

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/369374179>

# Profiling and antibiotic resistance of some human intestinal isolates

Article in *World Journal of Advanced Research and Reviews* · March 2023

DOI: 10.30574/wjarr.2023.17.3.0411

CITATIONS

0

READS

52

2 authors:



**Adetoyosi Daniels**  
Achievers University

31 PUBLICATIONS 32 CITATIONS

SEE PROFILE



**David Adedotun Ibiyemi**  
Achievers University

11 PUBLICATIONS 13 CITATIONS

SEE PROFILE

## Profiling and antibiotic resistance of some human intestinal isolates

Adetoyosi Olutosin Daniels <sup>1,\*</sup> and David Adedotun Ibiyemi <sup>2</sup>

<sup>1</sup> Associate Professor, Department of Biological Sciences, Achievers University, Owo, Ondo State, Nigeria.

<sup>2</sup> Senior Lecturer, Department of Chemical Sciences, Achievers University, Owo, Ondo State, Nigeria.

World Journal of Advanced Research and Reviews, 2023, 17(03), 520–532

Publication history: Received on 26 January 2023; revised on 10 March 2023; accepted on 13 March 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.17.3.0411>

### Abstract

The cases of antibiotic resistance in Enterobacteriaceae are of great global concern. This study aimed at contributing towards the fight against antibiotic resistance and ameliorate the management/treatment of Enterobacteriaceae-linked diseases.

Ten (10) rectal swab samples were collected from five (5) male and five (5) females and two isolates were identified from the samples collected. The isolates were identified through colonial, morphological and biochemical tests carried out following standard procedures. Isolates were investigated for their antibiotic resistance profile, Multiple Antibiotic Resistance indices (MARi), pathogenicity and their resistance genes were identified through molecular means using plasmid amplification and primers. Primers used include. *ermB*, *BlaTem*, *qnrB* genes

Result obtained showed the isolates to be *Escherichia coli* and *Klebsiella oxytoca*. *E. coli* showed Alpha ( $\alpha$ ) hemolysis, while *Klebsiella oxytoca* showed gamma ( $\gamma$ ) hemolysis. *E. coli* was resistant to 75% of the antibiotics used, *Klebsiella oxytoca* was sensitive to 42% of all the antibiotics. All the test organisms were resistant to all classes of Cephalosporins. The plasmid profiling revealed that all isolates have low molecular weight plasmids. The molecular fingerprinting of the isolates using gene primers viz -a viz *ermB*, *BlaTem*, *qnrB* genes showed *E. coli* to have resistance genes for macrolides (*ermB* gene) while none of the isolates had resistance factor against quinolones (*qnrB* gene). This study showed a high carriage of Enterobacteriaceae having phenotypic resistance with corresponding plasmid-borne resistance genes.

The need to understand how bacteria adapt to the antibiotic environment will lead to new therapeutic strategies for antibiotic-resistant infections. Interventions measures to minimize the abundance of antibiotic-resistant commensals and opportunistic pathogens may include faecal microbiota transplantation and the use of live biotherapeutics.

**Keywords:** Rectal swab; Resistance genes; Primers; Plasmids; *ErmB*; *BlaTem*; *QnrB* Hemolysis; Multiple antibiotic resistance index.

### 1. Introduction

The development of antibiotics in the 20<sup>th</sup> century is regarded as a major breakthrough, (Bud, 2007). Worldwide antibiotic use increased by 65% between 2000 and 2015 (Klein *et al.*, 2018), However, the long- or short-term use of antibiotics can have significant effect on the gut microbiome (Dethlefsen and Relman, 2011). The microflora of the intestinal microenvironment as a unit has important protective, metabolic, and trophic functions. Resident bacteria serve a central line of resistance to colonization by exogenous microbes and thus assist in preventing the potential invasion of the intestinal mucosa by an incoming pathogen (Canny and McCormick, 2008).

\* Corresponding author: Daniels AO

Human intestinal bacteria, apart from the beneficial activity, play a role as traffickers in antibiotic resistance genes. Intestinal bacteria not only exchange resistance genes among themselves but might also interact with bacteria that are passing through the colon, causing these bacteria to acquire and transmit antibiotic resistance genes (Salyers *et al.*, 2004). Antibiotic resistance of bacteria is a cause for concern. The World health organization (2018) predicted that antibiotic resistance will likely create a public health emergency if nothing is done.

