https://dx.doi.org/10.4314/dujopas.v11i1b.23
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 11 No. 1b March 2025*

Molecular Detection of Tet A and Tet B Genes in *Escherichia coli* Isolated from Selected Abattoirs in Northwestern Nigeria

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Abstract

Tetracycline is reported to be widely used as a therapeutic agent and as well as growth promoter during livestock production. Bacterial antibiotic resistance to tetracycline is known to be associated with certain resistance genes including the tet genes, either by protection of the enzymatic or ribosome modifications of the drug which could be transferred among bacteria via plasmid mediation. Tetracycline resistant E. coli isolates (n=35) from selected abattoirs in Northwestern Nigeria were analyzed using polymerase chain reaction (PCR) for the detection of some of the tetracycline resistant genes such as tet*(A) and* tet*(B). Results obtained from agarose gel PCR showed that resisitant gene tetA was detected in 11 (31.4%) of the bacteria whereas those of tetB resistance gene was found in 26 (74.3%) of the E. coli isolates indicating that it was predominant. Seven (7) of the isolates had both* tet*(A) and* tet*(B) genes (20.0%) whereas, none of tetA and tetB genes was found in 5 (14.3%) of the E. coli. The presence of these tet genes in the both bacteria suggested that they could be responsible and be the major determinant of resistance to the emergence of bacterial resistance to tetracycline in the environment such as abattoirs. These tet genes could be possibly transferred to other organisms, humans and abattoir environments, resulting in infections and as well pose public health challenges and in*

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control of infectious diseases which might deter progress in health outcomes and increase cost of treatment on the societies. Good hygienic and manufacturing practices in the slaughterhouses, and indiscriminate use of antibiotics during livestock production especially the commonly used tetracycline is indispensable in order to minimize risks that could be associated with antimicrobial resistant bacteria.

Keywords: Abattoir, Bacteria, Livestock, Resistance, Tetracycline gene.

INTRODUCTION

Abattoir is referred to as a special facility structured and licensed for inspecting and slaughtering animals before they are released for public consumption (Alonge, 2005; Brantz, 2008). Tetracycline is known to be a broad-spectrum antibiotic which inhibits the bacterial growth by interfering with synthesis of protein. It is useful as therapeutic agent in human, veterinary medicine and aquaculture industry and also used as growth promoter in animal husbandry (Gaskins *et al*., 2002; Olowe *et al*., 2013; Avijit *et al*., 2016). The indiscriminate use of antibiotics in many countries potentially promotes the emergence and transmission of antibiotic resistant bacteria and resistant genes in various environment (Davies and Davies, 2010; Amangelsin *et al*., 2023; Mohammed *et al*., 2024). Antibiotic resistance has been reported to be associated with global public health threat in both medical sciences and public health practice, posing challenges to the control of infectious diseases. This has deterred the progress on health outcomes, increasing cost of infectious disease treatment on societies (Leung *et al*., 2011; Capita and Alonso-Calleja, 2013).

Bacterial resistance to tetracycline is known to be governed by *tet* genes which are usually involved in either active efflux, enzymatic modification, or ribosomal protection of the drug (Paulsen *et al*., 1996; Robert, 2012). According to Roberts (2005), not less than 40 different tetracycline resistant genes have been characterized. Several earlier reports on tetracycline resistant genes in both humans and animals have been reported (Koo and Woo 2011 and Skocková *et al*., 2012), even from farm products, especially cow milk, chicken and aquaculture (Jones *et al*., 2006; Skockova *et al*., 2015., Wang *et al*., 2016; Kallau *et al*., 2018 and Mahmound *et al*., 2020; Ahamed *et al*., 2023). It has been established that *tet*(A) and *tet*(B) genes are mostly present in gram-negative bacteria and are reported to cause resistance to tetracycline antibiotics and their diversity of distribution is dependent on some environmental conditions including waste, underground water, and soil (Olowe *et al*., 2013; Wang *et al*., 2016; Birkegard *et al*., 2017; Mohammed, *et al*., 2024) and horizontal gene transmission mechanism is reported to be employed among bacteria in the environment (Urumova, 2016). These genes are known to produce new proteins associated with mobilizable plasmids and transposons (Roberts, 2005; Roberts, 2012). This study is focused on detecting *tet*(A) and *tet*(B) genes in selected *E. coli* isolated from some abattoirs in Northwestern Nigeria.

