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Detection of antibiotic resistance genes from airborne bacterial isolates from public primary schools in Benin City, Nigeria.

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Abstract: The detection of resistance genes from airborne bacterial isolates from public primary schools in Benin City, Nigeria was evaluated in this study. Indoor air samples were collected once daily and monthly across wet and dry seasons using Passive air sampling technique. Isolates were identified using the 16sRNA methods, and sequences were deposited in the gene bank. Antibacterial susceptibility was evaluated using Kirby-Bauer disc diffusion method and the multiple antibiotic resistance (MAR) index, plasmid profiles and presence of resistance genes (t*etA, tetS* and *ermB*) were evaluated. Data were analyzed using a student's t-test at a 0.05 probability level. The results revealed that bacteria count ranged from 3.39±0.49 x102 cfu/m3 - 10.42±2.61 x102 cfu/m3 (wet season) and from 4.18±0.14 x102 cfu/m3 - 12.75±1.61 x102 cfu/m3 (dry seasons). Most classrooms had intermediate contamination levels. The bacteria isolates obtained include *Bacillus aryabhattai, Arthrobacter nicotianae, Pontibacter rhizospherae, Bacillus aereus, Exiguobacterium profundum, B. cereus, Pontibacter korlensis, Vagococcus fluvialis, Arthrobacter arilaitensis, Exiguobacterium acetylicum, Alcaligenes faecalis, B. stratosphericus, B. subtilis,* and *B. pumilus. B. aerius* and *A. arilaitensis* were resistant to all antibiotics and all bacterial isolates were found to have higher MAR index values greater than 0.2. Each isolate had at least one plasmid while a few possessed multiple plasmids. *A. nicotianae, B. aerius, V. fluvialis,* and *B. subtilis* expressed all resistant genes evaluated in the study. The study revealed that a plethora of bacteria isolated from the indoor air of public primary school classrooms have antimicrobial resistance genes (ARGs) and are of public health importance.

1. Introduction

Antimicrobial resistance (AMR) is a global health threat undermining the progress made in achieving sustainable development goals (SDGs). The microbial resistance to drugs is rapidly escalating due to antimicrobial resistance genes (ARGs), posing uncertainties regarding the future effectiveness of existing antimicrobial drugs, especially against bacterial isolates (Vikesland *et al*., 2019). AMR can make infections more difficult to treat, leading to adverse health outcomes and the death of an estimated 4.95 million patients in 2019 alone, with antibacterial-resistant genes accounting for 1.27 million deaths (Murray *et al*., 2022). Sadly, Bacterial pathogens with ARGs are widespread in the clinical environment. Still, reports suggest that microbes in water, domesticated animals, soil, and air (indoor and outdoor) can act as reservoirs for ARGs (Becsei *et al*., 2021). Several studies across the globe have evaluated the microbiological quality of indoor and outdoor air with fascinating results. Some of the reports revealed that the (indoor and outdoor) air is home to a plethora of bacterial isolates

which are of public health significance, and their overall importance cannot be overemphasized (Bragoszewska and Biedron, 2018; Ologbosere and Ogofure, 2020; Ologbosere *et al*., 2021; Ugbo *et al*., 2022). There have been reports concerning the need to study antimicrobial resistance in bacteria because it has been projected that by 2050, death due to antibiotic-resistant bacteria will be more than death due to non-communicable diseases like cancer; therefore, there ought to be policy and regulatory changes if the prediction is to be avoided (O'Neill, 2016). Reports have also shown that the outdoor, in addition to the indoor environment, represents one of the largest reservoirs of ARGs, and one effective way to control their spread is via environmental stewardship (Singer *et al*., 2016). Regulatory agencies and partners must work assiduously to invoke policy changes that would terminate the problem of AMR via environmental stewardship to control and curtail the spread of ARGs in and within the environment. The proximity and interaction between the indoor and outdoor anthropogenic environment and the host-driven spread of antimicrobial resistance make the outdoor environment the largest source of ARGs (Surette and Wright, 2017; George *et al*., 2022). Therefore, routine screening for ARGs from bacterial isolates is essential in understanding the global prevalence, identification of hot spots, tracking resistance mechanisms and informing treatment decisions. There are a few reports in Benin City, Nigeria, concerning the detection of resistance genes from environmental isolates but no data on the detection of resistance genes from public primary schools in Benin City. This study was carried out to evaluate and detect the presence of specific resistance genes in antibiotic-resistant bacteria isolated from the indoor air of selected public primary schools in Benin City, Nigeria.

2. Methodology

2.1 Study Site and Location

Three government-owned public primary schools were selected in this study. The schools were located on longitude 60^0 20' 0" North, latitude 05^0 38' 0" East with a mass of 19,794 km^2 in Benin City, Nigeria. The schools have an average of 70 pupils (children) per class. The schools are located in urban and suburban areas of the City.

2.2 Sample Collection and Procedure

Passive air sampling was used to collect air samples from the selected classrooms. Plates containing tryptone soy agar were exposed for 45 mins according to the method delineated (Bragoszewska and Biedron, 2018). The plates were exposed 1 m above the floor to approximate the children's breathing levels. Samples were collected from Classrooms (Primaries 1, 3 and 5) in triplicates from three (3) selected Government owned Primary Schools in Benin City. The three (3) Public Schools were identified as Schools A, B and C. The sampling was carried out once daily and monthly across wet and dry seasons (May 2018 - September 2018 for the wet season and October 2018 - March 2019 for the dry season).

