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Assessment of Airborne Extended Spectrum β-Lactamase Producing Enterobacteriaceae Isolates from Motor Parks in Benin City, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CEO and FMA conceived, designed the experimental plan and performed the lab experiments, also wrote the article. Authors CEO, FMA, OBIU and POA collected and analyzed the data. Authors CEO and SEO contributed in reagents preparation, performed methodology and analytical tools. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was aimed at assessing airborne bacteria and extended spectrum β-lactamase producing Enterobacteriaceae isolated from the motor parks in Benin City.
Methodology: Airborne bacteria samples were collected in the motor parks (Central, Ugbowo, Agip, 19th Street, and Ekosodin Park), in Benin City using the settled plate method on Nutrient and

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MacConkey agar. Temperature, relative humidity, and airborne bacteria of the different parks were determined. The bacterial isolates were identified using phenotypic method. The antibiotic susceptibility profile was investigated by the disc diffusion method. The Enterobacteriaceae isolates were screened for Extended-spectrum β-lactamase (ESBL) production potentials. Virulence factor analysis and detection of ESBL genes (TEM, CTX-M, SHV, and OXA) were carried out on the selected ESBL producers.

Results: The relative humidity recorded at the parks at the point of agar plate exposure ranged from 82 – 90%. The airborne heterotrophic bacterial and coliform counts from the motor parks ranged from $9.50\pm0.63 \times 10^2$ – $1.18\pm0.02 \times 10^3$ cfu/m^3 and $2,48\pm0.41\times10^2$ – $3.30\pm0.41\times10^2$ cfu/m^3 respectively. The identified Enterobacteriaceae were *Citrobacter freundii, Serratia marcescens, Citrobacter koseri, Klebsiella pneumoniae, Morganella morganii, Klebsiella oxytoca, Shigella dysenteriae, Proteus mirabilis, Escherichia coli, Enterobacter cloacae* and *Citrobacter diversus*. The isolate with highest frequency of occurrence was *E. coli* with values of 30.48 %. The isolate *K. pneumoniae* had the highest MAR index of 0.8. The isolate *S. marcescens* had the highest virulent factor of 60% and possessed all the ESBL genes detected.

Conclusion: Strong infection control measures should be put in place because more ESBL-producing Enterobacteriaceae have emerged in the surroundings of specific parks in Benin City.

Keywords: Enterobacteriaceae, motor park, airborne, temperature, ESBL.

1. INTRODUCTION

Vehicular usage will continue to increase over the years due to Urbanization and transportation thereby resulting in having motor parks at different locations [1]. Motor Parks are mapped out locations within a town or city to coordinate the transportation system that involves the use of commercial vehicles for transporting individuals to their various destination either long or short journey [2]. These parks play a critical role in the management of vehicular traffic and congestion in cities. It is also used as a waiting, recreational and relaxation spot for travelers, hence, there is an in-flux and efflux of human activities. However, the activities in these motor park could cause problem the air resulting to environmental pollution. One of such environmental problems is the biological pollution. The major components of the biological pollution are the microorganisms such bacteria, viruses and fungi. These biological pollutants are released into the air of the motor parks through human and vehicular activities. These microorganisms can be airborne pathogens and when inhaled by humans may be detrimental resulting to human health risk [3].

The quality of air in the motor park environment is significant, especially concerning the health of the drivers, commuters, and people who visit the motor parks [4]. The transmission of pathogenic bacteria in motor park environments can occur through direct or indirect means. Direct transmission can happen in the motor park when an infected individual talks, coughs, or sneezes, releasing aerosols containing pathogenic bacteria into the air. These bioaerosols can be inhaled by nearby individuals, thus, leading to the transmission of infectious diseases. In motor parks, various surfaces and objects, such as door handles, seats, ticket counters, or shared equipment, can become contaminated with bacteria from infected individuals. When healthy individual touches these bacteria infested surfaces and then touch their face, mouth, or eyes, they can introduce the bacteria into their body, leading to potential infection, which are indirect means of transmission [5].

Bacteria containing airborne droplets may persist in the air for an extended period, especially if they are mixed with dust particles. The risk of inhalation of high airborne bacterial load can subsequent result to several respiratory infectious diseases [6]. Previous reports showed that there are thousands of bacteria in a liter of air especially in the motor park environment due to human activities [5,7]. Poor sanitation protocols and inadequate hygiene practices in motor parks can contribute to the spread of enteric flora and other pathogenic organisms [8].

