www.ajbrui.org

Afr. J. Biomed. Res. Vol. 26 (September 2023); 447- 450

Research article

Tetracycline Resistant *Vibrio* Species with *tet Genes* in Soil Samples from Two Dumpsites in Port-Harcourt, South-South, Nigeria

Otokunefor, K.[1], Ujah, N.P.[1], *Perewari, D.O.[2], and Agbagwa O.E.[1]

[1]*Department of Microbiology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria*

[2]*Department of Microbiology, Faculty of Science, Niger Delta University, Amassoma, Bayelsa State, Nigeria*

ABSTRACT

With consistent evolutions in the menace of antimicrobial-resistance, there is an ongoing need to continuously grow in understanding the developmental strategies of drug resistant pathogens and possible reservoirs of the resistance determinants. Resistance against tetracycline, a first line anti-cholera drug choice has been reported. With acquisition of resistance in *Vibrio* thought to be associated with exchange of extrachromosomal genetic material from closely related species, this study therefore explores the potential role of environmental *Vibrio* species from Rivers State, Nigeria as reservoirs of tetracycline -resistance genes. Soil samples were collected from two dumpsites in Rivers State, Nigeria and processed for the isolation and identification of *Vibrio* sp according to standard protocols. Identified *Vibrio* isolates were then screened for the presence of four *t*etracycline -resistance determinants (*tet* A, *tet* B, *tet* G and *tet* M) following DNA extraction by boiling. A total of 29.2% of isolates (14/48) possessed at least one of the four *tet* genes assayed for. For specific occurrence, *tet* B was the most occurring of the four (14.6%) and *tet* G the least occurring (2.1%). Both dumpsites had a similar level of occurrence of the *tet* genes (26.9% and 31.8%), but there was a higher representation (54.5%) of *tet* genes in isolates obtained from the subsoil than the surface (7.7%). Results indicate a potential role of these environmental *Vibrio* sp. as reservoirs of tetracycline -resistance genes. The presence of the *tet* M gene associated with a conjugative transposon adds more risk to this potential. More studies are needed to proffer a better understanding of the risks and relationships present.

Keywords: *Environmental Vibrio, reservoir, resistance, tetracycline , tet M, dumpsites*

Author for correspondence: Email: doubraotis@ndu.edu.ng ; Tel: +234 **

Received: March 2023; Accepted: July 2023

DOI: https://doi.org/10.4314/ajbr.v26i3.21

INTRODUCTION

Tetracycline is commonly used as one of the first line drugs of choice for the treatment of cholera based on recommendations by the World Health Organization (WHO) (Ahmadi, 2021). With the evolving menace of antimicrobial resistance, members of the *Vibrio* species, particularly *Vibrio* cholerae have not been spared. Worldwide, increasing reports of antimicrobial resistance among this group of bacteria have been made, with multidrug resistance reported (Adesiyan *et al*., 2020, Das *et al*., 2020, Gxalo *et al*., 2021).

Specifically, several studies have reported an increase in tetracycline resistance which appears to vary from region to region, studies to studies. Several reports of this resistance have been made of resistance rates of up to 100% (Kar *et al*., 2015, Jain *et al*., 2016, Khany and Farzami 2016). A 2019 study reported an upsurge in resistance rates to 40% by 2007 and subsequent decline to 8% by 2009 (Rijal *et al*., 2019). A recent review however gave a 50% average for tetracycline resistance (Ahmadi 2021). These variations are thought to be a function of the loss of the resistance genes in the absence of selective pressure.

The tetracycline resistance among *Vibrio* has been found to be associated with over 38 determinants, with *tet* A, *tet* B and *tet* C reported as the 3 most commonly occurring. The determinants act by three broad mechanisms; production of ribosomal protection proteins, enzymatic inactivation and application of efflux pumps (Khany *et al*., 2016). Acquisition of this and other resistance in *Vibrio* has been thought to be via the exchange of extrachromosomal genetic material from closely related species rather than via chromosomal mutations. Environmental *Vibrio* could therefore play a key role as reservoirs of these resistance determinants. This study examined *Vibrio* species from two dumpsites in Rivers State, Nigeria and further explored the potential role of these environmental *Vibrio* species as reservoirs of tetracycline resistance genes.