2.3 Molecular Identification of Airborne Bacteria Isolates

The boiling technique delineated by Chakravorty *et al*. (2007) was used to extract bacterial genomic DNA. Briefly, 2 ml of 24 h. culture was transferred to the Eppendorf tube and centrifuged for 5 min at 10,000 xg. The supernatant was discarded before adding 200 µl of sterile distilled water to the pellets and vortexed for 1 min. The vortexed samples were heated for 15 mins at 100 ^{0}C in a heating block. Final centrifugation followed this for 2 min. at high speed. The supernatant obtained from the second spin is collected as pure DNA and preserved appropriately for further use. 10 µl of the DNA was used for PCR amplification. A universal primer 27F universal primer with the sequence 5'-

AGAGTTTGATCMTGGCTCAG and the 1540R primer with the sequence 5'-TACGGYTACCTTGTTACGACT-3' (forward and reverse primers) were used for the amplification of the bacteria DNA before the samples were sent for sequencing of the amplified DNA fragments.

2.4 Polymerase chain reaction (PCR), Amplified DNA Integrity and Purification of Amplified DNA

The experiment used a thermocycler (GeneAmp 9700 produced by Applied Biosystem, USA). The mixture was a constitution of a colourless cocktail reaction containing 10 µl of 5xGoTaq, 1 µl of dNTPs mix (10 mM), 3 µl $MgCl_2$ (25mM), 1µl of 10pmol each 27F-5'-AGAGTTTGATCMTGGCTCAG-3' and - 1540R, 5′- TACGGYTACCTTGTTACGACT-3′ primers as well as 0.3 units of Taq DNA polymerase, which was made up to 42 µl containing 8µl of DNA template (Taq DNA polymerase was from Promega, USA) and sterile distilled water (SDW). The PCR profile consists of initial denaturation for 5 min at 94^0C; closely or briefly accompanied by a 30 cycle of 94^0C for 30 s, 50^0C for a minute and 72^0C for a minute and 30 seconds; and a final step of termination for 10 min at 72^0C. The DNA fragment was purified to remove the PCR reagents with ethanol, and the purified fragments were amplified on 1.5 % agarose gel. The amplified PCR product sizes were assessed by comparing their mobility with the molecular weight ladder (100bp); these were evaluated together in the gel with the experimental samples.

2.5 Sequencing, Blasting of Sequences and Deposition of Sequences in Gene Bank

The amplified bacterial DNA fragments were sequenced using Applied Biosystems's Genetic Analyzer (Model 3130 x l) sequencer. The manufacturer's instructions were succinctly followed in the manual (BigDye terminator), and caution was applied were necessary to obtain accurate results. Blasting was carried out on the sequences using the National Centre for Biotechnology Information (NCBI) blast website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The bacterium with the highest homology was chosen, and the sequences were deposited in the GenBank (https://submit.ncbi.nlm.nih.gov/subs/genbank/) to obtain ascension numbers for the isolated bacteria from indoor air of primary schools.

2.6 Antibiotic Susceptibility Testing and Determination of Multiple Antibiotic Resistance Index

The antimicrobial susceptibility of the bacteria isolates was evaluated using methods delineated by the Clinical and Laboratory Standard Institute (2020). Standard antibiotic discs (Oxoid, UK) were employed for this test, and the Kirby-Bauer disc diffusion method was used to evaluate the susceptibility or resistance of the isolates to the standard antibiotics from different classes. Freshly grown bacteria were cultured on Mueller Hinton agar for 18-24 hours. The inoculum corresponding to 1.5 x 10^8 cells/ml McFarland standard was streaked on the plates before the introduction of the discs (added with extreme care) with the aid of sterile forceps. The susceptibility results were recorded after incubation for a 24 h period at 37 ^{0}C. The inhibition zones around each disc (measured using a meter rule in diameter) were assessed and interpreted based on the (2020) CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S). The antibiotic discs used in the study with their corresponding codes and concentrations include tetracycline (30µg TET), cloxacillin (5µg CXC), ceftriaxone (30µg CTR), cefuroxime (30µg CRX), erythromycin (5µg ERY), amoxicillin/clavulanic acid (30µg AUG), ofloxacin (5µg OFL), ceftazidime (30 µg CAZ), and GEN= gentamycin (10 µg GEN). The multiple antibiotic resistance (MAR) index is a tool that identifies isolates of public health

significance based on resistance to antibiotics from different classes. It also determines whether the resistance is from places of high or low-risk areas and possible interaction of microorganisms in the biosphere via the exchange of genes or plasmids, as well as the possibility of evaluating antibiotic use, misuse and abuse. This tool becomes necessary for health risk assessment; an index of ≥ 0.2 and above indicates a 'high-risk' contamination source. The multiple antibiotic resistance (MAR) index was determined for isolates using the formula (Chitanand *et al*., 2010):

$$MAR\ index = \frac{y}{nx}$$

Where y = number of resistances scored, n = number of isolates, and x = number of antibiotics.

2.7 Plasmid Isolation and Profiling

Freshly grown cells on tryptone soy broth for 24 hr were transferred into 1000 µl Eppendoff tubes and labelled appropriately. The broth was centrifuged at 14000g to obtain the pellets. 200 µl of buffer P1 was added to the pellets, then 200 µl of buffer P2 was added and mixed to obtain a clear purple and viscous cell. Then 400 µl of buffer P3 was added and mixed to obtain a supernatant, which was transferred into a zymo-spin column in a collection tube. The zymospin column collection tube was centrifuged for 30 secs, and the flow-through in the collection tube was discarded. A 200 µl of Endo-wash buffer was added to the column and centrifuged for 30 secs. A 400µl of plasmid wash buffer was added to the column and centrifuged for 1min and was transferred into a clean 1.5ml microcentrifuge tube, and 30µl of DNA elution buffer was added to the column and centrifuged for 30secs to elute the plasmid DNA. Agarose gel (0.8%) was prepared, and two drops of ethidium bromide were added and allowed to gel. Then 10µl of the samples mixed with loading dye were loaded into each well. The DNA ladder (10µl) was also loaded on the last well. The electrophoresis was allowed to run at 100v for 40min, and the bands were visualized with a UV trans-illuminator (Atker *et al*., 2021).