Motor parks can become crowded and busy environments, especially when serving as waiting spots for commuters. The increased influx of people and the misuse of toilet facilities and hidden corners by drivers and commuters to ease themselves and exposed refuse bin in the park environment, can contribute to an untidy environment and create conditions that favours the spread of airborne bacteria, including Enterobacteriaceae [8,9].

Enterobacteriaceae are facultative anaerobes, non-sporing, rod shaped and Gram-negative enteric bacteria. They belong to the order Enterobacteriales, class Gammaproteobacteria, and phylum Proteobacteria based on their common genetic and phenotypic characteristics as well as evolutionary history [10]. To treat infections caused by Enterobacteriaceae, beta-lactam antibiotics like ciprofloxacin and gentamicin are frequently used. The production of beta-lactamases, inactivate beta-lactam antibiotics [11], which is the primary mechanism for beta-lactam antibiotic resistance of Enterobacteriaceae.

Antibiotic resistant bacteria include those that are resistant to different types of antibiotics have been implicated in urinary tract, the respiratory tract, wounds, and blood infections. Enterobacteriaceae that produce ESBLs have grown to be a significant global issue. Broad-spectrum antibiotics become less effective due to the spread of ESBLs, which has a considerable negative influence on patient outcomes [12]. The study was aimed at assessing airborne bacteria and extended spectrum β-lactamase producing Enterobacteriaceae isolated from the motor parks in Benin City.

2. MATERIALS AND METHODS

2.1 Sampling Area and Collection

The study was carried out in selected motor parks in Benin City. The motor Parks were Central, Ugbowo, Agip, 19th Street, and Ekosodin, all Benin City, when passengers converge to board vehicles to different locations. Sampling of these Parks was by settled plate method using nutrient agar, and MacConkey agar plates. In the designated points, each plate was positioned 1.0 m above the floor level and exposed to the air for a period of 15 minutes while the environmental parameters such as temperature, relative humidity and atmospheric pressure were measured. The samples were brought to the laboratory in a sterile container for incubation and bacteriological examination [13]. After sample collection, incubation was carried out at 37 ºC for 24-48 hrs

2.2 Enumeration and Identification of Bacterial Isolates

The incubated plates were examined with visible discrete colonies and counted. The airborne bacteria were evaluated as colony forming units per meter cube (cfu/m^3) based on the formula below [14].

$$\text{Cfu/m}^3 = \frac{5a\ x\ 10000}{bt}$$

where;

a: Number of colonies counted in the Petri dish
t: Time of exposure (15min)

b is equal to πr^2 (The radius of the petri dish used in this study was 5.07cm^2).

Colonies were randomly picked from the MacConkey agar plates, and sub-cultured on NA for purification. The bacterial isolates were stored in NA slants for further studies. The purified bacterial isolates were identified by morphology, Gram's reaction and biochemical test using the scheme in Bergey's manual of determinative bacteriology [15,16].

2.3 Detection of Virulence factor tests

2.3.1 Lipase activity

The bacterial isolates lipase activity was assayed on tryptone soy agar (TSA) plates supplemented with Tween 80 (1 %v/v). The plates of TSA were spot inoculated with the isolates and incubated at 37 ºC for 24 h. Clear halo surrounds the areas where the lipase producing organism has grown indicated the production of lipase.

2.3.2 Haemolysin production

This test determines the ability of the bacterium to produce hemolysins, toxins that can lyse red blood cells. The presence of haemolysin indicates the potential of the bacterium to damage host cells. All isolates were grown on sheep blood agar plates, which were then incubated at 30 °C for 24 h before being examined for a zone of hemolysis around colonies. According to Ryan et al. [17], the results were classified as α-haemolysis (greenish zones), β-haemolysis (clear zone), or γ-haemolysis (no haemolysis).

2.3.3 Protease activity

Protease activity of Isolates was assayed on 1% casein (v/v) supplemented TSA plates . The isolates were inoculated on 1% casein supplemented TSA plates and incubated at 37ºC for 24 – 48 h. Zone of clearance around the colonies as a result of casein hydrolysis was

considered positive result while no clearance indicated a negative result.

2.3.4 DNA degrading activity

The DNA degrading activity of isolates was tested using DNase agar plates. The isolates were inoculated on DNase agar plates and incubated at 37 °C for 24 - 48h. The degradation of DNA released methyl green that turned the medium colourless around the tested isolate. Where the DNA is not hydrolysed, the medium remains green [18].