MATERIALS AND METHODS

Area of study/Sample collection

The *E. coli* tested in this study were obtained from selected abattoirs: Zango Shanu located in Zaria, Kaduna State; Maiyanka in Kano, Kano State; Ultra-modern in Gusau, Zamfara State and Ultra-modern in Dutse, Jigawa State**.** Samples collected include effluent, water for washing carcasses, surface swabs including hides, raw meat, floors, walls, butchers' palms and knives.

Laboratory analyses

Each of the isolated *E. coli* was screened in Postgraduate Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria using Microbiological and biochemical standard methods (Tiwari *et al*., 2009; Cheesebrough, 2010).

The confirmed bacteria were tested against the selected antibiotics following standard disk diffusion method using commercial antimicrobial disks (CLSI, 2013).The antibiotics used include: erythromycin (15 µg), amoxicillin-clavulanic acid (30 µg), cefoxitin (30 µg), tetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), sulfonamides and trimethroprim (25 µg), chloramphenicol (30 µg), and vancomycin (30 µg). Bacterial suspension (inoculum) from overnight culture was made and standardized using McFarland turbidity of 0.5. A sterile wire loop was used to collect the inoculum and streaked uniformly all over the surface of prepared Mueller Hinton Agar plate after which each antibiotic disc was placed onto the inoculated plate using a sterile forceps. The plates were incubated at 37^0C for 18 hours (except for vancomycin) after which the diameter of each zone of inhibition was measured in millimeters. The cleared zone diameter of each disc was interpreted as susceptible, intermediate, or resistant using the recommended zone diameter and interpretative standards for enterobacteriaceae (CLSI, 2013).

Molecular detection of tetracycline-resistance genes

Tetracycline resistant genes *tet*(A) and *tet*(B) were investigated using PCR assay in Bioscience Laboratory of International Institute for Tropical Agriculture (IITA), Ibadan. Thirty-five (35) deoxyribonucleic acid (DNA) extracted from tetracycline resistant *E. coli* isolated from the selected abattoirs using Genomic DNA purification kit (GeneJET), following the manufacturer's protocols were analyzed for both resistant genes by transferring 3µl purified genomic DNA extract directly to a 7µl PCR mixture containing 1µl oligonucleotide primers (forward and reverse), 2.5mM deoxyribose nucleoside triphosphate (dNTP), 10×Tris-HCl buffer, 2.5mM $MgCl_2$, 0.1µl Taq DNA polymerase (Roche, Onatro, Canada), 0.8µl Tween 20 and 1.84µl ultra-pure water. A tube containing all the PCR mixture except DNA was used as control. Each tube was spin for about 5 seconds after which they were inserted in a PCR machine {Gene Amp PCR system 9700 (Applied Biosystems)}. The thermal cycling protocol consisted of denaturation at 94^0C for 5min, followed by 36 multiplication of DNA strands. The cycles of 3 steps which consisted of 90sec at 53^0C for annealing process and 45min at 72^0C for extension, and then 5min at 72^0C for the final extension. The PCR reaction products (5ul aliquot) were then separated using electrophoresis in 1.5% agarose gel in TAE (20mM acetic acid, 40Mm Tris-HCl, and 1mM EDTA) containing 0.5 µg/ml ethidium bromide. However, a molecular weight marker (Invitrogen) with 1kbp DNA ladder was used as a size standard. The products were then visualized using ultra-violet transilluminator. The primers employed in the process are as indicated in Table 1 below:

Table 1: Oligonucleotide Primers used for Polymerase Chain Reaction (PCR)