2.8 Determination of Resistant Genes

Three resistant genes, *tetA, tetS* and *ermB,* were evaluated for the bacteria isolates obtained from the indoor air of the public primary school using standard PCR techniques. The primers for erythromycin (*ermB*) and tetracycline (*tetA* and *tetS*) resistance were designed employing the methods of (Charpentier *et al*., 1993; Sutcliffe *et al*., 1996; Ng *et al*., 2001). The primers used for evaluating antibiotic resistance include *ermB* F-5'- GAAAAGGTACTCAACCAAATA-3', R-5'-AGTAACGGTACTTAAATTGTTTAC3' *tetA* F- 5'-GCTACATCCTGCTTGCCTTC-3' R- 3'-CATAGATCGCCGTGAAGAGG-5' *tet*-S F-5'-ATCAAGATATTAAGGAC-3' R 3'-TTCTCTATGTGGTAATC-5'

2.9 Statistical Analysis

Data obtained were analyzed using PhyloT, GraphPad Prism, Microsoft Excel and Genstat. Descriptive statistics were employed for preliminary analysis of the data obtained, and an unpaired student's *t*-test was used to evaluate differences in bacteria bioburden levels between seasons.

3. Results and Discussion

3.1 Estimation of Bacteria Bioburden and Identification of Bacteria in Indoor Air Environment

The bacteria burden obtained from the classrooms across the sampling periods is shown in Table 1. Between the dry and wet seasons of sampling, the lowest bacteria counts were observed in school B in the wet period (3.39±0.49 x 10^2 cfu/m^3), while the highest counts were obtained in school C in the dry period (12.75±1.61 x 10^2 cfu/m^3). There was no significant statistical difference ($p>0.05$) in the bacteria burden in the primary schools evaluated for dry and wet sampling seasons in all primary schools assessed in the study. The bioburden of the indoor air of the primary schools following the results mentioned above for the bacterial burden, the classrooms evaluated can be said to have an intermediate degree of contamination following the sanitary standards for non-industrial premises published in Andualem *et al*. (2019) and Ologbosere and Ogofure (2020). It was revealed that indoor bacteria burden between the range of 100 – 500 cfu/m^3 is regarded as intermediate contamination/pollution, while counts between the range of 500 – 2000 cfu/m^3 are considered high contamination/pollution. The values obtained in this study for the primary schools are in concert with several reports in the literature on the bacteriological quality of indoor air quality. Intermediate pollution levels for indoor air were reported in the findings of Shahida *et al*. (2017) and Ologbosere and Ogofure (2020). Similar results were also obtained by Fang *et al*. (2014), who evaluated the characteristics and concentration of culturable bacteria in residential homes in China and revealed that some homes had very low levels of bacterial contamination (< 50 cfu/m^3), while the majority of houses in the study had intermediate contamination level which was similar to the report obtained in this study. It is also worthy of note that for both wet and dry seasons, a particular classroom had a high level of contamination with a bacterial burden greater than 1000 cfu/m^3.

Table 1. Mean bacteria burden (x 10^2 cfu/m^3) of the different classrooms across different seasons

Primary Schools	Class	Wet period	Dry period	*p-value*
Primary School A	Pry 1	4.44±1.04	6.20±1.48	0.362
	Pry 3	3.96±0.86	5.25±1.38	0.453
	Pry 5	3.91±0.81	4.42±1.29	0.753
Primary School B	Pry 1	4.60±0.72	4.18±0.14	0.656
	Pry 3	5.14±0.82	4.18±0.73	0.414
	Pry 5	3.39±0.49	3.88±0.63	0.559
	Pry 1	10.42±2.61	12.75±1.61	0.481
Primary School C	Pry 3	6.72±0.81	11.60±2.91	0.172
	Pry 5	5.48±0.52	6.84±1.62	0.463

The molecular characterization and phylogenetic relatedness of bacterial isolates from indoor air in selected primary schools are shown in Figure 1. Twenty-one (21) bacterial isolates from six (06) genera were identified, with seven (07) bacterial isolates obtained from each primary school. The bacteria isolates obtained in this study include *Bacillus aryabhattai, Arthrobacter nicotianae, Pontibacter rhizospherae, Bacillus aereus, Exiguobacterium profundum, Bacillus cereus, Pontibacter korlensis, Vagococcus fluvialis, Arthrobacter arilaitensis, Exiguobacterium acetylicum, Alcaligenes faecalis, Bacillus stratosphericus, Bacillus subtilis,* and *Bacillus pumilus*. The identified bacteria isolates deposited in GenBank have been assigned accession numbers (MN120793, MN120794, MN120795, MN120796, MN120797, MN120798, MN120803, MN120804, MN120799, MN120806, MN120805, MN120807, MN120800, MN120801, MN120808, MN120809, MN120810, MN120811, MN120813, MN120812, and MN120802). The isolates had a percentage homology to the closest species within the

range of 98.12 to 99.85 %. The results obtained in this study were in agreement with the reports of Fang *et al*. (2014), Shahida *et al*. (2017) and Ologbosere and Ogofure (2020). These researchers isolated both Gram-positive and Gram-negative bacteria from the indoor air of residential homes and schools. The isolates obtained in the study mentioned above include *Bacillus* (mostly isolated in all studies), Arthrobacter, Pontibacter, and Exiguobacterium, which have been reported to be major contaminants of the air. The relationships of the bacteria isolates generated using PhyloT are shown in Figure 1. It was revealed that *B. subtilis, B. stratophericus, B. pumilus,* and *B. cereus* are more closely related but distantly related to *E. profundum* and *E. acetylicum*.

