Exogenous and cell-free nucleic acids in water bodies: A penchant for emergence of pandemic and other particulate nucleic acid associated hazards of public health concern in water nexus

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Globally, the origin of major exogenous and extra-chromosomal nucleic acids has remained vague, yet there are unremitting reports of emerging nucleic acid particles which are associated with disease cases and other health concerns. The presence of exogenous and extra-chromosomal nucleic acids was determined as potential predilection for emergence of pandemic in water nexus using metagenomics and molecular biology based techniques. Water samples (50) were collected from various water bodies in Oghara environs, filtered (0.45 µm Nitrocellulose filter paper) and treated to confirm the absence of viable microorganisms. Non-bacterial associated nucleic acids were extracted from decontaminated water specimen, Amplified using Polymerase Chain Reaction (PCR), detected using agarose Electrophoresis, while nucleic acid extracts were transferred and expressed in the recipient *Escherichia coli K-12* strain. The PCR detection result revealed various exogenous resistant genes including *Bla TEM* (8/50; 16%), *SHV* (10/50; 20%), *CTXM* (12/50; 24%), *AmpC* (16/50; 32%), *NDM-1* (11/50; 22%), *VIM* (6/50; 12%), *flor* (33/50; 66%), *str* (8/50; 16%), *aadA* (5/50; 10%), *tetA* (24/50; 48%), *intI* (39/50; 78%), *sulI* (9/50; 18%), *sul2* (38/50; 76%), *tmp* (21/50; 42%), *CTXM-9* (18/50; 36%) and *CatII* (16/50; 32%). The result also shows the presence of resistant extra-chromosomal genes phenotype (13/50; 26%) with resistant determinants in the various sampled water. The detection of such antibiotic resistant genes amongst cell free and/or exogenous nucleic acids is an indication that present in the water environment are potential nucleic acid based agents of public health relevance. Such nucleic acid particles are also potential emerging agents of particulate disease situation. A redirected and focus-based interest on removal of such particulate cell free and/or exogenous and extra-chromosomal nucleic acids possesses potential for control of future emerging disease cases.

Key words: Cell free exogenous nucleic acids; extra-chromosomal nucleic acids; antibiotic resistant genes; water nexus; recipient *Escherichia coli* K-12 strain.

INTRODUCTION

In recent times, there has been consistent report of diverse disease cases arising from cell free nucleic acids or non-cellular/particulate nucleic acids both in the form of extra-chromosomal nucleic acids, mobile genetic elements (MGEs),

phage, exogenous nucleic acids (e.g. exDNA) and membrane vesicles. The knowledge of cell-free DNA (cfDNA) occurrence has been reported in blood and other body fluids by various investigators since over six decade (Mandel and Metais, 1948), however its clinical relevance only became known after Leon et al. (1977) described increased levels of cfDNA in the study of some cancer patients. Since then, cfDNA and other exogenous nucleic acids have been globally applied as tumor-derived marker in diverse disease prognosis and diagnosis both in public health system, human and the environmental nexus (Woegerbauer et al., 2020; Toyofuku et al., 2019; Norton et al., 2013; Fernando et al., 2010; Hung et al., 2009; Chan et al., 2005; Ziehler et al., 2002; Lo et al., 1998). The origin of these genetic and nucleic acid based components in water nexus has remained hesitant with diverse speculations from various investigators of related studies. Some of the investigators have opined that non-cellular nucleic acids originated from partial breakdown of some cell associated nucleic acids from diverse organism following: (i) drug or antibiotic treatment, (ii) simultaneous turgor pressure, (iii) antimicrobial-stressed environment, etc (Woegerbauer et al., 2020; Toyofuku et al., 2019, 2017; Devos et al., 2017; Fulsundar et al., 2014). The various members of exogenous nucleic acids find their way into the environment via release from worn-out tissues, urine, waste disposal and leftovers of partially degraded chromosomal nucleic acids. Other antimicrobial and chemical treatment of cellular/biological based components in the environment also release into the water nexus in addition to wastewater release diverse non-cellular/particulate nucleic acids both in the form of extra-chromosomal nucleic acids, mobile genetic elements (MGEs), phage, exogenous nucleic acids (e.g. exDNA) and membrane vesicles. These nucleic acids in the environment has been linked with the occurrence of antibiotic resistant genes (ARGs) both in the public health system and in the environment (Igere et al., 2020; Mane et al., 2018; Manaia et al., 2018; Perovic et al., 2018; Petrovich et al., 2018). In addition, the environmental water nexus has been shown to encourage the transfer of these nucleic acid components via horizontal gene transfer (HGT) into some organisms (Woegerbauer et al., 2020; Toyofuku et al., 2019; Sørensen et al., 2005). It is important to note that the detection of such cell-free nucleic acids is an adequate risk assessment determinant especially as it has been associated with potential outbreak causal agent. The detection of such cell-free components applies molecular biology techniques including *in situ* nucleic acid hybridization, purification of nucleic acids, polymerase chain reaction (PCR), gene cloning and microarrays. Other modified method is the wastewater-based epidemiology (WBE) or wastewater-based epidemiological studies on particulate nucleic acids which involves a multifacet process, whereby diverse nucleic acids are source tracked by the evaluation of wastewater. This method has been applied for members of viral particles such as norovirus, polio, bacteriophage T2, adenovirus, enteroviruses, and ECHO virus (Mao et al., 2020). Countries such as Australia, Italy, Spain, Netherlands, China, Japan, etc. have employed WBE methods in the detection of SARS-CoV-2 amongst municipal wastewater as well as wastewater release. Although there have been related reports of such exogenous nucleic acids, there is dearth of information of metagenomic analysis of such non-cellular/exogenous nucleic acids in water nexus. This study investigated exogenous and extra-chromosomal nucleic acids in water bodies: a penchant for emergence of pandemic and other particulate nucleic acid associated hazards of public health concern in water nexus.