Primer	Sequence (5'-3')	Gene	AS (bp)	Reference
Tet A	GGT TCA CTC GAA CGA CGT CA CTG TCC GAC AAG TTG CAT GA	*tet A*	577	Randall *et al*., 2004
Tet B	CCT CAG CTT CTC AAC GCG TG GCA CCT TGC TGA TGA CTC TT	*tet B*	634	Randall *et al*., 2004

AS: Amplicon size; bp: Base pair

RESULTS

The presence of *tet*A resistant gene 11 (31.4%) is shown on Plate I and *tet*B resistance gene 26 (74.3%) in *E. coli* is indicated in Plate II. Both genes were present in 7 (20%) of the total isolates while in only 5 (14.3%), neither *tet* A nor *tet* B genes was present.

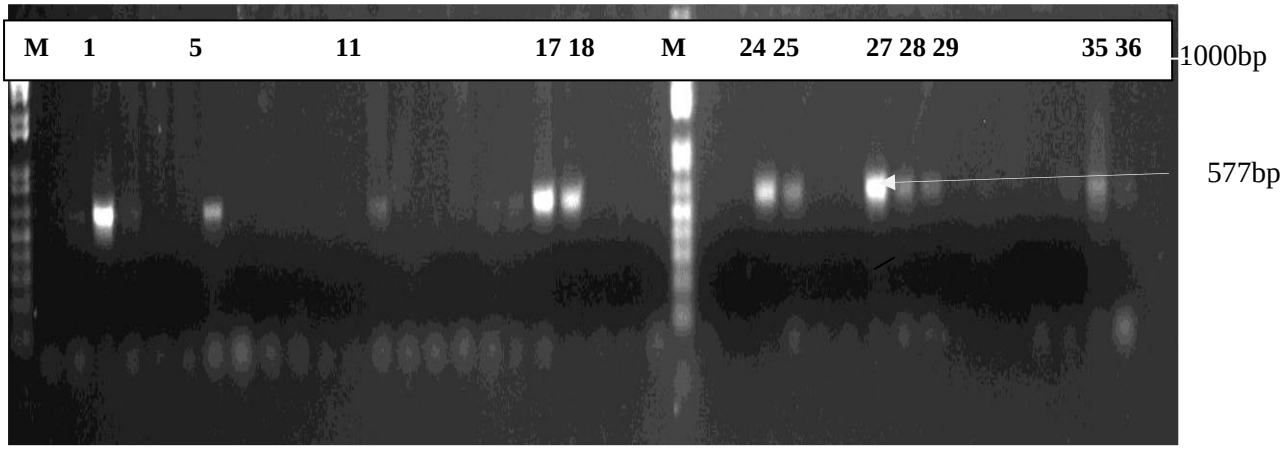

Plate I: Agarose gel Showing PCR Product of tet A Resistance Gene from the isolated *E. coli*.
Lanes 1, 5, 11, 17-18, 24-25, 27-29, and 35; *Lane* 36 = Negative control; Lanes M = DNA 1000bp Marker