MATERIALS AND METHODS

Sample collection and processing: Soil samples were collected from two different dumpsites in Rivers State, Nigeria. The two locations were the Rivers State Waste Management Authority (RIWAMA) dumpsite (GPS Location: 4˚ 54'12''N 6˚ 57'52''E) and the Alakahia dumpsite (GPS Location: 4˚ 53'14.3''N 6˚ 55'19.9''E) which has existed for more than a decade. These samples were collected from two depths at every sampling point (TS: Surface and SS: 15 cm).

Isolation and Identification of *Vibrio* species: These samples were then analysed for the presence of *Vibrio* species according to CDC recommendations (CDC 2023). This first involved creating a suspension of the soil sample in alkaline peptone water (APW) in a 1 in 10 ratio. A 1 ml aliquot of this was then diluted 10-fold in APW and pre-enriched for 6 hours at 28°C. Culture was carried out on Thiosulfate-citrate-bile salts-sucrose (TCBS) agar and the identities of characteristic *Vibrio* colonies confirmed using standard biochemical tests (Cheesbrough 2006, Cowan 1993).

Screening for selected tetracycline resistance determinants: Next, molecular analysis was carried out on a subset of the *Vibrio* species to ascertain the presence of the selected tetracycline resistance determinants. This first involved the extraction of genomic DNA using the boiling method (Ajuga *et al*., 2021). The presence of the four tetracycline resistance genes (Table 1) was then determined using a standard PCR protocol. This involved an initial denaturation at 95°C for 5 minutes, and 35 cycles of denaturation at 95°C for 30 seconds, annealing 95°C for 30 seconds, elongation at 72°C for 45 seconds and final extension at 72°C for 6 minutes.

RESULTS

An assessment of the isolates showed that 29.2% of isolates (14/48) possessed at least one of the four tet genes assayed for. The specific occurrence of the individual genes however varied with tetB noted as the most commonly occurring of the four (14.6%) and tetG the least commonly occurring (Figure 1). Most isolates (11/14) possessed only one of the tet genes tested for, with 3 isolates having two of the genes each (tetA/B, tetA/M, tetB/M).

Table 1:
Selected primers for tetracycline resistance genes.

Gene	Primer name	Primer sequence (5' to 3')	Annealing Temp (°C)	Product size (bp)	References
tet A	*tet A* F *tet A* R	TACATCCTGCTTGCCTT AGATCGCCGTGAAGAG	62	205	Jurado-Rabadan *et al*., 2014
tet B	*tet B* F *tet B* R	CATTAATAGGCCCATCGCTG TGAAGGTCATCGATAGCAGG	58	929	Olowe *et al*., 2013
tet G	*tet G* F *tet G* R	GCTCGGTGGTATCTCTGCTC AGCAACAGAATCGGGAACAC	52	468	Al-Bahry *et al*., *2016*
tet M	*tet M* F *tet M* R	ACAGAAAGCTTATTATATAAC TGGCGTGTCTATGATGTTCAC	55	171	Aminov *et al*., 2001

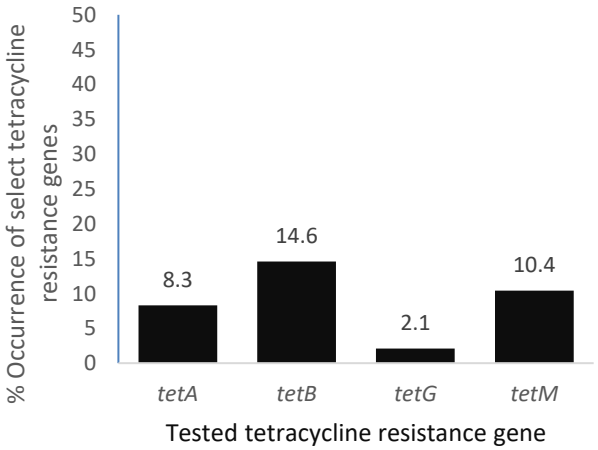

Figure 1:
Variable occurrence of select tetracycline resistance genes among *Vibrio* sp.