MATERIALS AND METHODS

Study area and samples

Fifty replicate samples of untreated and treated water sources as well as wastewater effluents were collected from various sites (Hospital effluents, municipal effluents, domestic effluents, abattoir effluents, poultry effluents especially birds, pigs, cow, goats and fish), over a period (5 months) between May and September 2020. It is important to note that the effluents from the sampled water systems are constantly released into the Ethiope River or water flow. The physicochemical parameters of sampled water

sources were analyzed using standard methods as previously described by Igere et al. (2020). Water samples were first concentrated via ultracentrifugation/filtration (Method A), while the extraction of nucleic acids was done by an adsorption-elution method using electronegative membranes (QIAamp Qiagen Inc, Valencia, CA, USA, www.Qiagen.com).

Whole nucleic acid (DNA/RNA) Extraction

The whole nucleic acid (DNA/RNA) was extracted using the modified method of nucleic acid and viral RNA extraction from wastewater by Qiagen. This method involves the recovery nucleic acids by first treating the water samples with 2.5 molar (2.5 M) magnesium chloride in 0.2-0.5 L of water, cold centrifugation at 4500 g for 10 min while the supernatant is taken through filteration using 0.45 nm Whatmann No. 1 filter paper or nitrocellulose paper. The pre-treated water samples were pasteurized at 60°C to inactivate any live bacteria, cold centrifugation the pasteurized samples at 4500 g for 10 min while the supernatant is taken through filtration using 0.45 nm Whatmann No. 1 filter paper or nitrocellulose paper. Nucleic acids (DNA/RNA) were extracted from pre-treated sample using the method of www.Qiagen.com. The advantages of these methods include an initial processing time of about 1 h and the need only for cheap, widely available equipment and reagents. There are also drawbacks, such as the clogging of the filters that may increase processing time. It is important to note that this method may be applied for SARS-CoV-2 detection and show that the test can be used on samples from outside the lab. About 5 µg of each purified DNA extract was used for amplification, detected by agarose gel electrophoresis and visualized in documentation system.

Nucleic acid (DNA/RNA) quantitation

The purity and concentration of extracted DNA from various water sources was ascertained by DNA quantitation prior to downstream reactions such as PCR using the following expression. A pure DNA sample should have a ratio range of 1.5 to 2.0 particularly in TE buffer whereas a ratio range < 1.5 indicates contamination with protein-based material (www.Qiagen.com).

DNA/RNA concentration (ug/ul) = (Absorbance at 260 nm × Dilution coefficient × Dilution Factor)/1000

where Dilution coefficient for extracted DNA = 50 µg/µl and dilution coefficient for extracted RNA = 40 µg/µl.

To determine the purity of isolated DNA:

DNA purity = Absrobance at 260 nm (X) / Absorbance at 280 nm (Y)

Transformation of extracted exDNA onto *E. coli* K-12 strain

Extracted exogenous nucleic acids from water samples were transferred onto a recipient *E. coli* K-12 strain and susceptibility was determined. This technique was adopted as described by Yang et al. (2008); and the numbers of transferred exogenous resistant nucleic acids genes were subsequently determined.

PCR amplification of extracted exDNA

Specific primers sequences shown in Table 1 were retrieved and sent to Inqaba Biotechnical industries (Pty) Ltd. (Hatfield Pretoria, South Africa) for synthesis and reports. The resistance primer sequence were used as described in Table 1 using PCR (T100^{TM} thermal cycler, Bio-Rad, Hercules, Califonia, USA) and cycling conditions in a 200 µL microfuge tube are as follows: approximately 50 picomolar to 1 µM DNA extract; a final volume of 25 µL; a GoTaq ®G2 green master mix supplied in 2× Green GoTaq®G2 reaction buffer containing pH 8.5, dNTPs {400 µM each of dATP, dGTP, dCTP and dTTP}, 3 mM $MgCl_2$ and GoTaq®G2 DNA polymerase at optimal concentration as specified by Promega Corporation (USA; www.promega.com). Primer concentration of 0.5 µM; cycling condition of genes were 4 min at 94.0°C followed by 35 cycles of 94.0°C for 1 min, 56.0°C for 1 min and 72.0°C for 1min and a final extension step at 72.0°C for 8 min. Others are shown in Table 1. The agarose electrophoresis (electrophoresis machine CLS-AG100, Warwickshire, United Kingdom) was done at an appropriate voltage of 100 V for 50 min until the

Table 1. Primer pairs and amplification conditions.