2.3.5 Coagulase test

The enzyme Coagulase is produced by certain bacteria, such as *Staphylococcus aureus*. It promotes the formation of blood clots, which can help the bacterium evade the host's immune system and establish an infection. Plasma clothes as a result of coagulase, t changes fibrinogen into fibrin. Prepare a dilution of the plasma at a ratio of 1 in 6 in saline (0.85% NaCl), and then put 1 ml of the diluted plasma into test tubes. To create a milky suspension, several isolates colonies were emulsified into 1 ml of diluted rabbit plasma. For four hours, the test tube was incubated at 30 °C in free air, by tilting the tube, check for clot development at 1, 2, and 4 h. Overnight, the negative tubes were left at room temperature for further examination. Any size fibrin clot was classified as positive, and no clot as negative

2.3.6 Gelatinase production

The isolates gelatinase production was assayed in a nutrient gelatin medium. The gelatin medium was inoculated with the isolates on and incubated at 37°C for 24 – 48 h. Clear zones in the media signified the presence of gelatin-liquefying bacteria. No clear zone indicates a negative result [18].

2.4 Antibiotics Susceptibility Profile

The Kirby-Bauer disc (Oxoid, Basingstoke UK) diffusion method was used to test resistance and sensitivity of bacterial isolates to different antibiotics commonly employed for their eradication. Bacteria grown actively between 18 - 24 h on Mueller-Hinton agar with inoculum corresponding to 10^6 cells (McFarland's standard) was streaked on Mueller-Hinton plates using a sterile inoculating loop. Then appropriate antibiotic discs were used to impregnate the media using sterile forceps according to the method of CLSI [19]. The antibiotics used were Chloramphenicol (30µg), Septrin (30µg), Sparfloxacin (10µg), Augmentin (10µg), Ciprofloxacin (30µg), Amoxacillin (30µg), Pefloxacin (Gentamycin (30µg), 30µg), Tarivid (10µg) and Streptomycin (30µg). The inoculated plates were incubated at 37 ºC for 24 h. Clear zones of inhibition, were measured with a ruler in millimeters (mm) and compared with a standard interpretation chart. Results were interpreted as susceptible (S), intermediate (I), and resistant (R), according to the standardized CLSI (clinical laboratory standard institute) charter. CLSI [19].

The bacterial isolate Multiple antibiotic resistance (MAR) index is defined as x/y, where 'x' indicates the number of antibiotics to which the test isolate displayed resistance while 'y' indicates the number of antibiotics to which the isolate was subjected [20].

2.5 Screening and Selection of Extended–Spectrum β-lactamase (ESBL) Producing Isolates

The Enterobacteriaceae isolates were screened for ESBL production using cefotaxime (30 µg) and ceftazidime (30 µg). When the zone of inhibition is greater than or equal to 27 mm with cefotaxime (30 µg) and greater than or equal to 22 mm with ceftazidime (30 µg), the isolates were considered as potential ESBL producers [19,20].

The potential ESBL producing isolates were selected for ESBL production by using ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) discs and cefotaxime (30 µg) and cefotaxime/clavulanic acid discs (30/10 µg) at appropriate distances on a Mueller-Hinton agar (MHA) plate. A difference in the zone of inhibition zone ≥ 5 mm for a combination disc (ceftazidime + clavulanic acid or cefotaxime + clavulanic acid) compared with ceftazidime or cefotaxime disc alone was confirmed as positive for ESBL production [19-21].

2.6 DNA Extraction for the PCR Analysis of TEM, SHV, CTX-M, and OXA Gene

The bacterial DNA extraction was carried out using a combination of lysis, filtration, and purification steps according to the method of Ahmed et al. [22].

Detection of ESBLs gene was carried out on the bacterial isolates by PCR amplification and gel electrophoresis methods. The genes and their primers were TEM (F: TGCAACAGTGCCTCTC GATA, R: CTCGTGCACCCAACTGATCT), CTX-M (F: CGCTGTTGTTAGGAAGTGTG, R: GGCTG GGTGAAGTAAGTGAC),SHV (F: GGTTATGCGT TATATTCGCC, R: GGTTAGCGTTGCCAGTGCT C), OXA (F: ATATCTCTA CTGTTGCATCTCC, R: AAACCCTTCAAACCATCC) [23-26].

Combine a 2.5 µl of 10x PCR buffer, 1 µl of 25mM $MgCl_2$, 1 µl of each forward and reverse primer, 1 µl of DMSO, 2 µl of 2.5mM dNTPs, 0.1 µl of 5 u/µl Taq DNA polymerase, 3 µl of 10 ng/µl DNA and 13.4 µl of nuclease-free water in a sterile microcentrifuge tube. Mix the contents thoroughly by briefly centrifuging the tube. The resulting PCR cocktail mix was ready for use in the PCR reaction. 36 cycles of initial denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and elongation at 72 °C for 45 seconds were performed after the first denaturation at 94 °C for 5 minutes. After which the temperature was maintained at 10 °C indefinitely after a last elongation step at 72 °C for 7 minutes. On 1.5 % agarose electrophoresis gels stained with ethidium bromide, amplified fragments were seen.