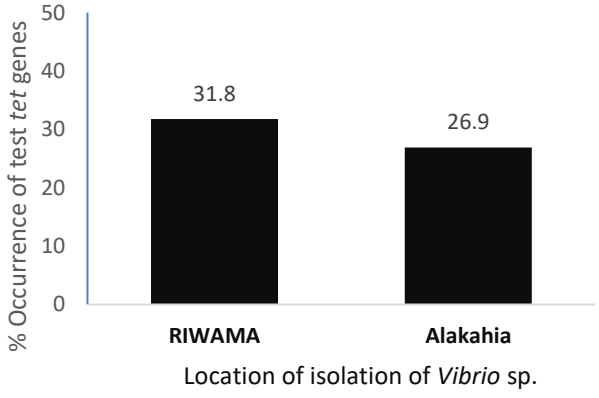

Figure 2:
Effect of isolation location on occurrence of *tet* genes

Biochemical analysis confirmed that 61% (59/97) of the isolates with the characteristics yellow or green colonies on TCBS were *Vibrio* sp, while 8.2% (8/97) of these *Vibrio* isolates were confirmed to be *Vibrio* cholerae. Molecular assessment of a total of 48 of these *Vibrio* isolates by PCR showed that 29.2% of isolates (14/48) possessed at least one of the four *tet* genes assayed for. The specific occurrence of the individual genes however varied with *tet* B noted as the most commonly occurring of the four (14.6%) and *tet* G the least commonly occurring (Figure 1). Most isolates (11/14) possessed only one of the *tet* genes tested for, with 3 isolates having two of the genes each (*tet* A/B, *tet* A/M, *tet* B/M).

Analyzing a possible effect of location on occurrence of the tested genes showed that both sites had a similar level of occurrence of the *tet* genes (Figure 2). The occurrence however showed higher levels of variation with respect to sampling points (Figure 3). With a higher representation of tested genes observed with isolates obtained at the subsoil locations (54.5%, 12/22).

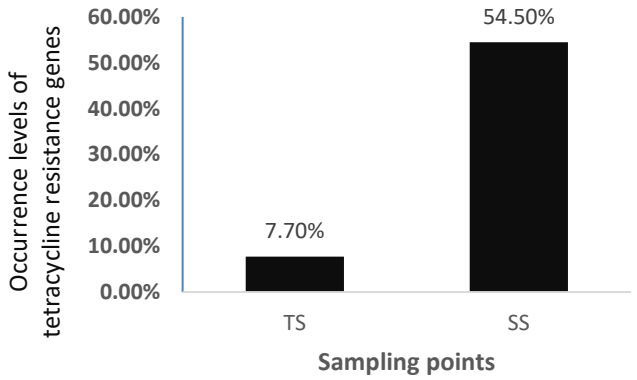

Figure 3:
Variable occurrence of *tet* genes in isolates obtained from different sampling point.

DISCUSSION

Several environments have been reported to possess the potential to serve as reservoirs of antibiotic gene markers. Specifically, tetracycline resistance genes have been reported among environmental strains of *Vibrio* sp from various parts of the globe. The occurrence of these resistance genes however varies, with one study assessing the occurrence of tetracycline resistance determinants in *Vibrio* sp from coastal waters in Thailand observing a zero occurrence of the tetracycline determinants (Jeamsripong *et al*., 2022). This was similar to findings by Tramuta and colleagues who noted an absence of tetracycline resistance determinants in *Vibrio* sp isolated from oysters (Tramuta *et al*., 2021). In contrast, Thiang and colleagues exploring the presence of 14 different tetracycline resistance determinants detected a 100.0% occurrence of at least one of the tetracycline resistance genes in the test population (Thiang *et al*., 2022). This was much higher than the 29.2% occurrence observed in this study. Unlike this present study which specifically studied *Vibrio* sp from dumpsites, the isolates from the Thiang study were obtained from marine aquaculture farms and this could perhaps inform the wide disparity in findings.