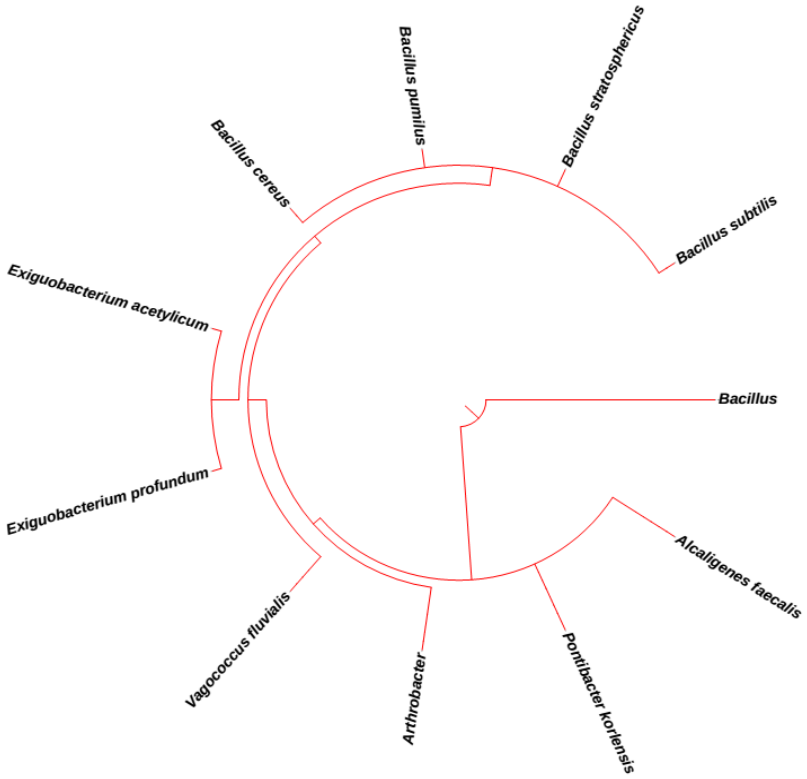

Figure 1. Phylogenetic relatedness of bacteria isolates obtained from indoor air.

3.2 Antibiotic Susceptibility and Multiple Antibiotic Resistance Index

The results of antibiotic susceptibility and multiple antibiotic resistance index of bacterial isolates from indoor air in selected Primary Schools are shown in Tables 2 and 3, respectively. *Bacillus aerius* and *Arthrobacter arilaitensis* were resistant to all antibiotics used in the study. *Pontibacter korlensis* was observed to be susceptible to tetracycline and ofloxacin antibiotics. However, most organisms were resistant to the antibiotics used in the study except for a few, such as *E. profundum*, which was susceptible to gentamicin and ceftazidime. *P. korlensis* was observed to be susceptible to tetracycline and ofloxacin antibiotics. The findings about the antibiogram of the isolated bacteria also agreed with the report of Chapin *et al*. (2004), who evaluated the multidrug resistant nature of airborne bacteria isolates from a concentrated swine feeding operation. The airborne isolates were multidrug resistant, just like the isolates obtained in this study. More so, the results by Bragoszewska and Biedron (2018) opined that multi-antibiotic-resistant bacteria of human origin were isolated from indoor air in office buildings. In this study, it was found that all identified bacterial isolates were found to be higher than the permissible limit of 0.20, with *B. aerius, A. faecalis, E. acetylicum,* and *A. arilaitensis* having multiple antibiotic resistance (MAR) indexes of 1.00.

Table 2: Antibacterial susceptibility testing of bacterial isolates from the indoor air in Public Primary Schools

Bacterial isolates (*n*)	AUG	CAZ	CRX	GEN	CTR	ERY	OFL	TET	CXC
A. faecalis (5)	2S	2S	2S	4S	2S	5R	2S	2S	5R
E. acetylicum (2)	2R	2R	2R	1R	1R	1R	1R	2R	2R
A. nicotianae (1)	1R	1R	1S	1S	1R	1R	1R	1R	1R
B. aryabhattai (1)	1R	1R	1R	1S	1R	1R	1S	1R	1S
P. rhizosphera (1)	1R	1R	1R	1S	1R	1S	1R	1R	1R
B. cereus (3)	3R	3R	3R	1S	1S	1S	3S	2S	3R
B. subtilis (1)	1R	1R	1R	1R	1S	1R	1R	1R	1R
B. *pumilus* (1)	1R	1S	1R	1R	1R	1S	1S	1R	1R
P. korlensis (1)	1R	1R	1R	1R	1R	1R	1S	1S	1R
B.stratosphericus (1)	1R	1R	1R	1R	1R	1R	1R	1S	1R
A. arilaitensis (1)	1R	1R	1R	1R	1R	1R	1R	1R	1R
V. fluvialis (1)	1R	1R	1R	1S	1R	1R	1R	1R	1R
E. profundum (1)	1R	1S	1R	1S	1R	1R	1R	1R	1R
B. aerius (1)	1R	1R	1R	1R	1R	1R	1R	1R	1R