Target gene	Primer name	Sequence 5' 3'	Expected Band size (bp)	Annealing Temp (°C)	Reference
TEM	*blaTEM-F* *blaTEM-R*	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	515	56	Sharma et al. (2013)
SHV	*blaSHV-F* *blaSHV-R*	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	390	55	Sharma et al. (2013)
AMPC	*ampC-F* *ampC-R*	TTCTATCAAACTGGCARCC CCYTTTTATGTACCCAYGA	545	45	Titilawo et al. (2015)
NDM	*blaNDM-1-F* *blaNDM-1-R*	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	621	52	Nordmann et al. (2011)
Cat	*catII-F* *catII-R*	ACACTTTGCCCTTTATCGTC TGAAAGCCATCACATACTGC	542	56	Titilawo et al. (2015)
STR	*str-F* *str-R*	CTTGGTGATAACGGCAATTC CCAATCGCAGATAGAAGGC	348	59	Titilawo et al. (2015)
	aadA-F *aadA-R*	GTGGATGGCGGCCTGAAGCC AATGCCCAGTCGGCAGCG	525	53	Titilawo et al. (2015)
VIM	*VIM-F* *VIM-R*	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390	52	Dallenne et al. (2010)
TetA	*TetA-F* *TetA-R*	GTAATTCTGAGCACTGTCGC CTGCCTGGACAACATTGCTT	950	55	Yolanda et al. (2004)
IntI	*intI-F* *intI-R*	GCTGGATAGGTTAAGGGCGG CTCTATGGGCACTGTCCACATTG	521	55	Hochhut et al. (2001)
FLOR	*flor F* *flor R*	TTATCTCCCTGTCGTTCCAGCG CCTATGAGCACACGGGGAGC	586	55	Hochhut et al. (2001)
Sul	*sul2 F* *sul2 R*	AGGGGGCAGATGTGATCGC TGTGCGGATGAAGTCAGCTCC	625	58	Hochhut et al. (2001)
CTX-M	bla_{CTX-MF} bla_{CTX-MR}	CGCTTTGCGATGTGCAG ACC GCG ATA TCG TTG GT	550	55	Tejashree et al. (2017)
	$bla_{CTX-M-10F}$ $bla_{CTX-M-10R}$	GCTGATGAGCGCTTTGCG TTACAAACCGTTGGTGACG	684	54	Oliver et al. (2001)
	$bla_{CTX-M-8/25F}$ $bla_{CTX-M-8/25R}$	AACRCRCAGACGCTCTACb TCGAGCCGGAASGTGTYATb	326	55	Mohammed et al. (2016)
	$bla_{CTX-M-2F}$ $bla_{CTX-M-2R}$	ATGATGACTCAGAGCATTCG TTATTGCATCAGAAACCGTG	884	54	Petroni et al. (2002)
	$bla_{CTX-M-9F}$ $bla_{CTX-M-9R}$	GTGACAAAGAGAGTGCAACGG ATGATTCTCGCCGCTGAAGCC	857	55	Sabate et al. (2000)

dye front was 2 cm from the end of the gel. The power was switched off and the electrophoresis tank was disconnected from the power pack. The gel was carefully removed from the casting tray and photograph using the Gel doc imaging system (Bio Rad, USA).

Limitation

There was no High-Throughput gene sequencing of samples, shotgun paired-end library construction, analysis of sized libraries using an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). metagenomic sequencing was not performed by Macrogen (Seoul, Korea) using the TruSeq DNA PCR-Free Kit (Illumina Inc., San Diego, CA, USA).

RESULTS

Nucleic acid quantitation

Using the Qiagen specification for DNA quantitation (www.Qiagen.com), the various nucleic acid extracts produced OD range of 1.7 to 1.8 affirming the presence of nucleic acids in the extract of sampled water.

Transformation of extracted exDNA onto *E. coli* K-12 strain

The transformation of exDNA onto *E. coli* K-12 and the post antibiotic susceptibility testing of transformant cells showed that wild cells which were previously sensitive to employed antibiotics produced diverse resistant markers shown in Table 2. The various resistant phenotypes include CTX^r, CXC^r, AMP^r, STR^r, CXM^r, TET^r, CHL^r, OFL^r, CAZ^r, CTX^r, ERY^r, AMP^r, PN^r, SXT^r, CXM^r, etc. It is important to note that such resistance phenotypes have been reported amongst potential pathogens in the environment (Igere et al., 2021; Igere et al., 2020). This report further affirms the origin of such resistance phenotype and its predominance in the water nexus and study area.

Table 2. Transferred exDNA resistant markers onto recipient strain.

