Detection of Tetracycline Resistance Genes and Susceptibility Profile of Bacteria Isolated from Clinical Samples

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ABSTRACT

The use of Antibiotics by humans for an extensive period of time has an impact not just on the disease being treated, but also on the microbial population in that environment. Antibiotic abuse in many developing nations have aided the emergence and widespread transmission of antibiotic resistant resistance genes in the environment. Tetracycline is a broad-spectrum antibiotic used to treat diseases in both humans and animals. Tetracycline is regulated by *tet* genes, which are engaged in active drug efflux, ribosome protection, or enzymatic drug modification. The use of Tetracycline's as a treatment option for treating oral and systemic disorders haves been limited due to the existence of these genes. Hence, this study aimed to detect tetracycline *tet* (A) and *tet* (B) resistance genes in bacterial isolated from clinical samples and to determine their susceptibility profile. An experimental design was used for this study. A total of forty human clinical isolates from blood, urine, throat, wound, and sputum were randomly selected for this study. The isolates were collected and sub-cultured in nutrient broth for analysis and PCR was used for the detection of genes. The result showed that antibiotics resistant genes were present in the all of the isolates. However, *tet*B had the highest occurrence (91.6%) of than *tet*A (8.4%). All the isolates showed high resistant to the various antibiotics including ceftazidime, cefuroxime, gentamicin, ofloxacin, amoxicillin, nitrofurantoin, cefixime, and ciprofloxacin. This could be due to the frequent use of broad-spectrum antibiotics in clinical settings which have led to the development of antibiotic resistant strains among bacteria. Antibiotics resistant genes (*tet*A & *tet*B) are present in bacteria isolated from clinical setting and may become a serious public health concern when these genes are transferred among organisms that affect both humans and animals. Hence, preventative measures should be employed to reduce the diffusion of these genes in the clinical setting since they present a serious public health concern when they are transferred among organisms that affect both humans and animals.

Key words: Antibiotics, Resistant genes, Susceptibility Profile, Tetracycline, Antibiotics, Resistant genes, Susceptibility Profile

INTRODUCTION

The breakthrough discovery of streptomycin, penicillin and cephalosporins in 1940s was quickly followed by the advent of antibiotic-resistant microorganisms. The fast growth in the number of antibiotic-resistant species, particularly strains carrying multi-drug resistance genes (Fluit *et al*. 2000), as well as the absence of tools to detect the presence of these resistance genes in pathogenic bacteria, have become major public health concerns across the world. Antibiotic-resistant microorganisms have grown at an alarming rate in the past few years. In 2019, the WHO estimated that 1.26 million deaths globally were directly cause by

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antibiotic resistant and 4.95 million deaths were attributed to drug-resistant infections (WHO, 2019). Antibiotic-resistant bacteria frequently spread in the environment as a result of the existence and transmission of resistance genes among microorganisms, as well as selection pressure to maintain these genes circulating in a population.

Tetracycline is a broad-spectrum antibiotic that suppresses protein synthesis by attaching to bacterial ribosomes. With rising resistance to tetracycline, its usage in clinical treatment in most affluent nations has increasingly decreased. However, it is still widely utilized in agricultural techniques (Zalewska et al., 2021). Ogwuche et al. (2021) revealed that Tetracycline is still used in human medicine in Nigeria, both clinically and as a "over-the-counter" (OTC) antibiotic. Over-the-counter use appears to be more prevalent than use in clinical practice. In a recent investigation of prescription patterns in a tertiary care hospital in Nigeria, tetracycline was found to be utilized as combination treatment in 11.7% of instances rather than on its own (Perewari et al., 2022). When the use of tetracycline in humans is combined with its use in animal husbandry, an explosion in tetracycline resistance could be expected. Tetracycline-resistant determinants have been widely reported from both clinical and nonclinical isolates of *E. coli* in various parts of Nigeria (Ogwuche *et al*., 2021).