Legend: Keys: (*n*)= number of isolates, CAZ = ceftazidime, AUG= augmentin, ERY= erythromycin, OFL= ofloxacin, GEN= gentamicin, CRX = cefuroxime, TET = tetracycline, S = susceptible, R = Resistant

Table 3: Multiple antibiotic resistance index and profile of bacterial isolates from indoor air

Isolates	Classes of Resistant Antibiotics	MAR index
A. faecalis	PEN, CEPH, AMG, TET, MAC, QUIN,	1
E. acetylicum	PEN, CEPH, AMG, TET, MAC, QUIN,	1
A. nicotianae	PEN, CEPH, TET, MAC, QUIN,	0.78
B. aryabhattai	PEN, CEPH, TET, MAC	0.66
P. rhizosphera	PEN, CEPH, TET, QUIN,	0.78
B. cereus	PEN, CEPH, AMG, TET, MAC	0.88
B. subtilis	PEN, CEPH, AMG, TET, MAC, QUIN,	0.88
B. *pumilus*	PEN, CEPH, AMG, TET	0.66
P. korlensis	PEN, CEPH, AMG, MAC	0.78
B. stratosphericus	PEN, CEPH, AMG, MAC, QUIN,	0.88
A. arilaitensis	PEN, CEPH, AMG, TET, MAC, QUIN,	1
V. fluvialis	PEN, CEPH, TET, MAC, QUIN	0.88
E. profundum	PEN, CEPH, TET, MAC, QUIN,	0.78
B. aerius	PEN, CEPH, AMG, TET, MAC, QUIN,	1

Legend: antibiotic classes and examples in parenthesis PEN - Penicillin (AUG, CXC), CEPH - Cephalosporins (CAZ, CTR, CRX), AMG - Aminoglycosides (GEN), TET - Tetracycline (TET), MAC - Macrolides (ERY), QUIN - Quinones (OFL)

Similar findings were observed in the report of Ogofure and Ologbosere (2023) who evaluated the MAR index of bacteria isolates from environmental sources and showed that they were also above the permissible limit of 0.2. Plates 1-3 show a gel picture of the presence of resistance genes in the airborne isolates. More so, the plasmid profiling of the isolates to assess the presence of one or multiple plasmids in the bacteria, which could be a factor of bacteria resistance, was also evaluated. Most of the isolates with high MAR index values had more than one plasmid. *Alcaligenes faecalis* possesses three plasmids, while *B. cereus* has one. *E. acetylicum, B. stratosphericus* and *P. korlensis* possessed one of the three evaluated resistance genes in the study.

3.3 Plasmid Profile and Antibiotic Resistant Genes

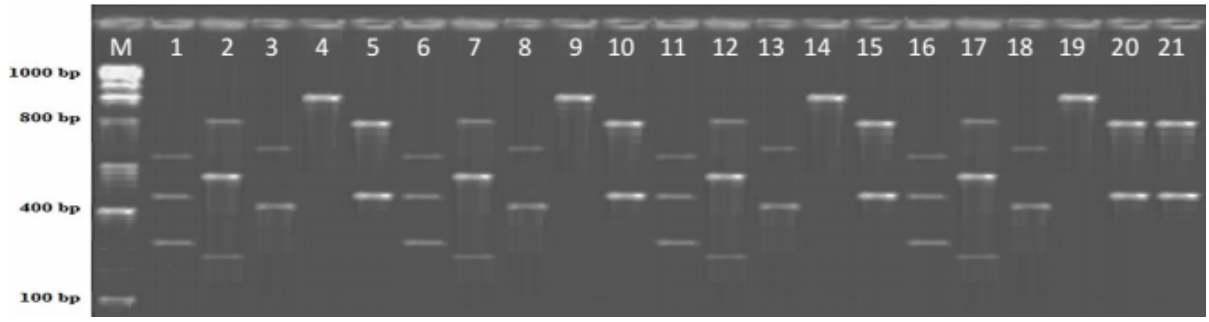

Plate 1. Plasmid profile of bacterial isolates

Lane 1	*Bacillus aryabhattai*	Lane 11	*Exiguobacterium acetylicum*
Lane 2	*Arthrobacter nicotianae*	Lane 12	*Alcaligenes faecalis*
Lane 3	*Pontibacter rhizosphera*	Lane 13	*Alcaligenes faecalis*
Lane 4	*Bacillus aerius*	Lane 14	*Alcaligenes faecalis*
Lane 5	*Exiguobacterium profundum*	Lane 15	*Alcaligenes faecalis*
Lane 6	*Bacillus cereus*	Lane 16	*Alcaligenes faecalis*
Lane 7	*Pontibacter korlensis*	Lane 17	*Bacillus stratosphericus*
Lane 8	*Vagococcus fluvialis*	Lane 18	*Exiguobacterium acetylicum*
Lane 9	*Bacillus cereus*	Lane 19	*Bacillus cereus*
Lane 10	*Arthrobacter arilaitensis*	Lane 20	*Bacillus subtilis*
		Lane 21	*Bacillus pumilus*