2.7 Agarose gel Electrophoresis

Agarose gel electrophoresis is a technique used for separating DNA fragments based on their molecular weight. Agarose powder was dissolve in 1X TBE buffer by boiling the mixture while stirring. After cooling slightly, ethidium bromide was added (a fluorescent dye used to visualize DNA bands). The agarose mixture was poured into the electrophoresis tank, a comb was placed in the gel to create wells for loading the DNA samples, and the gel was allowed to solidify. The plasmid DNA sample was mixed with loading dye, which helps to visualize the migration during electrophoresis. The DNA samples were loaded into the wells created in the gel using a micropipette (care was taken not to introduce any air bubbles). The electrode wires were connected to the power pack, ensuring that the negative terminal was connected to the end where the samples were loaded. The power pack was turned on, and the voltage was set to 60-100V. The electrophoresis was allowed to run until the desired migration was achieved. The gel was removed from the tank and place on a UV transilluminator. The DNA bands appear as bright fluorescent bands. Image of the gel was captured using BioRad Mini-Sub Get GT system. The migration distance of the DNA marker bands was used to estimate the molecular weight of the amplified genes by comparing it to the molecular ladder [27].

3. RESULTS AND DISCUSSION

3.1 Temperature, Relative Humidity and Pressure Obtained at the Parks

The temperature (^oC), relative humidity and atmospheric pressure readings at the various parks during plate exposure are shown in Table 1. The highest temperature value was recorded in Central park while the lowest was from Ugbowo Motor Park with values 29.8 and 25.9 ^oC respectively. Ugbowo motor park had the highest relative humidity of 90 % while Central park recorded the lowest relative humidity of 70 %. Ugbowo and Agip parks recorded the highest values of 1014 N/m^2, while Ugbowo, Agip, 19th street and Ekosodin parks respectively recorded the lowest values of 1008 N/m^2.

Survival of bacteria cells in the air environment and become viable is dependent on the environmental factors such as temperature, relative humidity and pressure [28]. The temperature and relative humidity from the different locations were not significantly different ($p > 0.05$). Tang [29] reported that temperatures above 24 ^oC decrease the survival rate of airborne bacterial. High temperatures of the environment increase inactivation bacteria cell, mainly through desiccation and protein denaturation of protein, while lower temperatures increase survival times [30]. The temperature effect is closely linked with relative humidity. The survival of most microbes is depended on relative humidity range of 55–75 % [31].

3.2 Bacteria Enumeration from the Parks

The heterotrophic bacterial counts on nutrient agar plates and coliform bacterial counts on MacConkey agar plates at the various parks are shown in Table 2. The highest bacterial load of $1.40 \pm 0.04 \times 10^3 cfu/m^3$ was recorded at Ekosodin motor park, while the lowest value of $8.40 \pm 0.63 \times 10^2 cfu/m^3$, was recorded at Ugbowo motor park. The highest coliform load value of $4.96 \pm 0.41 \times 10^2 cfu/m^3$ was recorded at Ekosodin park, while Ugbowo motor park recorded the lowest value of $1.93 \pm 0.48 \times 10^2 cfu/m^3$.

Table 1. Temperature (°C), Relative humidity (%) and atmospheric pressure (N/m^2) of the parks recorded at the point of agar plate exposure

Park	Temperature (°C)	Relative humidity (%)	Atmospheric pressure (N/m^2)
Central	26.9	82	1013
Ugbowo	25.9	90	1014
Agip	28.9	83	1014
19th Street	28.8	85	1012
Ekosodin	27.9	88	1009

Table 2. Heterotrophic bacterial and coliform counts (cfu/m^3) at the motor parks

Park	Heterotrophic bacteria	Coliform bacteria
Central	$1.18\pm0.02\times10^3$	$2.75\pm0.24\times10^2$
Ugbowo	$9.50\pm0.41\times10^2$	$2.48\pm0.41\times10^2$
Agip	$1.05\pm0.02\times10^3$	$3.17\pm0.24\times10^2$
19th Street	$1.06\pm0.02\times10^3$	$3.30\pm0.41\times10^2$
Ekosodin	$1.24\pm0.04\times10^3$	$4.96\pm0.41\times10^2$