Water sample	Extrachromosomal phenotypic resistant markers
Recipient Strain	Nil
RW1	CTX^r,AMP^r PN^r,CXM^r,TET^r
RW3	AMP^r PN^r,TET^r
RW6	CXC^r,AMP^r,TET^r,CHL^r
RW7	TET^r,CHL^r
RW10	AMP^r PN^r,STR^r,CXM^r,TET^r,CHL^r
AWWE1	AMP^r PN^r,CHL^r
AWWE2	PN^r,STR^r,CHL^r
AWWE3	CTX^r,CXC^r,AMP^r ,STR^r,TET^r,CHL^r
AWWE5	TET^r,CHL^r
AWWE6	CHL^r
AWWE7	AMP^r PN^r,TET^r
AWWE8	PN^r,STR^r
CAN2	AMP^r PN^r,SXT^r,TET^r
CAN3	CTX^r,CXC^r,AMP^r PN^r,STR^r,TET^r,CHL^r
CAN9	AMP^r PN^r,STR^r,TET^r,CHL^r
HWWE1	CXC^r,ERY^r,AMP^r PN^r,STR^r,CXM^r,TET^r,CHL^r
HWWE2	AMP^r,TET^r,CHL^r
HWWE3	CTX^r,CXC^r,AMP^r,STR^r,CXM^r,TET^r,CHL^r, OFL^r
HWWE4	CAZ^r,CTX^r,ERY^r,AMP^r PN^r,SXT^r,CXM^r,TET^r
HWWE5	CAZ^r,CTX^r,AMP^r PN^r,STR^r,SXT^r,TET^r,CHL^r
HWWE6	PN^r,STR^r,CXM^r,CHL^r
HWWE10	AMP^r,STR^r,SXT^r,CHL^r
PWWE3	PN^r,STR^r,CHL^r
PWWE6	CTX^r,CXC^r,AMP^r,CXM^r,SXT^r,CHL^r
PWWE9	CXC^r,AMP^r, PN^r,SXT^r,CHL^r
DWWE1	PN^r,STR^r,SXT^r,CHL^r
DWWE3	CTX^r,CXM^r,ERY^r, PN^r,SXT^r,CHL^r
DWWE6	PN^r,SXT^r,TET^r,CHL^r
DWWE10	CTX^r,CXC^r,AMP^r PN^r,CXM^r,TET^r,CHL^r/8

Gene amplification and detection of extracted exDNA

The molecular gene detection employing PCR technique and using the various target specific antibiotic resistance gene primer pairs/set (Table 1) revealed various resistant genes as shown in Figures 1, 2, 3, 4, 5, 6 and 7 showing that the extracted exogenous nucleic acids consist of diverse resistant genes in the sampled water. The sampled water RW1 harbored four resistant genes, AWWE3 harbored five resistant genes while a high prevalence of resistant genes were observed in water samples collected at HWWE1 (8), HWWE3 (9), HWWE4 (8), HWWE5 (8), HWWE6 (4), HWWE10 (5), etc., shown in Table 2. Some of the resistance genes observed were of high clinical relevance as it indicates fecal contamination and potential for spread of resistance via horizontal transfer was possible.

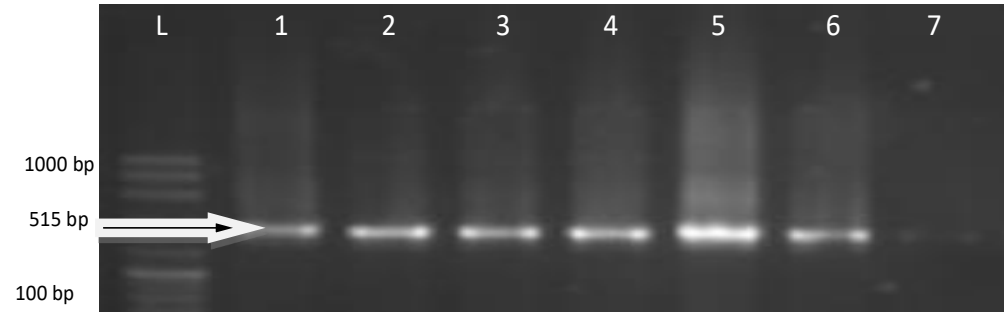

Figure 1. PCR products of positive *BlaTem gene*. L is a molecular marker as number 1 is a positive control, while numbers 2, 4, 5, 6, and 7 are positive detected exogenous specific gene.

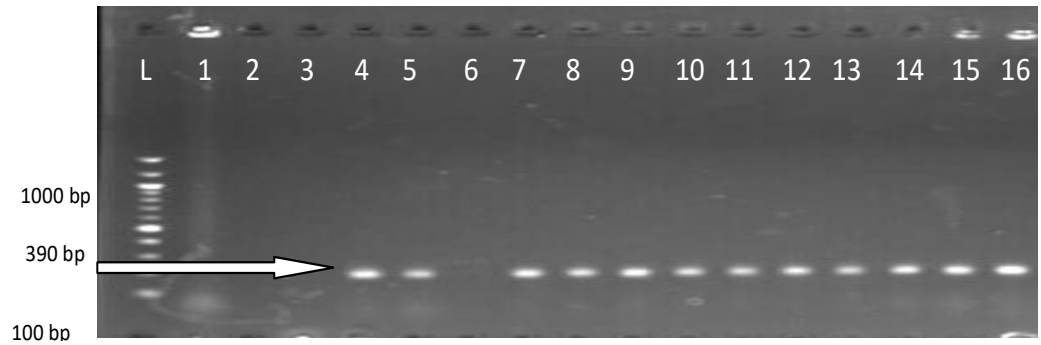

Figure 2. PCR products of positive *Blashv gene*. L is a molecular marker as numbers 1, 2, and 3 are negative detection, number 4 is a positive control, while numbers 5, 7-16 are positive detected exogenous specific gene and number 6 is a negative control.