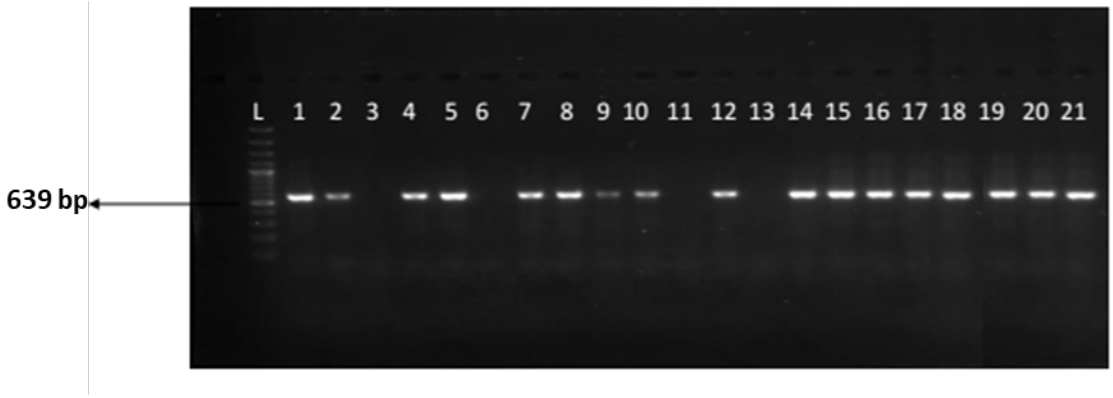

Plate 2: Agarose gel electrophoresis of the *ermB* gene PCR products amplified from 21 bacteria isolates

Lane 1	*Bacillus aryabhattai*	Lane 11	*Exiguobacterium acetylicum*
Lane 2	*Arthrobacter nicotianae*	Lane 12	*Alcaligenes faecalis*
Lane 3	*Pontibacter rhizosphere*	Lane 13	*Bacillus pumilus*
Lane 4	*Bacillus aerius*	Lane 14	*Alcaligenes faecalis*
Lane 5	*Exiguobacterium profundum*	Lane 15	*Alcaligenes faecalis*
Lane 6	*Bacillus cereus*	Lane 16	*Alcaligenes faecalis*
Lane 7	*Pontibacter korlensis*	Lane 17	*Bacillus stratosphericus*
Lane 8	*Vagococcus fluvialis*	Lane 18	*Exiguobacterium acetylicum*
Lane 9	*Bacillus cereus*	Lane 19	*Bacillus cereus*
Lane 10	*Arthrobacter arilaitensis*	Lane 20	*Bacillus subtilis*
		Lane 21	*Alcaligenes faecalis*

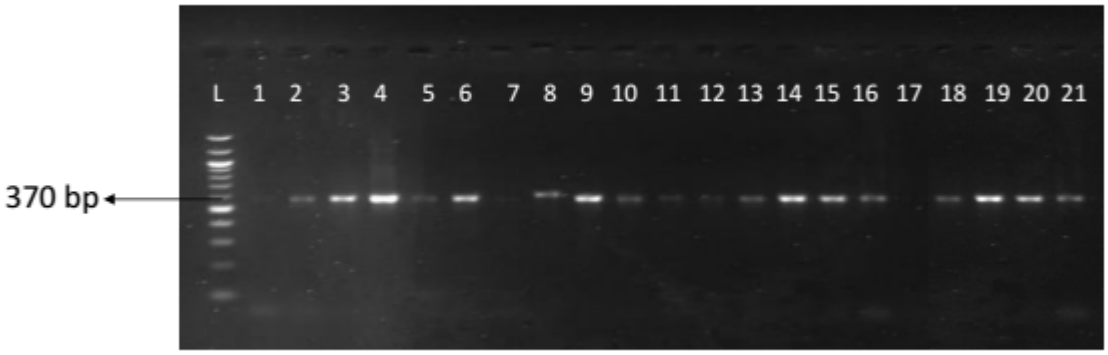

Plate 3: Agarose gel electrophoresis of the *TetS* gene PCR products amplified from 21 bacteria isolates

Lane 1	*Bacillus aryabhattai*	Lane 11	*Exiguobacterium acetylicum*
Lane 2	*Arthrobacter nicotianae*	Lane 12	*Alcaligenes faecalis*
Lane 3	*Pontibacter rhizosphere*	Lane 13	*Alcaligenes faecalis*
Lane 4	*Bacillus aerius*	Lane 14	*Alcaligenes faecalis*
Lane 5	*Exiguobacterium profundum*	Lane 15	*Alcaligenes faecalis*
Lane 6	*Bacillus cereus*	Lane 16	*Alcaligenes faecalis*
Lane 7	*Pontibacter korlensis*	Lane 17	*Bacillus stratosphericus*
Lane 8	*Vagococcus fluvialis*	Lane 18	*Exiguobacterium acetylicum*
Lane 9	*Bacillus cereus*	Lane 19	*Bacillus cereus*
Lane 10	*Arthrobacter arilaitensis*	Lane 20	*Bacillus subtilis*
		Lane 21	*Bacillus pumilus*

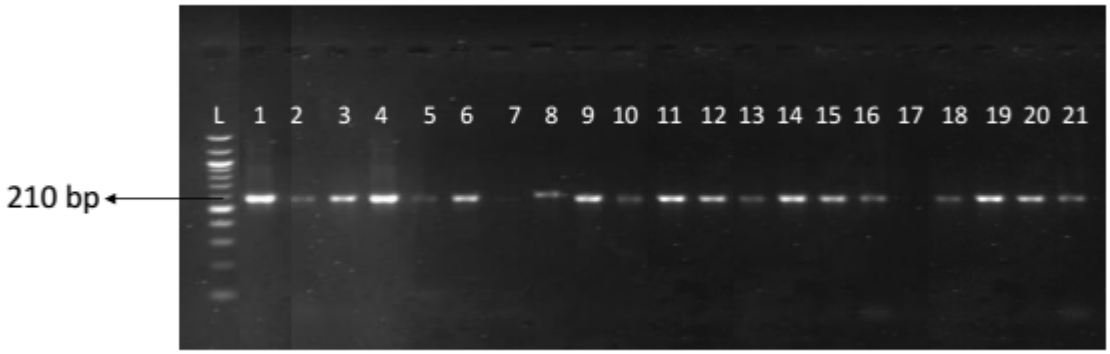

Plate 4: Agarose gel electrophoresis of the Tet*A* gene PCR products amplified from 21 bacteria isolates