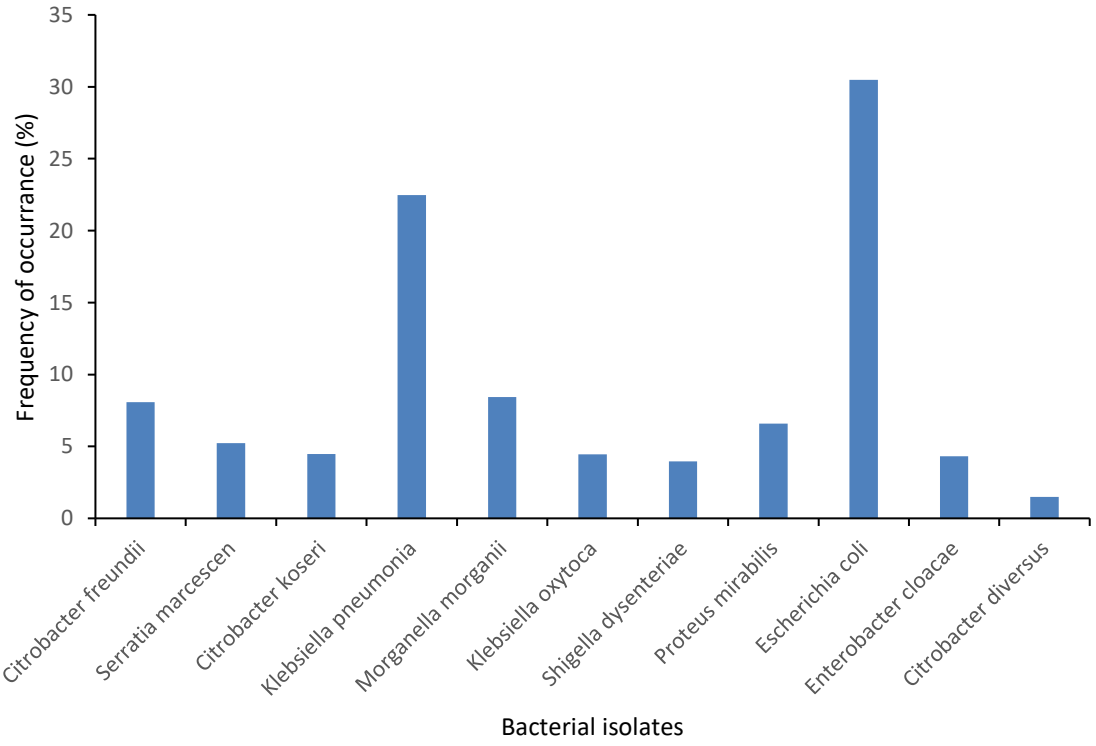

Fig. 1. Identified Enterobacteriaceae isolates from MacConkey agar and their % frequency of occurrence

3.3 Identification and Frequency of Occurrence of Enterobacteriaceae Isolates

Identification of Enterobacteriaceae isolates from macconky agar using cultural, morphological and biochemical characterization and frequency of occurrence of isolates is shown in Fig. 1. The identified isolates were *Citrobacter freundii*, *Serratia marcescens*, *Citrobacter koseri*, *Klebsiella pneumoniae*, *Morganella morganii*, *Klebsiella oxytoca*, *Shigella dysenteriae*, *Proteus mirabilis*, *Escherichia coli*, *Enterobacter cloacae* and *Citrobacter diversus*. The isolates with the highest and lowest frequency of occurrence were

Escherichia coli and *Citrobacter diversus* with values 30.48 and 1.49 % respectively.

The high bacterial counts recorded in the various motor parks may be due to its busy nature in all the location. The bacterial counts from this exceeds the permissible limit of 10^3 cfu/m^3 as recommended by WHO health risks of biological agents in an environment [32]. The highest count was observed in Ekosodin motor park. Statistically, there was no significant difference in the bacterial load obtained from the motor parks ($p< 0.05$). The variation of airborne bacterial load in locations might be due to environmental factors such as temperature, humidity, and particulate matter concentration [14]. In addition, human and vehicular activities could also contribute to bacterial counts [6].

The identified Enterobacteriaceae isolates were *Citrobacter freundii*, *Serratia marcescens*, *Citrobacter koseri*, *Klebsiella pneumoniae*, *Morganella morganii*, *Klebsiella oxytoca*, *Shigella dysenteriae*, *Proteus mirabilis*, *Escherichia coli*, *Enterobacter cloacae* and *Citrobacter diversus*. The frequency of occurrence of Enterobacteriaceae isolates in this study showed that *Escherichia coli* 30.48 % and *Klebsiella pneumoniae* 21.4 % were the most occurring, which was similar to the report of Mulisa *et al.* [33]. *Citrobacter diversus* 1.49 % showed the lowest frequency of occurrence.