Knowledge of the gut microbiome, an extraordinarily complex community of organisms, has improved dramatically since the introduction of metagenomics, however, inappropriate usage of antimicrobials has transformed the human healthy intestinal gut flora into a reservoir of antibiotic-resistant organisms, also called the gut resistome (Van Schaik, 2015). This problem is intensified by increasing prevalence of resistant bacteria in the community leading to the increase in the risk of cross-transmission (van Duin and Paterson, 2016).

The human gut is full of diverse bacteria, many of which harbor resistance against a broad range of antibiotics such as vancomycin-resistant Enterococci and carbapenem-resistant Enterobacteriaceae. Humans acquire AMR organisms and antibiotic-resistance genes (ARGs) from, and can spread them to other reservoirs, such as animals. Thus, the gut resistome—the collection of ARGs harbored by the microbiota—is an important piece of the AMR puzzle (Barron, 2022).

It is on the basis that gut microbiome can acquire resistance to antibiotics from continuous exposure to antibiotics and also from the environment that this research work was carried out.

---

## 2. Material and methods

### 2.1. Sampling

Human anal samples (10) were collected from both male and female human using sterile swabs. The volunteer's consents were sought before they were given the swab sticks. The swab tubes were labelled with assigned code for individual (A,- J). The inoculated swab sticks were collected and transferred into the laboratory and kept under refrigeration until use.

### 2.2. Media used

All media (McConkey, Eosin-Methylene Blue agar, Thiosulphate-citrate-Bile Salts Sucrose agar, SSA, De Man Rogosa and Sharpe agar and Nutrient Agar) were prepared according to the manufacturer's specification.

### 2.3. Isolation and Swab Culture

Each swab stick was dipped into test tube containing peptone water and shaken gently to dislodge the microbial colonies on it into the peptone water which serves as the stock for serial dilution. A 1 ml aliquot of the stock was pipetted into a second test tube containing 9ml of peptone water. A 1ml aliquot was pipetted from the second test tube into a third one containing 9ml of peptone water. This procedure was repeated until a dilution of  $10^{-6}$  was attained. This dilution ( $10^{-6}$ ) was used to inoculate already prepared agar using the spread plate method.

### 2.4. Preparation of Isolates

A 1ml aliquot of the stock culture was pipetted into agar plates containing different media. An L-shaped glass spreader was dipped into alcohol. The glass spreader (hockey stick) was passed over a Bunsen burner to sterilize. The sample was spread evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at the same time. After spreading, the plates were allowed to stand for a few minutes for the surface to dry. The inoculated plates were then Incubates 37°C for 24 hours.

### 2.5. Preparation of pure culture

Discrete colonies were picked and used to streak already gelled agar plates and incubated at 37°C for 24hr after which the pure cultures were subjected to colonial, morphological and biochemical characterization.

### 2.6. Gram Staining

The isolates were subjected to Gram staining following the laid down procedures of Cheesebrough, (2000).

## 2.7. Biochemical Tests

The isolates were subjected to the following biochemical tests following Standard methods. Sugar fermentation, Citrate utilization Test, Oxidase Test, Indole Test, Urease Test, Methyl red Test & Voges Proskauer Test (Tankeshwa, 2022).

## 2.8. Pathogenicity Test

Blood agar was prepared by weighing blood agar powder and dispensed into a conical flask. 100ml of distilled water was added and shaken thoroughly. The medium was then sterilized in an autoclave. Afterwards, the medium was poured into plates and allowed to gel. The plates were then inoculated with the isolate by streaking method and incubated at 37°C for 24hrs. The pathogenicity test was confirmed by determining  $\alpha$  or  $\beta$  hemolytic zone of the isolates on the plate. i.e. ability of the isolates to lyse red blood cells.