Tetracycline resistance is mediated by about forty acquired tetracycline-resistant genes that encode for either e¥ux pumps, enzymatic inactivation, or ribosomal protection genes. The *tet*A gene encodes the *tet*A e¥ux pump, a well-known modulator of tetracycline resistance in Enterobacteriaceae family, including as *E. coli*. The *tet*B-mediated resistance can also encode for an e¥ux pump. (Grossman, 2016; Haeili *et al*., 2022). It is well-known as the determinant with the broadest host range of tetracycline-resistant genes, owing to its interaction with a conjugative transposon. The implications of these resistance mechanisms include that antibiotic stocks are ineffectual in illness treatment and management, resulting in longer hospital stays and a higher budgetary burden. Given the widespread distribution of tetracycline-resistant genes (tet) in humans, the environment, and animals, the usefulness of this medicine in clinical practice is uncertain (Perewari *et al* ., 2022). However, the continuous use of tetracycline in therapeutic settings may constitute a significant hazard to public health. This study was conducted to identify tetracycline-resistant genes in bacteria isolated from clinical samples and to characterize their susceptibility profile.

MATERIALS AND METHODS

Collection of Clinical Samples

A total of forty (40) bacterial isolates previously isolated and stored at the Babcock University Microbiology Laboratory were randomly selected comprising of clinical isolates (from blood, urine, throat, wound and sputum isolated). These isolates were collected and sub-cultured in nutrient broth for analysis.

DNA Extraction

Bacterial DNA was extracted using Quick-DNA^{TM} miniprep plus kit (Zymo research, Biolab, USA) according to manufacturer protocol. 1% Agarose electrophoresis was used to check the quality of the DNA before polymerase chain reaction (PCR).

Detection of Tetracycline-resistant genes

The presence of Tetracycline-resistant genes (*tet* A & *tet* B) were detected in the isolates using PCR. Briefly PCR mixture (25 µl) contained 12.5 µl solution of OneTaq Quick-Load 2X Master Mix with Standard Buffer (New Eng-land BioLabs), 0.8 µl nuclease free H2O, 2.0 µl of DNA template (dNTPs) and 0.5 of 10 µl of each specific forward and reverse primers (Tet A & B) synthesized by Inqaba Biotech, South Africa targeting each gene. Simple and multiplex PCR were done using the method described by Ciesielczuk *et al.*

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(2013). PCR was carried out with a negative control containing all the reagents without template DNA. Amplification reaction was achieved using G-STORG PCR system (Gene Technology LTD). The cycling conditions were: initial denaturation at 94^{o}C for 3 minutes, denaturation 94^{o}C for 30 sec, annealing temperature (as listed in table 3) for 30 sec, extension 68^{o}C for 30 sec and final extension 68^{o}C for 5 min. The progamme was set for 30 cycles.

Agarose gel Electrophoresis

Agarose gel powder 0.3 grams was weighed and dissolved in 25ml of 1X TBE_buffer (1X: 89 mM Tri base, 19mM boric acid, 2 mM EDTA) solution then mixed and melted in a microwave at boiling temperature for 3 minutes. The solution was allowed to cool at 50-60^{o}C than 4µl of a 10 µl mg/ml solution of ethidium bromide (EtBr) was added and swirled then poured into a gel casting tray containing fixed comb. The gel was allowed to stay for 20 minutes to get solidify, after it was transfer to the gel tank filled and TBE buffer. The PCR samples (150µl) were then loaded using a micro pipette into the wells which run from negative to positive (Ezeamagu *et al*., 2018). The gel was run at 100V for 15 minutes and was viewed using the UV-Irradiation (TMW -20 Trans Illuminator, Alpha Innotech cooperation, USA) Malek *et al*. (2015).

Data Analysis

The data from the PCR were computed in PAleontological Statistics (PAST), Ryan et al. (1995), and the results are presented in tables and figures and analyzed using a descriptive method.

Antibiotic Sensitivity Testing (ABST)

Agar diffusion method was used to determine the antibiogram and was interpreted following the Clinical Laboratory Standard Institute (CLSI, 2017). The antibiotics disc (Abtek Biologicals Limited Gram-negative discs); used in the study include: ceftazidime (30 µg); cefuroxime (30 µg); gentamicin (10 µg); ofloxacin (5µg); amoxicillin/clavulanate (30µg); nitrofurantoin (30µg); cefixime (5µg); and ciprofloxacin (5 µg); Curtly, a single colony of the pure isolate was inoculated into a test tube containing 1 mL of nutrient broth and was incubated overnight at 37°C. The incubated broth was then standardized to match 0.5 McFarland standards. A sterile swab stick was dipped into the standardized suspension and streaked over the surface of a prepared Mueller Hilton Agar plates. The antibiotic disc was placed on the agar surface observing a distance of 30 mm edge to edge. The plates were incubated at 37°C for 24 h. The apparent zone of inhibition was measured with a meter rule to the adjacent diameter in millmeter.