The prevalence of isolated bacteria highlighted the fact that these isolates are ubiquitous. These genera of bacteria have been indicated to be some of the common bacteria genera isolated from air. Previous studies suggested that though these genera are predominantly soil flora, human and vehicular activities play a dominating role in the dissemination of airborne bacteria within the confered environment [6,34]. Ohahim et al. [8] also reported that these environmental bacteria found in the motor parks are most likely transported through wind as bioaerosols. The predominant isolate *E. coli*, in part agreed with the report of Dick and Wekhe [3] as one of the common bacteria isolated from airflora of a secondary school. The presence of *E. coli* suggested contamination by animal fecal matter or polluted water within the surrounding [35]. This isolate is known to cause urinary tract infection and diarrhoea in humans [36]. The wide range of Enterobacteriaceae isolated from the park is not surprising due to the fact that the locations may be unclean where wind and traffic can easily raised some dust particles that will sustain the organisms in the air [37].

3.4 Antibiotics Resistance

The results of the antibiotics susceptibility profile carried out on Enterobacteriaceae isolates (Table 3) showed that all the isolates were sensitive to the antibiotic Chloramphenicol (CH) while all the isolates were resistance to Amoxacillin (AM) expect *K. oxytoca* and *C. diversus*. The most sensitive isolates was *C. diversus*. The multiple antibiotics resistance (MAR) index of the isolates showed that *K. pneumoniae* had the highest MAR index of 0.8 while *C. diversus* had the lowest of 0.1.

From the result, 7 of the isolates were resistant to at least six (5) of the antibiotics, revealing that Enterobacteriaceae, Gram negative bacteria, showed more resistant to the tested antibiotics. This may be as a result of their unique outer membrane that prevent some antibiotics gaining access into the cell. Also, Gram negative bacteria have the ability to exchange genetic material among species with same strain or different species [38]. Most of the Enterobacteriaceae had a MAR index score greater 0.3, suggesting high resistance to all the tested antibiotics. The MAR index values reported in the study showed an indication that there is an abused antibiotics in these locations due to indiscrimate use of the drugs [20,39]. Factors responsible for the resistance could be, drug misuse, wrong antibiotic treatment, and prolong use of antimicrobial agents thus, increasing the rate of drug-resistant bacteria [40].

3.5 Screening and Selection of ESBL Producing Enterobacteriaceae

A difference in the inhibition zone of diameter greater than 5 mm for a combination disc (ceftazidime + clavulanic acid or cefotaxime + clavulanic acid) compared with ceftazidime or cefotaxime disc alone was confirmed as ESBL production (Table 4). Six Enterobacteriaceae namely *Citrobacter freundii*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, and *Escherichia coli* were selected for further studies.

3.6 Virulence Factor

The virulence factor (pathogenicity) analysis carried on ESBL producers is shown in Table 5.

Table 3. Antibiotic susceptibility profile of Enterobacteriaceae isolates

Isolate	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	MAR
Citrobacter freundii	S	S	R	S	R	R	S	R	R	S	0.5
Serratia marcescens	R	S	R	S	R	R	S	R	R	S	0.6
Citrobacter koseri	S	S	S	S	R	R	S	S	S	S	0.2
Klebsiella pneumoniae	R	S	R	R	R	R	R	R	R	S	0.8
Morganella morganii	S	S	R	R	R	R	S	R	R	S	0.6
Klebsiella oxytoca	R	S	S	S	R	S	S	S	S	S	0.2
Shigella dysenteriae	S	S	S	S	S	R	S	R	R	S	0.3
Proteus mirabilis	R	S	R	R	R	R	S	S	R	S	0.6
Escherichia coli	R	S	R	S	R	R	S	R	S	R	0.6
Enterobacter cloacae	S	S	R	R	R	R	S	R	S	S	0.5
Citrobacter diversus	S	S	S	S	S	S	S	S	S	R	0.1

SXT – Septrin (30µg); CH – Chloranphenicol (30µg); SP – Sparfloxacin (10µg); CPX - Ciprofloxacin (30µg); AM – Amoxacillin (30µg); AU – Augmentin (10µg); CN – Gentamycin (30µg); PEF – Pefloxacin (30µg); OFX – Tarivid (10µg); S – Streptomycin (30µg).