## 2.9. Antibiotic Sensitivity test

Identified isolates were tested against standard antibiotics using the method described by Kirby – Bauer (1996) pour plate method, after which the rings of standard antibiotics was placed on the surface of gelled agar. The antibiotic ring must touch the surface of the agar plate properly and allow the inoculate plate to incubate at 37°C for 24 hrs. Antibiotics used include; Amoxicillin (30  $\mu$ g) Cefotaxime (30 $\mu$ g), Ceftriaxone (45  $\mu$ g), Cefexime (25  $\mu$ g), Levofloxacin (5  $\mu$ g), Ciprofloxacin (45  $\mu$ g) Imipenem (10  $\mu$ g), Cefuroxime (25  $\mu$ g), Ofloxacin (5  $\mu$ g), Erythromycin (15  $\mu$ g) Gentamycin (10  $\mu$ g), Azithromycin (15  $\mu$ g). Augmentin (30  $\mu$ g), Nitrofurantoin (300  $\mu$ g),

## 2.10. Multiple antibiotics resistance index

The multiple antibiotics resistance index for the resistant bacteria isolates was determined according to the procedure described by Krumpman (1983). This is essentially to determine the degree of bacteria resistance to antibiotics. These indices will be determined by dividing the numbers of antibiotics to which the organisms were resistant to (a) number of antibiotics tested (b) resistance to two or more antibiotics is taken as multiple antibiotics resistance MAR greater than 0.2 shows high antibiotic resistance index.

## 2.11. Plasmid Profiling of Isolates

The antibiotic resistant isolates were subjected to plasmid profiling to determine if the resistance factors are plasmid mediated.

### 2.11.1. Plasmid Isolation

Plasmids were isolated using the QIAGEN Plasmid Purification mini kit. Gel Integrity. The integrity of the extracted plasmid was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3 $\mu$ l of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 10 $\mu$ l of each PCR product and loaded into the wells after the 100bp-10kbp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the plasmid were estimated by comparison with the mobility of the molecular weight ladder that was ran alongside experimental samples in the gel. Extracted plasmid was then used as the template for PCR amplification.

## 2.12. Molecular identification

Primer sequences were as earlier documented by (Barghouthi, 2011). Reaction cocktail used for all PCR per primer set included (Reagent Volume  $\mu$ l) - 5X PCR SYBR green buffer (2.5), MgCl<sub>2</sub> (0.75), 10pM DNTP (0.25), 10pM of each forward and backwards primer (0.25), 8000U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2  $\mu$ l template was added. Buffer control was also added to eliminate any probability of false amplification Table below shows the primer sequence and PCR profile used in amplifying each fragment. PCR was carried out in a Gene Amp 9700 PCR System Thermal cyler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

### 3. Results and discussion

#### 3.1. Morphological and biochemical characterization

The isolates were identified based on Gram status and their reaction to some biochemical tests. Table 3.1 showed the different isolate identified based on their biochemical properties and confirmed using the Bergey's manual of determinative bacteriology.

Isolate 1 was a Gram-negative short rod that was indole positive, citrate negative, oxidase negative methyl red positive VP negative and urease negative. The organism was able to ferment glucose, fructose galactose and lactose with evidence of gas and acid production. The suspected organism was *Escherichia coli*.

Isolate 2 was a Gram-negative rod, indole positive, citrate positive, oxidase negative, methyl red negative VP positive, urease positive and catalase positive. The organism was able to utilize glucose, sucrose, galactose, fructose and lactose. The suspected organism was *Klebsiella oxytoca*.

#### 3.2. Pathogenicity test

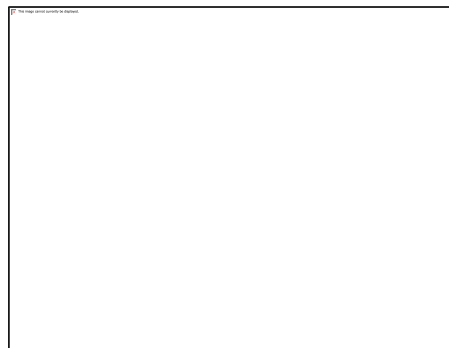
The pathogenicity test result showed the varying degree of blood hemolysis (Fig 1 and 2). *E. coli* showed alpha hemolysis signifying partial hemolysis where the organism can partially breakdown red blood cells. *Klebsiella oxytoca* showed gamma hemolysis showing that there was no hemolysis meaning the two organisms could not break down red blood cells. An  $\alpha$ -hemolytic reaction occurs when the hemoglobin in the red blood cells is reduced to methemoglobin, causing a greenish discoloration on the agar surrounding the colonies and the absence of hemolysis or discoloration is referred to as  $\gamma$ -hemolysis.