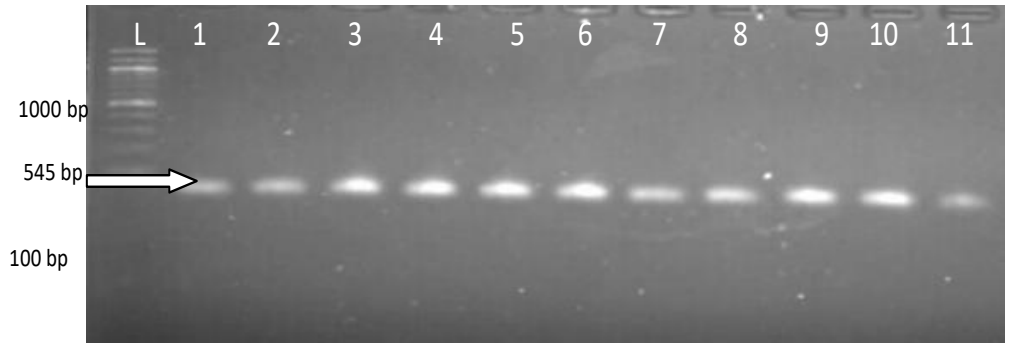

Figure 3. PCR products of positive *ampC gene*. L is a molecular marker as number 1 is a positive control, while numbers 2 - 11 are positive detected exogenous specific gene.

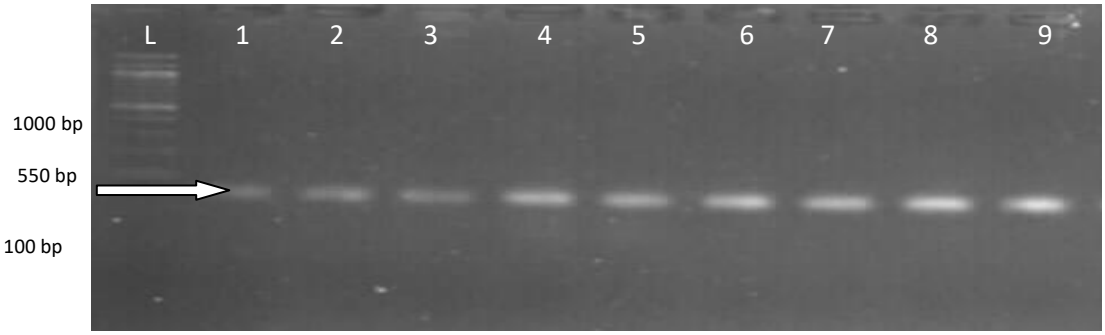

Figure 4. PCR products of positive *Blactxm gene*. L is a molecular marker as number 1 is a positive control, while numbers 2 - 9 are positive detected exogenous specific gene.

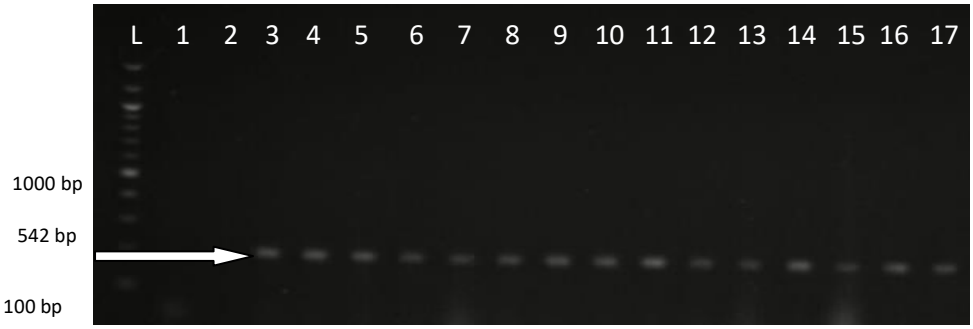

Figure 5. PCR products of positive *catII gene*. L is a molecular marker as numbers 1, 2 are negative control while number 3 is a positive control, whereas numbers 4 - 17 are positive detected exogenous specific gene.

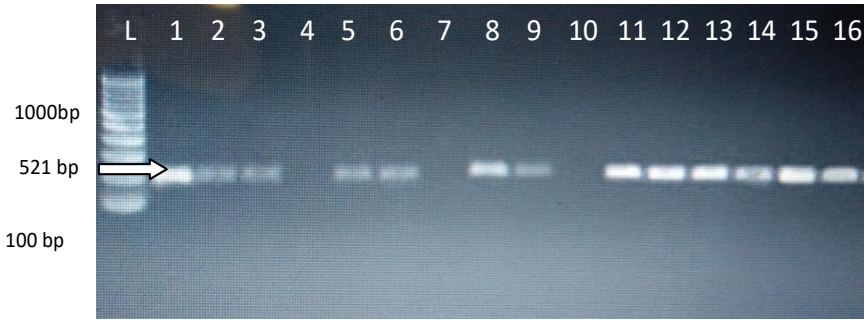

Figure 6. PCR products of positive *IntI gene*. L is a molecular marker as number 1 is a positive control, 4, 7 and 10 are negative while numbers 2, 3, 5, 6, 8, 9, 11-16 are positive detected exogenous specific gene.