Table 4. Screening of ESBL producing Enterobacteriaceae

Isolate	ceftazidime (30 µg)	cefotaxime (30 µg)	ceftazidime (30 µg) + Clavulanate (30 µg/ 10 µg)	cefotaxime (30 µg) + clavulanate (30 µg/ 10 µg)
Citrobacter freundii	10.50	10.60	17.40	17.20
Serratia marcescens	9.10	9.90	17.90	18.10
Citrobacter koseri	10.70	10.90	15.10	15.00
Klebsiella pneumoniae	8.50	8.30	17.20	17.00
Morganella morganii	9.10	8.80	17.00	17.10
Klebsiella oxytoca	22.80	25.20	ND	ND
Shigella dysenteriae	24.00	27.50	ND	ND
Proteus mirabilis	10.20	10.40	17.60	17.50
Escherichia coli	9.20	8.70	17.50	17.30
Enterococcus cloacae	10.80	10.60	15.70	15.30
Citrobacter diversus	22.50	25.80	ND	ND

ND = Not Defined

Table 5. Virulence factor analysis of ESBL positive bacterial isolates

Isolate	DNAse	Coagulase	Gelatin	Lipase	Haemolysis	Percentage %
Citrobacter freundii	-	-	-	-	-	0%
Serratia marcescens	+	-	+	+	-	60%
Klebsiella pneumoniae	-	-	-	-	-	0%
Morganella morganii	-	-	-	-	+	20%
Proteus mirabilis	+	-	+	+	-	60%
Escherichia coli	-	-	-	-	-	0%

This reveals the presence of phenotypic factors that aid in pathogenicity of the isolates. The isolates *Serratia marcescens* and *Proteus mirabilis* have the highest virulent factors of 60%.

Some of the factors such haemolysis, protease lipase and DNase have been regarded as criteria for pathogenicity in Enterobacteriaceae [18]. Studies of the virulence factor (pathogenicity) carried out showed that *Proteus mirabilis* (60%), *Serratia marcescens* (60%), are more likely to cause infection in immunocompromised individuals and could pose a threat to the quality of air at the motor park. *Morganella morganii* (20%), *Citrobacter freundii* (0%), *Klebsiella pneumoniae* (0%), and *Escherichia coli* (0%) showed less virulence.

3.6 ESBL Genes of the ESBL Producers

The electrophoresis of different DNA fragments of the ESBL (Fig. 2) positive isolates were amplified base on their size and charge,

enables for the typing of the ESBL producers, which were genetically linked to resistant gene(s) production. The TEM genes expressed amplified DNA products at 717bp (Fig. 2A), CTX-M genes expressed amplified DNA products at 569bp (Fig. 2B), SHV genes at 867bp (Fig. 2C), and OXA genes at 619bp (Fig. 2D) respectively. *Klebsiella pneumoniae* expressed 100% of all the genes (TEM, CTX-M, SHV, and OXA), while *Proteus mirabilis* showed 50 % (TEM, and CTX-M) of the genes is shown in Table 6.

Table 6. Summary of ESBL gene production by ESBL positive bacterial isolates

	Isolate	ESBL gene				
		TEM	CTX-M	SHV	OXA	%
1	*Citrobacter freundii*	+	+	+	–	75
2	*Serratia marcescens*	+	+	+	+	100
3	*Klebsiella pneumoniae*	+	+	+	–	75
4	*Morganella morganii*	+	+	+	–	75
5	*Proteus mirabilis*	+	+	–	–	50
6	*Escherichia coli*	+	+	+	–	75