Few other studies indicated the total occurrence of tested genes but rather focused on individual gene occurrences. Reports focusing specifically on occurrence of *tet* A gene noted rates ranging from 4% to 64.28% (Morita *et al*., 2020; Okoh and Igbinosa 2010; Adesiyan *et al*., 2022; Faja *et al*., 2019, Hu *et al*., 2020, Thiang *et al*., 2022). These studies differed in their source of isolates. The 8.3% occurrence of *tet* A gene observed in this study was much lower than the 25% and 28% observed by the two other studies which focused specifically on environment strains of *Vibrio* sp in general (Okoh and Igbinosa 2010; Adesiyan *et al*., 2022). A key variation might lie in the fact that whereas these previous studies explored isolates from aquatic environments, the present study looked specifically at dumpsites, a terrestrial environment.

One interesting finding of this study was a larger representation of the *tet* B resistance determinants in the test isolates. While a higher occurrence of the *tet* A gene is the more commonly observed phenomenon, a higher occurrence of the *tet* B gene is not unheard of. Several studies have reported this, albeit in Escherichia coli (Tuckman *et al*., 2007; Gholami-Ahangaran *et al*., 2021; Ahangaran *et al*., 2020). Unlike both *tet* A and *tet* B genes which encode for resistance associated with efflux pumps, the *tet* M gene is linked with ribosomal protection (Perewari *et al*., 2022). It is not often that the *tet* M gene is assayed for in studies analysing the molecular basis of tetracycline resistance as it is not one of the predominant mechanisms associated with this resistance. Results of this study were striking with the more common occurrence of *tet* M than *tet* A (10.4% versus 8.3%). Hu and colleagues had reported an 11.1% occurrence of this gene in strains of *Vibrio* parahaemolyticus with *tet* M in their study occurring more commonly than *tet* B. These findings could make a case for the constant inclusion of this gene in studies assessing tetracycline resistance genes especially considering the association of this gene with a conjugative transposon. The presence of this gene in this test population could therefore increase the risk of these environmental strains as reservoirs of tetracycline resistant especially with the notoriety of *tet* M in its ability to move between different types of organisms (Perewari *et al*., 2022).

In conclusion, results from this study analyzing the occurrence of specific tetracycline resistance genes in *Vibrio* species isolated from dumpsites indicate a potential role of these environmental *Vibrio* sp. as reservoirs of tetracycline resistance genes. The presence of the *tet* M gene adds more risk to this potential. More studies need to focus on this gene to ascertain the occurrence and distribution in *Vibrio* sp. in general and *Vibrio* cholerae in particular to proffer a better understanding of the risks and relationships present.

Acknowledgements:
The African Biosciences Ibadan, Oyo state provided research support for the molecular aspect of this work, and the authors are grateful to them.

REFERENCES

Ahmadi M. H. (2021): Global status of *tetracycline* resistance among clinical isolates of *Vibrio* cholerae: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 10, 115.

Adesiyan I. M., Bisi-Johnson M. A., Okoh A. I. (2022): Incidence of antibiotic resistance genotypes of *Vibrio* species recovered from selected freshwaters in Southwest Nigeria. *Sci Rep*. 2022; 12(1): 1-11.

Das B., Verma J., Kumar P., Ghosh A., Ramamurthy T. (2019): Antibiotic resistance in *Vibrio* cholerae: Understanding the ecology of resistance genes and mechanisms. *Vaccine*. 2020 Feb 29;38 Suppl 1:A83-A92. doi: 10.1016/j.vaccine.2019.06.031. Epub 2019 Jul 2. PMID: 31272870.