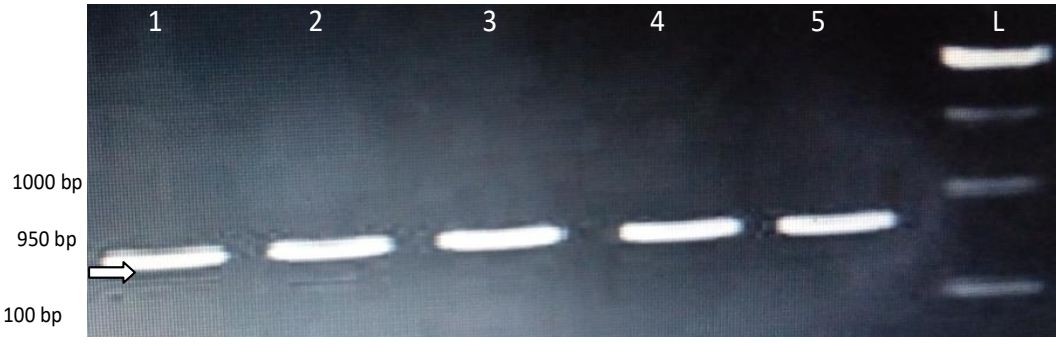

Figure 7. PCR products of positive *TetA gene*. L is a molecular marker as number 1 is a positive control, while numbers 2, 4, 5, 6, 7 are positive detected exogenous specific gene.

Diversity of detected genes

Using the principal component analysis (PCA) and principal coordinate analysis (PCoA), the detected genes were shown to be diverse and are distributed within the sampled water sources and the environment. Figures 8, 9, and 10 show the diversity and distribution of the targeted genes within the sampled area and the association of the various detected genes during the study.

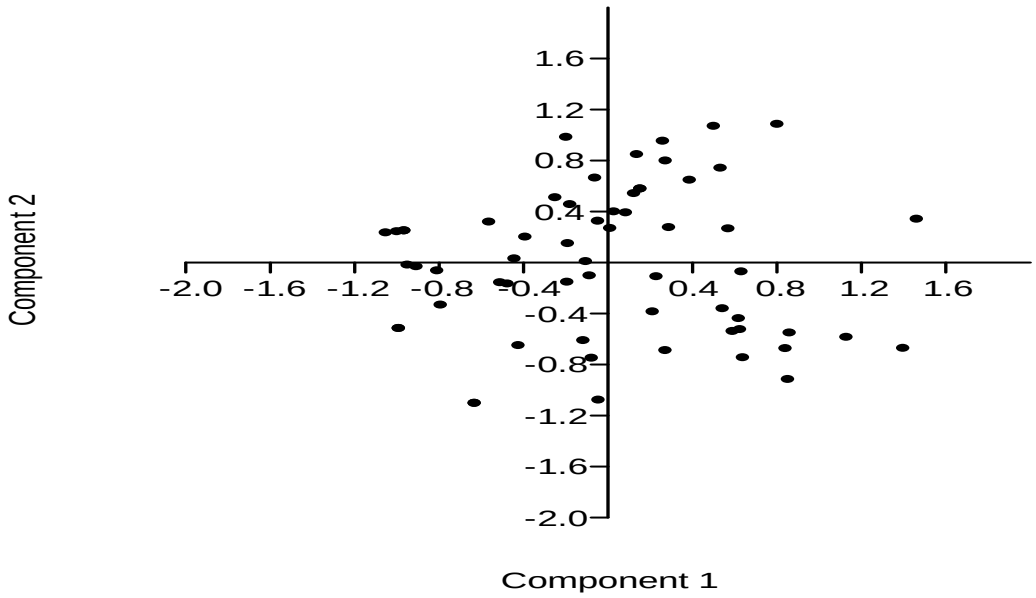

Figure 8. PCA scatter diagram showing the distribution of targeted resistant genes based on the detection.