RESULTS

In this study, a total of 40 bacteria were obtained of which 9, 24, 2, 2, 3 and 40 isolates were recovered from blood, urine, throat, sputum, wound. The percentage occurrences of bacteria were 40%, 25%, 22.5% and 12.5% for *Escherichia coli, Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, respectively.

Detection of Tetracycline-resistant genes

In this study Tetracycline resistant gene (*tet*A& *tet*B), were investigated in clinical isolates. The result showed that all the genes were present in the isolates from different clinical sources (Figure 1). However, *tet* B had the highest occurrence (91.6%) of than *tet*A (8.4%) among the isolates.

Antibiotics Sensitivity

A total of eight antibiotics were used in this study which include ceftazidime (CAZ), cefuroxime (CRX),

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gentamicin (GEN), cefixime (CXM), ofloxacin (OFL), amoxicillin/clavulanate (AUG), nitrofurantoin (NIT), ciprofloxacin (CPR) . The result showed that most of the isolates were highly resistant to CAZ, CRX, AUG, CPR, CXM, and GEN (Figure 2). However per sources the isolates from blood, urine, wound and throat showed high resistance to the following antibiotics CAZ, CRX, AUG, CPR, AUG, and OFL (Figure 3).

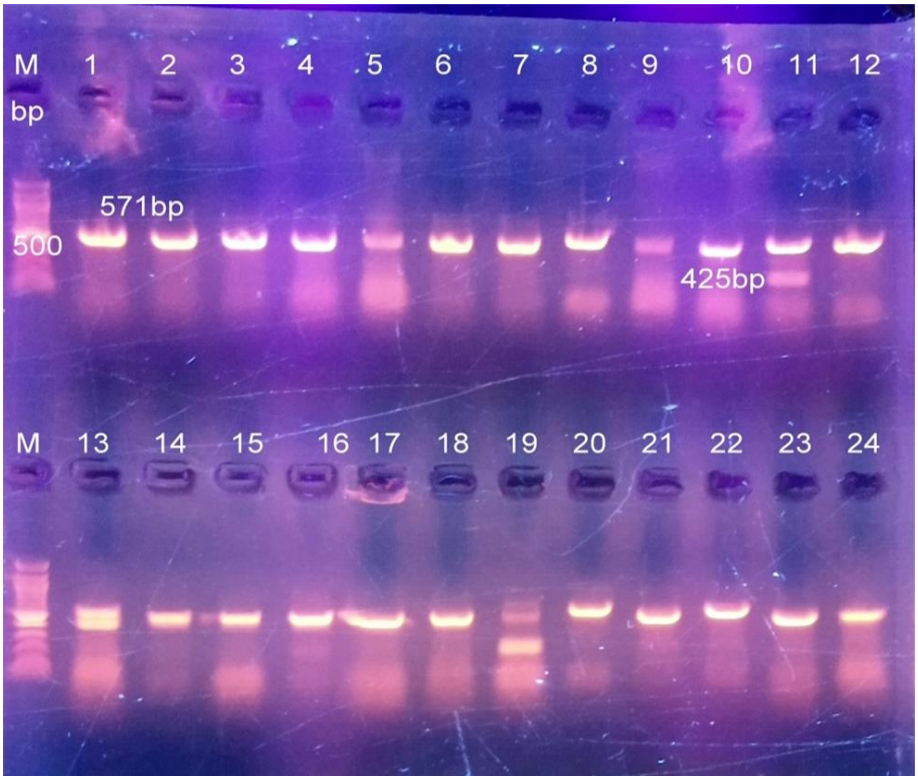

Figure 1: Electropherogram for Detection of *tet*A & B genes in clinical isolates M-Molecular ladder (100bp), *tet*A positive isolates: 11, and 19, Negative controls: *tet*B positive isolates: 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23 and 24