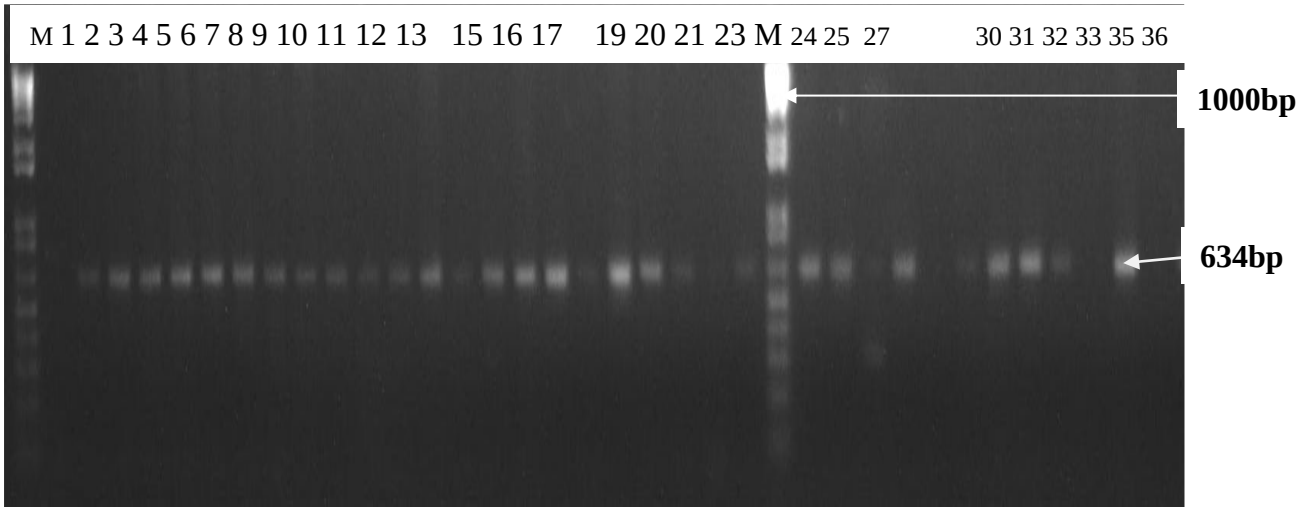

Plate II: Agarose gel Showing PCR Product of tetB Resistance Gene from the Isolated Bacteria
Lane 1= 100bp DNA Marker; Lanes 2-13, 15-17, 19-21, 23-25, 27, 30-32, and 35= *E. coli*; Lane M=DNA Marker; 36: negative control

DISCUSSION

High rate of tetracycline resistance by some of the isolated *E. coli* obtained in this research could be associated with indiscriminate use of the drug in therapy and as well in promotion of food efficiency during animal production (Walsh *et al*., 2003). Inappropriate prescribing and dispensing of antimicrobial agents, lack or inadequate regulation of the quality and use of antimicrobials and also little or no enforcement have facilitated unauthorized dispensing by persons who are not or poorly trained, contributing to the indiscriminate use of these drugs (Togoobaatar *et al*., 2010).

Tet genes are known to cause emergence of bacterial resistance to tetracycline antibiotics (Skockova *et al*., 2015; Wang *et al*, 2016; Birkegard *et al*., 2017; *et al*. Ralph, 2022; Mohammed *et al*., 2024) however, the presence of both tet A and tet B genes which was the focus of this study suggested that they are the major determinant of resistance to tetracycline in the selected

abattoir environments (Urumova, 2016). *Tet*(A) genes are known to associate with plasmids, easily transferred between gram negative bacteria (Roberts, 2012; Anderson *et al*., 2014; Amangelsin *et al*., 2023). More so, they are significantly higher in pathogenic than non-pathogenic bacteria (Smith *et al*., 2007; Zibendeh *et al*., 2016; Ahamed *et al*., 2023) and are mostly prevalent (Avijit *et al*., 2016).

Tet A genes are known to be common in the family enterobactriaceae and freely spread in the environment compared to tetB genes (Tao *et al*., 2010; Koo and Woo, 2011; Mahmound *et al*., 2020), associated with plasmids and are easily transferable between gram negative bacteria therefore enhancing wider and faster spread. This is not in line with the results obtained in this research probably due to the fact that they were present in lesser number 11 (31.4%) of the *E. coli* which might result in less widespread at such abattoir environments. More so, tet A genes are known to be significantly higher in pathogenic than non-pathogenic bacteria (Zebendeh *et al*., 2016, Ralph *et al*., 2022), this suggested that only few of the isolated *E. coli* in question are less pathogenic (31.4%). They might easily spread in the environment under low selection pressure of tetracycline drugs (Skockova *et al*., 2012).