Lane 1	*Bacillus aryabhattai*	Lane 11	*Exiguobacterium acetylicum*
Lane 2	*Arthrobacter nicotianae*	Lane 12	*Alcaligenes faecalis*
Lane 3	*Pontibacter rhizosphera*	Lane 13	*Alcaligenes faecalis*
Lane 4	*Bacillus aerius*	Lane 14	*Alcaligenes faecalis*
Lane 5	*Exiguobacterium profundum*	Lane 15	*Alcaligenes faecalis*
Lane 6	*Bacillus cereus*	Lane 16	*Alcaligenes faecalis*
Lane 7	*Pontibacter korlensis*	Lane 17	*Bacillus stratosphericus*
Lane 8	*Vagococcus fluvialis*	Lane 18	*Exiguobacterium acetylicum*
Lane 9	*Bacillus cereus*	Lane 19	*Bacillus cereus*
Lane 10	*Arthrobacter arilaitensis*	Lane 20	*Bacillus subtilis*
		Lane 21	*Bacillus pumilus*

Bacillus aryabhattai, Bacillus pumilus, and *Pontibacter rhizosphera* had two (*erm B* and *tet A* genes) of the three resistant genes (*erm B, tet S,* and *tet A* genes) evaluated in the study. *Arthrobacter nicotianae, Bacillus aerius, Vagococcus fluvialis* and *Bacillus subtilis* expressed all resistant genes evaluated in the study. Antibacterial resistance has been the revelation of the 20th century, which has made treatment of infection a cumbersome task and resulted or culminated in losses of billions of

dollars yearly in the United States and other parts of the world. The common antibiotics resistance evaluated in this study includes genes which code for erythromycin resistance (*erm B* gene) and tetracycline (*tet A* and *tet S* genes). The *erm* family of genes, according to Weisblum (1995), is amongst the variety of genes which code for or confer resistance to macrolide antibiotics using several mechanisms, which could be via active efflux of erythromycin, glycosylation or lactone ring cleavage by erythromycin esterase (Del-Grosso *et al*., 2007).

Table 4. Resistance genes in bacterial isolates from indoor air in selected primary school

Lanes	Molecular Identity	*ermB* gene	*tetS* gene	*tetA* gene
1	*Bacillus aryabhattai*	+	-	+
2	*Arthrobacter nicotianae*	+	+	+
3	*Pontibacter rhizosphera*	-	+	+
4	*Bacillus aerius*	+	+	+
5	*Exiguobacterium profundum*	+	-	+
6	*Bacillus cereus*	-	+	+
7	*Pontibacter korlensis*	+	-	-
8	*Vagococcus fluvialis*	+	+	+
9	*Bacillus cereus*	+	+	+
10	*Arthrobacter arilaitensis*	+	+	+
11	*Exiguobacterium acetylicum*	-	-	+
12	*Alcaligenes faecalis*	+	-	+
13	*Bacillus pumilus*	-	-	-
14	*Alcaligenes faecalis*	+	+	+
15	*Alcaligenes faecalis*	+	+	+
16	*Alcaligenes faecalis*	+	+	+
17	*Bacillus stratosphericus*	+	-	-
18	*Exiguobacterium acetylicum*	+	-	-
19	*Bacillus cereus*	+	+	+
20	*Bacillus subtilis*	+	+	+
21	*Alcaligenes faecalis*	+	+	+

Resistance to tetracycline antibiotics involves several mechanisms, which include antibiotic efflux pump, for which *tet A, tet B, tet C,* and *tet E* genes are responsible. At the same time, there is a target modification mechanism with ribosomal protection, which is coded for by *tet M, tet S, tet O* and *tet W* genes (Hedayatianfard *et al*., 2014). The majority of the isolates obtained in this study could employ more than one mechanism for antibiotic resistance as a function of their ability to express more than one gene evaluated for resistance in the study. The findings in this study were found to be consistent with the report of Hedayatianfard *et al*. (2014), who opined that *Arthrobacter arilaitensis,* amongst several other bacterial isolates from fish farms, were able to express *tet A* and *tet M* genes for antibacterial resistance.

Conclusion

The study has revealed that a plethora of bacteria isolated from the indoor air of public primary school classrooms have ARGs and are of public health importance. It further strengthens the argument that indoor air can be a reservoir of multi-resistant bacteria. It is imperative to note that with the myriads of MDR bacteria in the indoor air, measures should be put in place to ensure the safety of the pupils via cross ventilation and reduction of the number of pupils per class in addition to routine screening to evaluate the level of contamination.

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References

Akter S., Chowdhury AMMA, Mina S.A. Antibiotic Resistance and Plasmid Profiling of Escherichia coli isolated from human sewage samples. *Microbiology Insights* 14 (2021), 117863612110168. doi:10.1177/11786361211016808

Andualem Z., Gizaw Z., Bogale L., Dagne H. Indoor bacterial load and its correlation to physical indoor air quality parameters in public primary schools. *Multidisciplinary Respiratory Medicine* 14, 2 (2019). https://doi.org/10.1186/s40248-018-0167-y

Becsei Á., Solymosi N., Csabai I., Magyar D. Detection of antimicrobial resistance genes in urban air. *Microbiology Open* 10, e1248 (2021). https://doi.org/10.1002/mbo3.1248

Bragoszewska E., Biedron I. Indoor air quality and potential health risk impacts of exposure to antibiotics resistant bacteria in office rooms in Southern Poland. *International Journal of Environmental Research and Public Health* 15 (2018) 1-17.