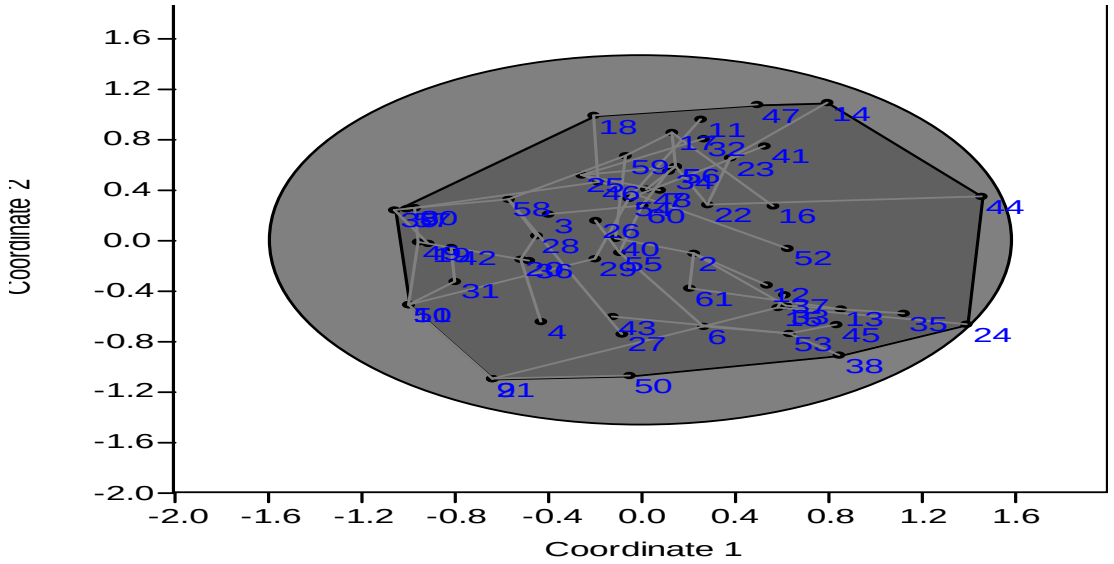

Figure 9. A PCoA scatter diagram showing the cluster of detected resistant gene fragments.

DISCUSSION

It is important to note that sharing and reception of exogenous nucleic acids or cell free nucleic acids in the form of extra-chromosomal DNA, mobile genetic elements (MGEs), phage, exogenous nucleic acids (e.g. exDNA) and membrane vesicles amongst microorganism is a natural phenomenon. The study involves determination and affirmation of exogenous and extra-chromosomal nucleic acids in water bodies as a penchant for emergence of pandemic and other particulate nucleic acid

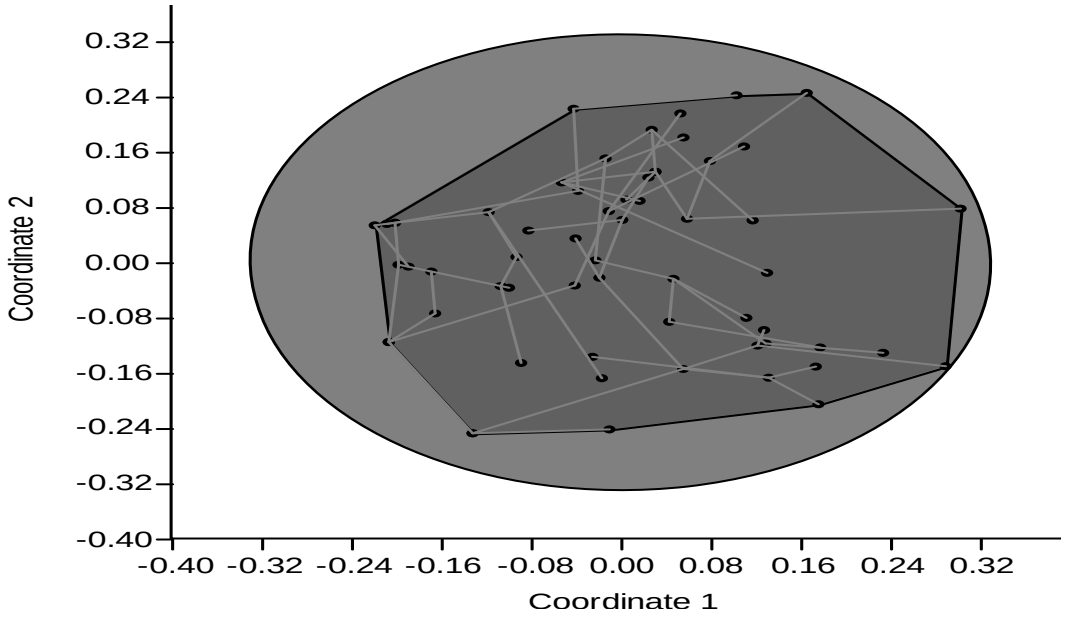

Figure 10. A PCoA scatter diagram showing the cluster of detected resistant gene fragments and their association.