#### 3.3. Antibiotic susceptibility

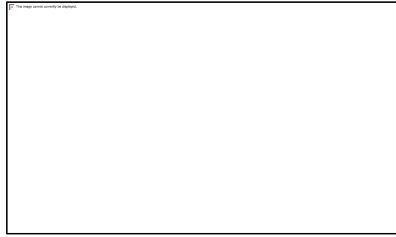
The result of the antibiotic susceptibility test of the isolates (Gram positive) is presented in table 3.2. *E. coli* was resistant to 75% of the antibiotics used but was sensitive to Amoxicillin (6mm), Ceftriaxone (11mm) and Ofloxacin (12mm). *Klebsiella oxytoca* was sensitive to 42% of the antibiotic used. These are Amoxicillin (7mm), Levofloxacin (11mm), Ciprofloxacin (14mm), Imipenem (7mm), Ofloxacin (9mm), Gentamycin (10mm). It was resistant to Cefotaxime, Ceftriaxone, Cefexime, Cefuroxime, Erythromycin and Azithromycin.

All the test organisms were resistant to Cefotaxime. Majority of the bacteria were resistant to the cephalosporin used.

For Gram negative antibiotic discs, the result obtained in presented in table 3.3. *E. coli* was resistant to 33% including Cefotaxime, Cefuroxime, Ampliclox and Cefexime of the antibiotics used while it was sensitive to Amoxicillin (9mm), Imipenem(10mm), Ofloxacin(13mm), Gentamycin (11mm), Nalidixic Acid (14mm), Nitrofurantoin (8mm), Ceftriaxone S.(16mm) and Levofloxacin (9mm).



**Figure 1** *E. coli* plate showing  $\alpha$  - hemolysis



**Figure 2** *Klebsiella oxytoca* showing no hemolysis (Ø)

**Table 1** Morphological, colonial and biochemical identification of isolates

Isolate	Morphology	Gram status	indole	citrate	oxidase	Methyl red	Voges Proskauer	urease	Catalase	Sugar fermentation					Suspected Organism
										Glucose	sucrose	Galactose	Fructose	Lactose	
1	Short rods	-	+	-	-	+	-	-	+	A/G	A	A/G	A/G	+	E. coli
3	Long rod	-	+	+	-	-	+	+	+	A/G	+/G	+/G	A	A/G	<i>Klebsiella oxytoca</i>

**Table 2** Antibiotic susceptibility test result of isolates (Gram positive)

Isolates/Antibiotics	Zones of inhibition in mm	
	<i>E. coli</i>	<i>Klebsiella oxytoca</i>
Amoxicillin.	6	7
Cefotaxime	R	R
Ceftriaxone	11	R
Cefexime	R	R
Levofloxacin	R	11
Ciprofloxacin	R	14
Imipenem	R	7
Cefuroxime	R	R
Ofloxacin	12	9
Erythromycin	R	R
Gentamycin	R	10
Azithromycin	R	R

Legend, R- Resistant

*Klebsiella oxytoca* was sensitive to 50% of the antibiotics used. These are Imipenem (10mm), Ofloxacin (13mm), Gentamycin (14mm), Nalidixic Acid (11mm), Nitrofurantoin (6mm). It was however resistant to Amoxicillin C, Cefotaxime, Cefuroxime, Ampliclox and Cefexime.

**3.4. Multiple Antibiotic Resistance Index of isolates.**

Resistance to two or more antibiotics is taken as multiple antibiotics resistance and MAR greater than 0.2 shows high antibiotic resistance index. The result is recorded in table 3.4 and 3.5.

For the Gram-positive antibiotic disc, The MARI for *E. coli* was 0.75, *K. oxytoca* had MARI of 0.5 (Table 3.4). For Gram negative antibiotic discs, *E. coli* had MARI of 0.33, *Klebsiella oxytoca* had MARI of 0.5 (Table. 3.5).