Gxalo O., Digban T. O., Igere B. E., Olapade O. A., Okoh A. I., Nwodo U. U. (2021): Virulence and antibiotic resistance characteristics of *Vibrio* isolates from rustic environmental freshwaters. *Front Cell Infect Microbiol*. 2021; 19(11): 732001

Kar S. K., Pal B. B., Khuntia H. K., Achary K. G., Khuntia, C. P. (2015): Emergence and spread of *tetracycline* resistant *Vibrio* cholerae O1 El Tor variant during 2010 cholera epidemic in the tribal areas of Odisha, *India*. *International Journal of Infectious Diseases*, 33, pp.45-49.

Jain M., Kumar P., Goel A. K. (2016): Emergence of *tetracycline* resistant *Vibrio* cholerae O1 biotype El Tor serotype Ogawa with classical ctxB gene from a cholera outbreak in Odisha, Eastern India. *Journal of pathogens*, 2016.

Khany A., Farzami M. R. (2016): Determination of *tetracycline* resistance genes in *Vibrio* cholerae O1 biotype El Tor serotype Inaba strains isolated from outbreaks occurred in Iran in 2013. *International Journal of Medical Research & Health Sciences*, 5(5), pp.121-126.

Rijal N., Acharya J., Adhikari S., Upadhaya B. P., Shakya G., Kansakar P., Rajbhandari P. (2019): Changing epidemiology and antimicrobial resistance in *Vibrio* cholerae: AMR surveillance findings (2006–2016) from Nepal. *BMC infectious diseases*, 19(1), 1-8.

CDC (2023): laboratory methods for the diagnosis of *Vibrio* cholera. https://www.cdc.gov/cholera/pdf/laboratory-methods-for-the-diagnosis-of-*Vibrio*-cholerae-chapter-5.pdf (Accessed 28th January, 2023).

Cheesbrough M. (2006): District laboratory practice in tropical countries Part II. Cambridge University Press, UK 2006

Cowan S. T., Steel K. J. (1993): Manual for the identification of medical bacteria, 3rd edition. London: Cambridge University Press, UK 1993.

Ajuga M. U., Otokunefor K., Agbagwa O. E. (2021): Antibiotic resistance and ESBL production in Escherichia coli from various sources in Aba metropolis, Nigeria. *Bulletin National Research Centre*. 2021; 45(1): 1-9.

Jurado-Rabadan S., de la Fuente R., Ruiz-Santa-Quiteria A. J., Orden A. J., de Vries L. E., Agerso Y. (2014): Detection and linkage to mobile genetic elements of *tetracycline* resistance gene *tet* (M) in Escherichia coli isolates from pigs. *BMC Vet Res* 2014;10.1186/1746-6148-10-155.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4105395/

Olowe O. A., Idris O. J., Taiwo S. S. ((2013): Prevalence of *tet* genes mediating *tetracycline* resistance in Escherichia coli clinical isolates in Osun State, Nigeria. *Eur J Microbiol Immunol* (Bp). 2013;3(2):135-140.

Al-Bahry S., Al-Sharji N., Yaish M., Al-Musharafi S., Mahmound I. (2016): Diversity of *Tetracycline* Resistant Genes in Escherichia coli from Human and Environmental sources. *The Open Biotechnol J* 2016;10:289–300.

Aminov R. I., Garrigues-Jeanjean N., Mackie R. I. (2001): Molecular ecology of *tetracycline* resistance: development and validation of primers for detection of *tetracycline* resistance genes encoding ribosomal protection proteins. *J Appl Environ Microbiol* 2001;67(1):22-32. doi:10.1128/AEM.67.1.22-32.2001.