associated hazards of public health concern in water nexus. The spectrophotometric analysis of the nucleic acid extract revealed that extracts contain nuclei acids which may either be DNA and/or RNA (www.Qiagen.com). It is important to note that the water samples were of environmental origin. Observing such exogenous or cell free nucleic acids in environmental water sample leaves the question of the sources of nucleic acids in the environment. In earlier studies by various investigators, it was reported that nucleic acid based components in water nexus has its origin traced to wastewater release, water treatment, disease treatment, as well as other waste disposal strategies applied within communities (Woegerbauer et al., 2020; Toyofuku et al., 2019, 2017). These components has remained hesitant with diverse speculations, as some investigators have opined that non-cellular nucleic acids originated from partial breakdown of some cell associated nucleic acids from diverse organism following: (i) drug or antibiotic treatment, (ii) simultaneous turgor pressure, (iii) antimicrobial-stressed environment, etc (Woegerbauer et al., 2020; Toyofuku et al., 2019, 2017; Devos et al., 2017; Fulsundar et al., 2014). Studies of Mandel and Metais (1948) and Leon et al. (1977) have also helped to affirm the origin since it is reported in blood and other body fluids by various investigators since over six decade. In recent times, their (exogenous nucleic acids) clinical relevance has been further affirmed as it is being used as a marker in the study of some cancer patients (Mandel and Metais, 1948; Leon et al., 1977). Globally, other investigators have also applied cfDNA and other exogenous nucleic acids as tumor-derived marker in diverse disease prognosis and diagnosis both in public health system, human and the environmental nexus (Woegerbauer et al., 2020; Toyofuku et al., 2019; Norton et al., 2013; Fernando et al., 2010; Hung et al., 2009; Chan et al., 2005; Ziehler et al., 2002; Lo et al., 1998). The observation of these exogenous nucleic acids in water indicates that it must have found its way into the environment via release from worn-out tissues, urine, waste disposal and leftovers of partially degraded chromosomal nucleic acids, etc.

The transformation result for *E. coli* K-12 strain also reveals positive extra-chromosomal DNA present in the sampled water which showed ranges of antibiotic resistant markers (Table 2) during the post antibiotic susceptibility detection and/or antibiotic susceptibility testing of the recipient strain/transformat. Table 2 shows the numerical occurrence of resistant markers and the resistant markers in the various sampled water sources including Pen^r, Tet^r, Aug^r,Cxc^r, Gen^r, Str^r, Chl^r and amp^r. This is an indication that the water samples possess exDNA which harbour

potential transferred onto other organisms. Such transferable nucleic acids are referred to as plasmids or extra-chromosomal DNA. This is a potential source for spread of resistant determinants onto other organisms in water bodies including bacteria, fungi and virus. It may also be a potential source to resistance epidemic as well as pandemic if appropriate attentions are not given to their removal from the water environment or nexus. Some of the nucleic acid extracts also showed sensitivity to the antibiotics used indicating negative test on the transformation technique employed. It was observed that twenty-one water samples were negative to the transformation process. This does not affirm that cell free nucleic acids were absent from the water sample because the PCR technique revealed amplicons of resistant genes (Figures 2, 3, and 4). It may be attributed to the inability of recipient strain to take-up exogenous DNA and/or the absence of some of the factors responsible for transformation of exogenous DNA or extra-chromosomal DNA onto recipient strains. Previous studies of Igere et al. (2020, 2021) also observed the presence of resistant genes amongst bacteria strains which were suggested to have an origin from environment and extra-chromosomal nucleic acids.

The molecular detection further confirms the presence of diverse resistant genes including the notorious NDM-1 gene harbored by plasmid or extrachromosomal DNA as previously revealed in the study of Wei et al. (2015). Other genes detected during the study include *Bla TEM* (8/50; 16%), *SHV* (10/50; 20%), *CTXM* (12/50; 24%), *AmpC* (16/50; 32%), *NDM-1* (11/50; 22%), *VIM* (6/50; 12%), *flor* (33/50; 66%), *str* (8/50; 16%), *aadA* (5/50; 10%), *tetA* (24/50; 48%), *intI* (39/50; 78%), *sulI* (9/50; 18%), *sul2* (38/50; 76%), *tmp* (21/50; 42%), *CTXM-9* (18/50; 36%) and *CatII* (16/50; 32%). In a previous study by numerous investigators (Bhagat et al., 2020; Kumar et al., 2020a, b, c), it was reported that present in the environment and water nexus are diverse resistant determinant which include antibiotics resistance genes, anti-parasite resistant genes, anti-fungal resistant genes and antiviral resistant genes which pose threat to environmental wellness. In a related meta-genomics study conducted in India, Europe, and South Africa, diverse resistant genes were also reported amongst wastewater release from a water treatment system indicating the vulnerability of the surrounding settlement to potential health hazards (Ram et al., 2020; Kumar et al., 2019) especially amongst poor populations that may not have access to adequate water sources. The ministry of water resource and safe water regulatory organization needs to redirect interest to these water sources as a strategy for the control of future spread of pandemic.

Conclusion

In recent times, there had been continuous reports of emerging pathologic situation arising from diverse organisms with difficult to manage clinical presentation including antimicrobial resistance (antibacterial, antifungal, antiviral and antiparasite resistance) and emergence of high profile particulate nucleic acid organisms. The observation of such cell free nucleic acids in water nexus is a potential penchant for the emergence for such disease situation especially as there had been in recent studies the distribution of extra-chromosomal DNA associated with antibiotic resistance and sharing of genes. There is ardent need for the removal of such exogenous or cell free nucleic acids from water bodies. This should also include the removal of exogenous nucleic acid as well as extra-chromosomal DNA from wastewater before their release into the diverse water bodies as well as receiving water shed. We are of the opinion that a redirected and research-based interest on the removal of such particulate cell free and/or exogenous and extra-chromosomal nucleic acids possess potential for control of future emerging disease cases and pandemics.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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