates were picked and transferred into test tubes containing 4ml sterile distilled water. The turbidity of bacterial isolates was standardized using the already prepared McFarland standard. Standardized bacterial isolates were swabbed horizontally and vertically on the surface of prepared Muller-Hinton agar plates in duplicates. Plates were allowed to dry for five minutes. after which discs containing the antibiotics were spread on the surface of the Muller-Hinton agar plates using sterile forceps. Plates were later incubated at 37 ºC for 18-24 hours. Observable zones of inhibition were measured using a rule graduated in mm and were interpreted as resistance, intermediates and susceptible using the guidelines outlined by the [10]. These were then used to prepare the antibiogram chart.

3. RESULTS AND DISCUSSION

In this study, bacterial isolates of the following genera, *Staphylococcus, Bacillus, Providencia, Pseudomonas, Escherichia, Enterobacter and Klebsiella* species were identified from the indoor air of the public toilets. The bacteria in this study could be pathogenic and responsible for a number of infections such as urinary tract infections, foodborne infections, and other related diseases. This agreed with [11] who reported some possible diseases caused by similar bacteria. For instance, foodborne diseases are caused by *Staphylococcus* and *Escherichia species*, Urinary tract infections are associated with *E. coli* and *P. vermicola*), Pneumonia is caused by *K. pneumoniae*, and Diarrhoea is caused by *E. coli*. *Escherichia coli* has been reported to be the most common cause of UTIs with some clones that may also be associated with gastrointestinal infections [12]. Furthermore, *Bacillus* species are known to withstand high temperatures and are confined to adapt in an unfavourable condition with the help of endospores. Thus, they could withstand heat and cause foodborne illness .

The antibiogram table presented in Table 1 showed that the bacterial isolates were resistant to more than one antibiotic and thereby exhibited multidrug resistance. The result showed that *Staphylococcus* species were completely (100%) resistant to amoxicillin while the level of resistance to pefloxacin, gentamycin, ampiclox, Rocephin and streptomycin was recorded in the following order; 30, 60, 50, 20 and 70%, respectively. Thus, the staphylococcal isolates were highly resistant to streptomycin and gentamycin. The resistance of *Bacillus* species to the antibiotics showed that they were highly resistant to ampiclox, streptomycin, Rocephin and septrin, and percentage resistance was recorded as 80, 70, 60 and 60%, respectively. *Providencia* species were 100% resistant to pefloxacin and Augmentin and were also very resistant to ampiclox (70%) and gentamycin (62.5%). About 37.5% resistance to ciprofloxacin was also recorded. Some levels of resistance by *Pseudomonas* species to gentamycin, ciprofloxacin, septrin, augmentin and sparfloxacin in the order of 47.1, 17.6, 29.4, 47.1 and 47.1 %, respectively were also observed. *Enterobacter* species were 100% resistant to gentamycin and had 18.2% resistance to augmentin and tarivid, respectively. The antibiogram table also showed that *Klebsiella* also had some level of antimicrobial resistance to the antibiotics. The resistance of bacteria to some of the antibiotics commonly in use is an increasing problem in the developing world [13]. The resistance of bacteria to Ampicillin, Amoxycillin, Chloramphenicol, Streptomycin, Cotrimoxazole, Tetracycline and Gentamycin have been reported in different parts of Africa where *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Proteus mirabilis* have been reported to be multidrug-resistant [14]. In a study by [1] also, the resistance of *Staphylococcus* species to amoxicillin and streptomycin were reported. The improper use of antibiotics, the erroneous prescription by unqualified medical agents with poor diagnoses have been reported to be among the main factors leading to antibiotics resistance in African countries [13]. Another factor which could also lead to resistance to antibiotics by microorganisms is the alteration of drug target sites or mutation of nucleic acid sequence. Resistance to antimicrobial agents could be acquired via the production of β-lactamases (enzymes) that hydrolyze the β-lactam ring of the penicillin and cephalosporin antibiotics thereby making them inactive against the bacterium. Also, the incorporation of phosphate, acetyl or adenylyl groups inactivates the aminoglycoside antibiotics [14].

Table 1. Antibiogram of Bacteria Isolated from the Study Sites (% Resistance)

Bacterial Isolates	PEF	CN	APX	Z	AM	R	CPX	ST	SXT	E	AU	OFX	CH	SP
	R (%)	R (%)	R (%)	R (%)	R (%)	R(%)	R(%)	R(%)	R(%)	R(%)	R (%)	R (%)	R (%)	R (%)
Staphylococcus sp(10)	30	60	50	0	100	20	0	70	NA	0	NA	NA	NA	NA
Bacillus sp(10)	0	0	0	30	80	60	0	70	60	0	NA	NA	NA	NA
Providencia sp (8)	100	62.5	NA	NA	75	NA	37.5	0	20	NA	100	12.5	40	37.5
Pseudomonas sp(17)	0	47.1	NA	NA	0	NA	17.6	0	29.4	NA	47.1	0	0	47.1
Escherichia sp(20)	0	0	NA	NA	0	NA	0	0	0	NA	0	0	0	0
Enterobacter sp (11)	0	100	NA	NA	0	NA	0	0	0	NA	18.2	18.2	0	0
Klebsiella sp(20)	15	15	NA	NA	0	NA	20	20	20	NA	15	0	20	20

KEYS: Pef: Pefloxacin, CN: Gentamycin, APX: Ampiclox, Z: Zinnacef, AM: Amoxicillin, R: Rocephin, CPX: Ciprofloxacin, ST: Streptomycin, SXT: Septrin, E: Erythromycin, OX: Oxacillin, AU: augmentin, OFX:Tarivid, CH: Chloramphenicol, SP:sparfloxacin, NA: not applicable

Plate1. Sample plates of a bacterial isolate showing zones of inhibition

4. CONCLUSION

This study has shown the presence of bacterial isolates which could be pathogenic. The antibiogram showed that there are resistant and multidrug-resistant bacterial isolates in public toilets and this is a serious public health challenge. Ciprofloxacin and Tarivid are the most effective drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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