The higher number of *E. coli* tetracycline resistance having *tet*(B) genes than those having *tet*(A) genes was in agreement with those of Sawant, *et al*. (2007) in faecal isolates of cattle, Kozak *et al*. (2009) in beef cattle, slaughterhouse premises in India Aradhye *et al*. (2014) and Avijit (2016) but in contrast with the results obtained by Olowe, *et al*. (2013), Keshvad *et al*. (2014) and Zibandehi *et al*. (2016). The reason might be associated with variation in sources which included water, effluent, and swabs of various surfaces in abattoir environment in this study compared to food sources such as chicken, fish and cow milk sources from other studies. It was found that most *E. coli* isolated from surfaces of abattoir environment are non-pathogenic, and likely harboring tet B genes which could also serve as an indication of higher selection pressure, overuse and misuse of tetracycline drugs in handling of cattle that are usually slaughtered in these abattoirs (*Tuckman et al*., 2007; Koo and Woo, 2011; Skockova *et al*., 2012). However, in previous clinical surveys, the *tet*(B) gene was found to be the most tetracycline resistance determinant and it was proved to have most range due to the ability to reside on highly mobile genetic elements that effectively transfer them among bacterial genera (Aradhye *et al*., 2014). Nevertheless, it was reported that the *tet*(A) gene has the ability to freely spread in farm animals hence, a determinant in it's easy spread in the environment compare to *tet*(B) gene (Koo and Woo, 2011; Skockova *et al*., 2012).

Antibiotic efflux pumps among the specific mechanisms involved in tetracycline resistance in *tet*(A) and *tet*(B) was identified among the isolates. Some previous studies have demonstrated the location of *tet* genes on plasmids and could be transferred horizontally (Szczepanowski *et al*., 2004; Rychlik *et al*., 2006; Urumova, 2016; Long *et al*., 2022). Bacterial resistance to tetracycline is known to occur as a result of active efflux of the drug from cells, altered ribosomal target site that prevents binding of the drug or production of modifying enzymes that tend to inactivate the drug (Urumova, 2016; Long *et al*., 2022).

Some of the *E. coli* 7 (20%) were positive for both genes which implied that they harbour more than one tetracycline resistance genes (Skockova *et al*., 2012; Olowe *et al*., 2013). Similar result was also obtained by other researchers (Gow *et al*., 2008; Aradhye *et al*., 2014) but in contrast with those of Aslam *et al*. (2009) who found only tetC gene. These bacteria are among those that were isolated from effluent and are likely to be highly resistant to tetracycline. However, the five *E. coli* that are void of any of the two tet genes implies that they might harbor other types of tet genes which are known to be found mostly in non-pathogenic bacteria, and are

thereby said to be less tetracycline resistant (Carlota *et al*., 2024). Some of these bacteria were isolated from water for washing meat, butchers' knives and palms.

CONCLUSION

Isolation of tetracycline resistant *E. coli* from the abattoir environment is an indicator of poor hygienic practices which could deteriorate the produced meat quality, constituting public health concern especially to the consumers.

Detection of the two resistance genes (*tetA* and *tetB*) from some isolated *E. coli* in this study suggested that they could be the major determinant resistance to tetracycline in the environment of abattoirs in question. Presence of those harbouring *tet*A genes could be a clear evidence that these pathogens are significantly higher in virulence and could be widely distributed. The progression in multiple antibiotic resistance of *E. coli* might increase virulence and consequently multiply the rate of pathogenic strains. However, only few isolates are found to harbour both genes and fewer harboured none.

It is recommended that the meat producers and veterinarians are to be enlightened on strategies to prevent and control antimicrobial resistance. There should be appropriate health management practices such as good hygiene in order to protect against enteric and other pathogens, use of vaccines in cattle rearing to reduce the frequent use of antimicrobial agents which might invariably reduce rate of antimicrobial resistance in such cattle. It is imperative to enact and improve regulations for the use of antibiotics, create national surveillance system for antibiotic resistance, mechanism of monitoring prescription and use of antibiotics in human, veterinary, and industrial sectors in the country. More studies on community-acquired resistance genes should be promoted since significant percentage of patronizers depend on meat produced in abattoirs. This might suggest the need for Good Manufacturing Practices (GMPs) hence, minimizing the risks of contamination associated with microbial resistant-bacteria.

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