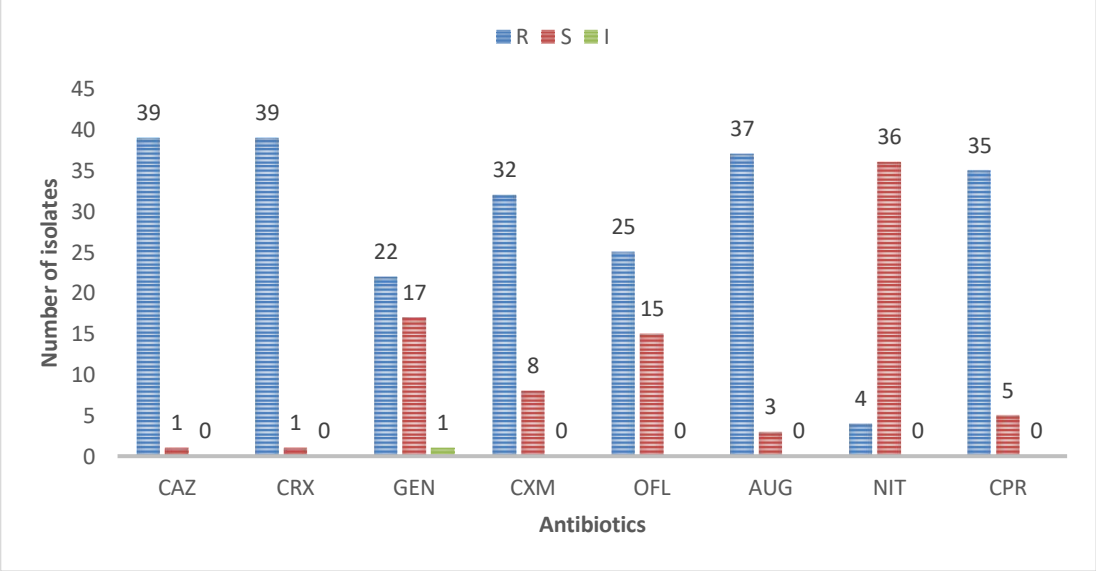

Figure 2: Clinical isolates sensitivity profile

Key: Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ciprofloxacin (CPR) Resistant(R), Susceptible (S), Intermediate (I)

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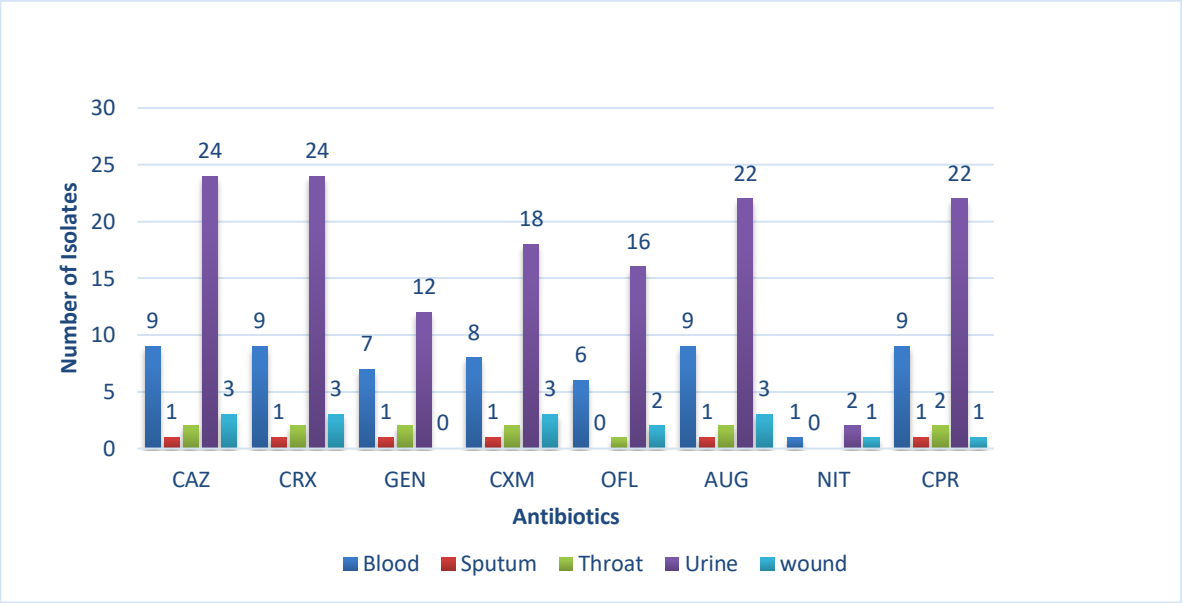