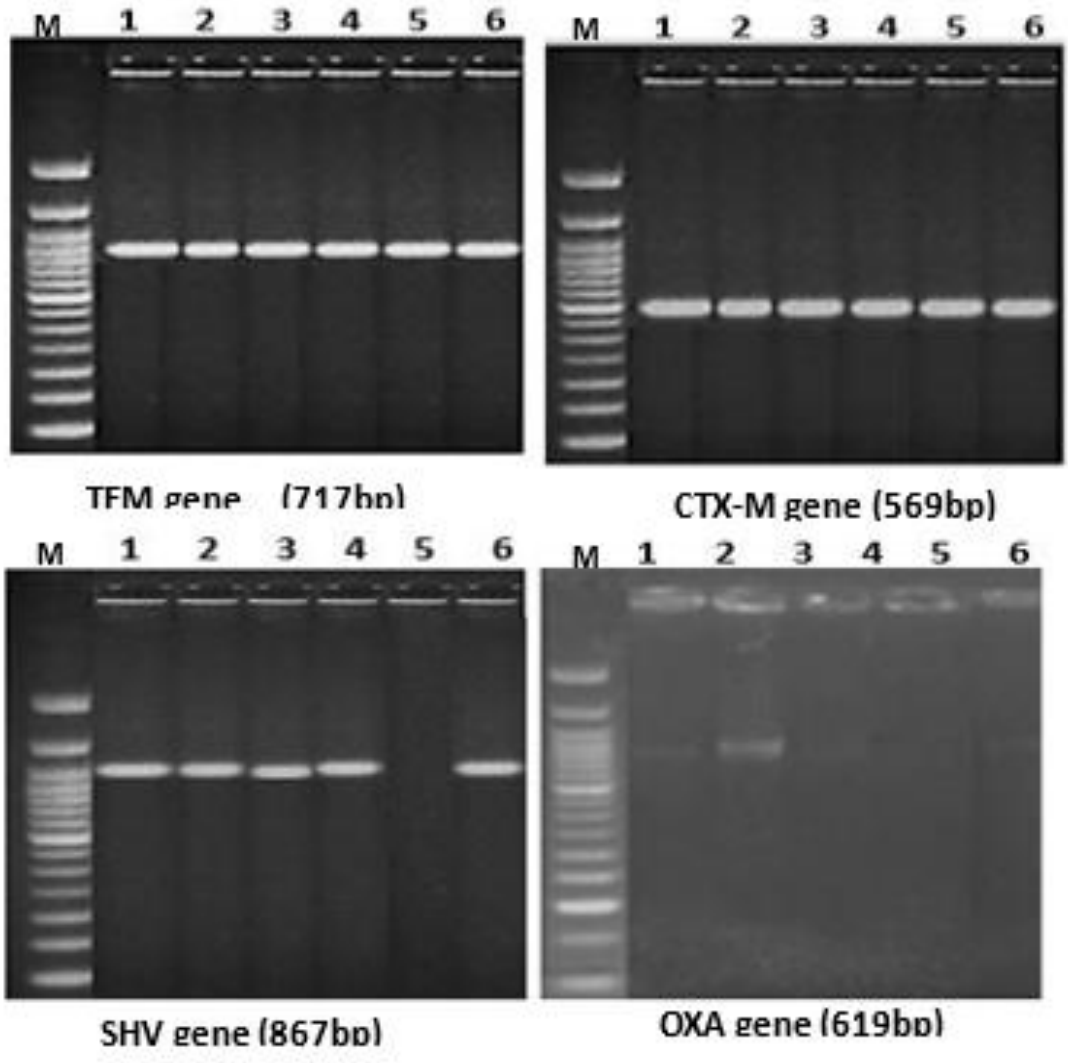

Fig. 2. ESBL genes of selected Enterobacteriaceae from various motor parks

Key:M = molecular marker. Lane 1 = C. freundii, Lane 2 = S. marcescens, Lane 3 = K. pneumonia. Lane 4 = M. morganii, Lane 5 = Proteus mirabilis and 6 = Escherichia coli

The resistance observed in ESBL producing Enterobacterioceae in this study, could be as a result of the bacterial isolates coming from source(s) where people are exposed to enteric flora in places with poor sanitation, or high rate of indiscriminate and widespread antibiotics usage, in accordance with report of Ogbolu et al., [41].

The ESBL genes TEM, CTX-M, SHV and OXA are genes coding for resistance of β-lactam antibiotics. The resistant genes TEM and CTX-M were detected in all the enterobacteriaceae isolates with potential to produce ESBL. The two gene were reported by Liu et al [35] as most predominant ESBL genes in retailed food samples in China. It was observed that *Serratia marcescens* contains all the ESBL genes (TEM, CTX-M, SHV and OXA), while *Proteus mirabilis* showed the absence of the SHV gene. The findings were similar to the investigation of Daam et al [26] who reported CTX-M as the common resistant gene among ESBL-producing Enterobacteriaceae. Variation of ESBL resistant genes distribution among the isolates suggests the need for surveillance to know the antimicrobial therapy of choice. These bacteria resistant genes are known to be plasmid-mediated and can easily be transferred among the isolates thus pose a serious health risk [27,42-45].

4. CONCLUSION

The findings of this study revealed presence of Enterobacteriaceae, in motor parks where the count was more than WHO standard values. This indicates that these contaminated air samples serve as potential reservoirs for pathogens. There is a significant risk of contracting infections through inhalation of contaminated air if proper hygiene measures are put in place. The results also showed that some of the isolates from the motor parks harboured beta lactamase genes posing a great public health concerns.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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