Jeamsripong S., Thaotumpitak V., Anuntawirun S., Roongrojmongkhon N., Atwill E. R., Hinthong W. (2022): Molecular Epidemiology of Antimicrobial Resistance and Virulence Profiles of Escherichia coli, Salmonella spp., and *Vibrio* spp. Isolated from Coastal Seawater for Aquaculture. *Antibiotics*, 11(12), p.1688.

Tramuta C., Decastelli L., Bianchi D. M., Boteva C., Pitti M., Masotti C., Serracca L., Battistini R. (2021): First Detection of Ampicillin Resistant Gene (blaTEM) Isolated from *Vibrio* species in Northern Italy. *Austin J Public Health Epidemol*. 2021; 8(5): 1114.

Thiang E. L., Chai C. Y. S., Lee C. W., Takada H., Wang A. J., Chai L. C., Bong C. W. (2022): *Tetracycline* Resistance and Prevalence of *Tetracycline* Resistance Genes in Bacteria from Marine Aquaculture Farms in Peninsular Malaysia. Sains Malaysiana 51(2) (2022): 345-357 http://doi.org/10.17576/jsm-2022-5102-02

Morita D., Takahashi E., Morita M., Ohnishi M., Mizuno T., Miyoshi S. I., Dutta D., Ramamurthy T., Chowdhury G., Mukhopadhyay A. K., Okamoto K. (2020). Genomic characterization of antibiotic resistance-encoding genes in clinical isolates of *Vibrio* cholerae non-O1/non-O139 strains from Kolkata, India: generation of novel types of genomic islands containing plural antibiotic resistance genes. *Microbiology and Immunology*, 64(6), pp.435-444.

Okoh A. I., Igbinosa E. O. (2010): Antibiotic susceptibility profiles of some *Vibrio* strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa. *BMC Microbiol*. 2010; 10(1): 1-6.

Faja O. M., Abd Sharad A., Younis K. M., Alwan M. G., Mohammed B. J., Ahmad A. (2019): Isolation, detection of virulence genes, antibiotic resistance genes, plasmid profile, and molecular typing among *Vibrio* parahaemolyticus isolated in Malaysian seawater from recreational beaches and fish. *Veterinary world*, 12(7), p.1140.

Hu Y., Li F., Zheng Y., Jiao X., Guo L. (2020): Isolation, molecular characterization and antibiotic susceptibility pattern of *Vibrio* parahaemolyticus from aquatic products in the southern Fujian Coast, China. J. Microbiol. Biotechnol. 30(6), 856–867. https://doi. org/10.4014/jmb.2001.01005.

Tuckman M., Petersen P. J., Howe A. Y., Orlowski M., Mullen S., Chan K., Bradford P. A., Jones C. H. (2007): Occurrence of *tetracycline* resistance genes among Escherichia coli isolates from the phase 3 clinical trials for tigecycline. Antimicrobial agents and chemotherapy, 51(9), pp.3205-3211.

Gholami-Ahangaran M., Karimi-Dehkordi M., Miranzadeh-Mahabadi E., Ahmadi-Dastgerdi A. (2021): The frequency of *tetracycline* resistance genes in Escherichia coli strains isolated from healthy and diarrheic pet birds. *Iran J Vet Res.* 2021 Fall; 22(4):337-341. doi: 10.22099/ijvr.2021.38454.5592. PMID: 35126542; PMCID: PMC8806167.

Ahangaran M. G., Zinsaz P., Pourmahdi O., Ahmadi-Dastgerdi A., Ostadpour M., Soltani M. (2022): *Tetracycline* Resistance Genes in Escherichia coli Strains Isolated From Biofilm of Drinking Water System in Poultry Farms. *Acta Veterinaria Eurasia*, 48(1), pp.64-69.

Perewari D. O., Otokunefor K., Agbagwa O. E. (2022): *Tetracycline* -Resistant Genes in Escherichia coli from Clinical and Nonclinical Sources in Rivers State, Nigeria. *International Journal of Microbiology*, 2022.