Figure 3: Antibiotics Resistance profile of clinical isolates per source

Key: Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ciprofloxacin (CPR)

DISCUSSION

The presence of pathogenic bacteria such as *E. coli*, *S. enterica* and *K. pneumoniae* indicates that they are widely disseminated in the clinical setting. The occurrence of Enterobacteriaceae in this study was similar to a study conducted by Malek *et al*. (2015) who also detected a high prevalence of *E. coli* and *K. pneumoniae* from clinical samples including blood, urine and wound swap.

In this study all the isolates showed highly resistant to CAZ, CRX, AUG, CPR, CXM, and GEN (Figure 2). However, per sources the isolates from blood, urine, wound and throat showed high resistance to the following antibiotics CAZ, CRX, AUG, CPR, AUG, and OFL (Figure 3). However, the extent of antibiotics resistance differed depending on the sample type. With higher levels noted from clinical samples, this could be due to the frequent use of broad spectrum antibiotics in clinical sittings which have led to the development of antibiotic resistant strains among bacteria. In addition to that, the use of medication without a proper prescription is an important issue in antibiotics abuse. Ayukekbong *et al.* (2017) mentioned several factors that accounts for antibiotic abuse in Sub-Saharan Africa including inappropriate prescription practices, inadequate patient education, limited diagnostic facilities and unauthorized sale of antimicrobials. It has been reported that bacteria acquire resistance by horizontal gene transfer of mobile genetic elements and the frequent usage of the antibiotics promotes the selection of existing resistance mechanisms (Stokes and Gillings, 2011). Antibiotics abuse related to self-medication can habitually result in insufficient quantity of the antibiotics and can contribute extensively to bacteria resistant profile (Ezeamagu *et al*., 2018).

Antibiotic resistant genes (ARGs) have been reported by the World Health Organization (WHO) as a problem because of their increasing prevalence and extensive distribution (WHO, (2014). This is a risk to public health (Rodriguez-Mozaz et al., 2015). The alarming rate of ARGs has been acknowledged as a result of the massive use of antibiotics in clinical and agriculture settings (Chang et al., 2015). In this study two (2) antibiotics resistance genes Tetracyclines (*tet*A and *tet*B) were investigated in clinical isolates. The result showed that both of the genes were present in the isolates. The occurrence and dissemination of tetracycline-resistant genes could be due to the misuse of tetracycline in clinical settings in the more developed

countries, recent years have seen a reduction of information on tetracycline from this setting with the focus now on tetracycline in animals. However, *tet*B had the highest occurrence (91.6%) of than *tet*A (8.4%) (Figure 1). The findings of this study correspond with a study conducted by Cicek et al. (2022). The result of their study showed that *tet*B gene (found in 30 of 46 isolates) is most commonly found in *E.coli* isolates grown in urine samples. This is in contrast with previous reports noting *tet*A as the most predominant tetracycline-resistant gene among organisms (Montaz *et al*., 2012; Jahantigh *et al*., 2020). The *tet*A in particular is widely found in most *E. coli* strains isolated from urine, stool, poultry, and soil (Al-bahry *et al*., 2016; Olowe *et al*., 2013). It has been postulated that *tet*A genes occurred more easily in the environment as compared to other tet racycline determinants (Skockova et al., 2021).

CONCLUSION

Antibiotics resistance genes (*tet*A & *tet*B) are present in bacteria isolated from clinical setting and may become a serious public health concern when these genes are transferred among organisms that affect both humans and animals. The use of Tetracycline's as a treatment option for treating oral and systemic disorders has been limited due to the existence of these genes which may greatly influence patient treatment outcomes during infection. Hence, preventative measures should be employed to reduce the diffusion of these genes in the clinical setting. Based on the findings of this study, it is recommended that the Ministry of Health (MOH) increases its effort in combating unauthorized sale of antibiotics and design antibiotic surveillance programs.

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