Chapin A., Rule A., Gibson K., Buckley T., Schwab K. Airborne multidrug-resistant bacteria isolated from a concentrated swine feeding operation. *Environmental Health Perspectives* 113(2) (2004)137-142.

Charpentier E., Gerbaud G., Courvalin P. Characterization of a new class of tetracycline-resistance gene tet(S) in Listeria monocytogenes BM4210. *Gene* 131 (1993) 27–34.

Chitanand M.P., Kadam T.A., Gyananath G., Totewad N.D., Balhal D.K. Multiple antibiotic resistance indexing of coliforms to identify high-risk contamination sites in aquatic environment. *Indian Journal of Microbiology* 50 (2010) 216–220

Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing (30th edn.). CLSI supplement M100. (2020) Wayne, PA: *Clinical and Laboratory Standards Institute* 291 pp.

Del-Grosso M., Northwood J.G.E., Farrell D.J., Pantosti A. (2007). The Macrolide Resistance Genes erm(B) and mef(E) Are Carried by Tn2010 in Dual-Gene Streptococcus pneumoniae Isolates Belonging to Clonal Complex CC271. *Antimicrobial Agents and Chemotherapy* 51(11), 4184–4186

Fang Z., Gong C., Ouyang Z., Liu P., Sun L., Wang X. (2014) Characteristic and concentration distribution of culturable airborne bacteria in residential environments in Beijing, China. *Aerosol and Air Quality Research* 14, 943–953.

George P.B.L., Rossi F., St-Germain M.-W., Amato P., Badard T., Bergeron M.G., Boissinot M. (2022) Charette S.J., Coleman B.L., Corbeil J. Antimicrobial resistance in the environment: towards elucidating the roles of bioaerosols in transmission and detection of antibacterial resistance genes. *Antibiotics* 11, 974. https://doi.org/10.3390/antibiotics11070974

Hedayatianfard K., Akhlaghi M., Sharifiyazdi H. (2014) Detection of tetracycline resistance genes in bacteria isolated from fish farms using polymerase chain reaction. *Veterinary Research Forum* 5(4), 269 - 275

Murray C.J., Ikuta K.S., Sharara F., Swetschniski L., Aguilar G.R., Gray A., Han C., Bisignano P., Rao P., Wool E. Global burden of bacterial antimicrobial resistance in 2019: A systemic analysis. *Lancet* 399 (2022) 629–655.

Ng L.K., Martin I., Alfa M., Mulvey M. (2001) Multiplex PCR for the detection of tetracycline resistant genes. *Molecular and Cellular Probes* 15(4), 209-15. doi 10.1006/mcpr.2001.0363. PMID: 11513555.

Ogofure A.G., Beshiru A., Igbinosa E.O. (2022) Evaluation of different agar media for the antibiotic susceptibility testing of some selected bacterial pathogens. *University of Lagos Journal of Basic Medical Sciences* 8(2), 34-41.

Ogofure A.G., Ologbosere O.A. (2023) Microbiological and Proximate Properties of Healthy and Diseased/spoilt (Broken) Tomatoes (Lycopersicum esculentum L.) Sold in Open Markets in Benin City: Public Health Implications. *Journal of Materials and Environmental Science* 14(04), 395 – 409.

Ologbosere O.A., Ogofure A.G. Bacteriological Quality of Aerial Ambient Air in Selected Creche and Daycare Centers in Ugbowo, Benin City. *NIPES Journal of Science and Technology Research* 2(3) (2020) pp.103 -112

Ologbosere, O.A., Ukpebor E.E., Ekhaise F.O. (2021) Antibiotic Susceptibility Pattern of Airborne Bacterial Isolates in Public Schools in Benin City, Nigeria. *NISEB Journal* 21(2), http://www.ojs.klobexjournals.com/index.php/nisebj

O'Neill J. (Chair) Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. London: *Review on Antimicrobial Resistance* (2016) 88 pages.

Shahidah N., Hasnah S., Shuhaili S., Syamzany A., Mohd-Shukri M.A. Indoor airborne bacteria and fungi at different background area in nurseries and daycare centres environments. *Journal Clean WAS* 1(1) (2017) 35-38.

Singer A.C., Shaw H., Rhodes V., Hart A. (2016) Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. *Frontiers in Microbiology* 7, 1728.

Surette M., Wright G.D. (2017) Lessons from the Environmental Antibiotic Resistome. *Annual Review of Microbiology* 71, 309-329.

Sutcliffe J., Grebe T., Tait-Kamradt A., Wondrack L. (1996) Detection of erythromycin-resistance determinants by PCR. *Antimicrob. Agents Chemother.* 40, 2562–2566.

Ugbo E. N., Ugadu I. O., Ugbo A. I., Nnabugwu C. C., Okata-Nwali, D. O. (2022) Analysis of airborne bacteria in environmental exposed tiger nuts (Cyperus esculentus) sold by street vendors in Abakaliki, Nigeria. *Journal of Materials and Environmental Science* 13(03), 281-290

Vikesland P., Garner E., Gupta S., Kang S., Maile-Moskowitz A., Zhu N. I. (2019) Differential drivers of antimicrobial resistance across the world. *Accounts of Chemical Research* 52(4), 916–924. https://doi.org/10.1021/acs.accounts.8b00643

Weisblum B. (1995) Erythromycin Resistance by Ribosome Modification. *Antimicrobial Agents and Chemotherapy* 39(3),577-583.