**Table 3** Antibiotic susceptibility test result of isolates (Gram negative)

Isolates/Antibiotics	Zones of inhibition in mm	
	<i>E. coli</i>	<i>Klebsiella oxytoca</i>
Amoxicillin C.	9mm	R
Cefotaxime	R	R
Imipenem	10	10
Ofloxacin	13	13
Gentamycin	11	14
Nalidixic Acid	14	11
Nitrofurantoin	8	6
Cefuroxime	R	R
Ceftriaxone S.	16	R
Ampliclox	R	R
Cefexime	R	R
Levofloxacin	9	11

Legend: R- Resistant

**Table 4** MARI of Gram positive bacteria

Isolates	Number of antibiotics isolates are resistant to	Total number of antibiotics tested	MARI
<i>E. coli</i>	9	12	0.75
<i>Klebsiella oxytoca</i>	6	12	0.5

**Table 5** MARI of Gram negative bacteria

Isolates	Number of antibiotics isolates are resistant to	Total number of antibiotics tested	MARI
<i>E. coli</i>	4	12	0.33
<i>Klebsiella oxytoca</i>	6	12	0.5

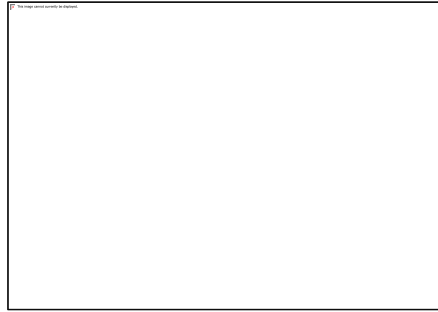
### 3.5. Plasmid profile of isolates

The isolates subjected to plasmid profiling showed all the isolates to have large plasmid borne resistant factors (Fig 3). All isolates have plasmid of 450kbp showing that they all have plasmids.

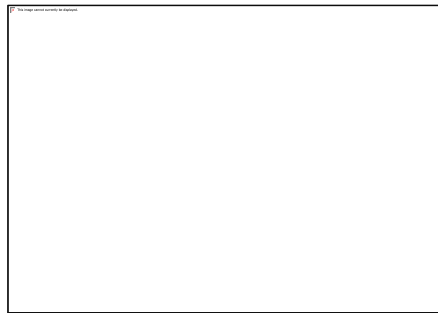
### 3.6. Molecular fingerprints of isolates.

Figure 4 shows the molecular fingerprints of the isolates using primers. *E. coli* was positive for resistance to *ermB* (macrolides) with a basepair of 450 kbp while *Klebsiella oxytoca* was negative for the gene.

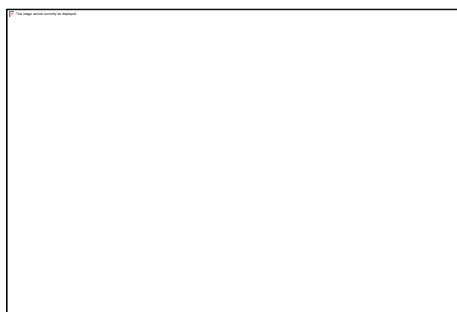
Fig 5 shows that only *E.coli* was positive to *BlaTem* (Betalactamase) gene with a bandwidth of 258bp while, *K. oxytoca* was negative for the gene. However, Fig 6 shows no positive reaction to *qnrB* gene (quinolones)



**Figure 3** The plasmid profile of the two isolates namely (1) *E. coli*, in well 1 and *Klebsiella oxytoca* in well 3

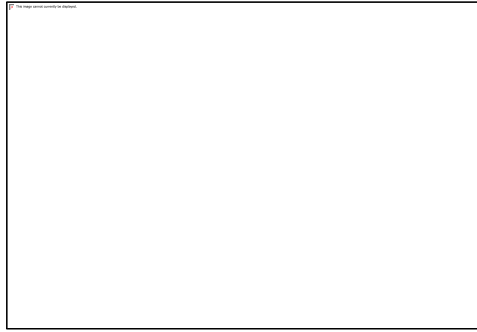


**Figure 4** Agarose gel electrophoresis of the PCR products of *ermB* gene amplified from selected bacteria isolates. (Band size approximately 450bp indicates positive to *ermB*). Gel indicates positive *ermB* amplification in *E. coli* but absent in *K. oxytoca*.



**Figure 5** Agarose gel electrophoresis of the PCR products of *BlaTem* gene amplified from selected bacteria isolates. (Band size indicates approximately 258bp indicates positive to *BlaTem*) Gel indicates positive *BlaTem* amplification in *E. coli* but absent in *Klebsiella oxytoca*





**Figure 6** Agarose gel electrophoresis of the PCR products of *qnrB* gene amplified from bacteria Isolates (Band size approximately 469bp positive to *qnrB*). Gel indicates no positive amplification

#### 4. Discussion

Rampant use of antibiotics in both community and hospitals has transformed the human healthy intestinal gut flora into a reservoir of antibiotic-resistant organisms. Bacteria in the gut not only acquire ARGs (Antibiotic resistance genes), but also contribute to the transfer of ARGs to other bacteria in the gut (Ravi *et al.*, 2014).

*Escherichia coli* is the most prevalent facultative anaerobic species in the gastrointestinal tract of human. It is often the main culprit of infections in the gastrointestinal tract (Rossi *et al.*, 2013). As well as other parts of human and animal organisms (Zhang *et al.*, 2020). In more precise terms, *E. coli* typically causes urinary infections (Isla *et al.*, 2017), but it can also lead to many other serious infections and conditions, such as: appendicitis (Song *et al.*, 2018) pneumonia (Park *et al.*, 2019), meningitis (Zhao *et al.*, 2018) endocarditis (Akenzuwa, 2018) and gastrointestinal infections (Sarowska, 2019). In this study, *E. coli* isolated showed alpha hemolysis (Fig 1) indicating that it was only able to partially break down red blood cells. The ability of *E. coli* to produce hemolysin is part of its virulence factor. Invasive strains frequently produce virulence factors such as  $\alpha$ -hemolysin (HlyA), which causes hemolysis by forming pores in the erythrocyte membrane (Skals *et al.*, 2019).

*E. coli* isolated in this study showed high level of resistance to some standard antibiotics used (Table 3.2 and 3.3). It was resistant to cephalosporins, gentamycin, erythromycin, imipenems and azithromycin. In their work, Aibinu *et al.*, (2004) recorded high level of resistance of *E. coli* to cloxacillin, amoxicillin, ampicillin, erythromycin, cotrimoxazole, streptomycin and tetracycline which is in agreement with the observation in this study. However, this result is contrary to the result obtained by Akingbade *et al.*, (2014) who observed high level of susceptibility to ceftriaxone, ciprofloxacin and ofloxacin and very high susceptibility was registered to gentamycin and ceftazidime but it has been observed that antibiotic susceptibility of bacterial isolates is not constant, but dynamic and varies with time and environment (Hassan, 1995). Gupta *et al* (2019) also recorded a high level of resistance of *E. coli* to cephalosporins. Similarly, Gashaw *et al* (2012) reported resistance of *E. coli* to 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporin, ciprofloxacin, tetracycline, meropenem and clavulanic acid.

The MARi of the organism were 0.75 and 0.33 for Gram negative and Gram-positive antibiotics discs used respectively (Table 3.4 and 3.65). Research has indicated that drug-resistant *E. coli* can be transmitted to human beings from the environment through direct or indirect contact (e.g., consumption of contaminated food and water) (Reinthaler *et al.*, 2013). Evidences accrue that food derived from a variety of animal sources contains large numbers of resistant bacteria and resistant genes (Marshall and Levy, 2011) and these can easily be transmitted to human through consumption.

The result obtained in the plasmid profiling of *E. coli* showed that the resistance factors to specific antibiotic were plasmid borne (Fig 5) and the molecular fingerprint revealed that it bore *ermB* gene (Fig 6). Holmberg *et al.* (1984) reported that plasmid analysis was at least as specific as phage typing. Uma *et al.*, (2009) observed that there is high frequency of resistance among *E. coli* strains with plasmids than without plasmids. In Fig 8, *E. coli* did not possess resistant gene for *qnrB* (aminoglycosides) although the report of the antibiotic susceptibility test showed resistance to antibiotics belonging to the aminoglycosides. This suggest that not all antibiotic resistance genes are located on the plasmid. Some are located on the chromosomes. Aja *et al.*, (2002) suggested that resistance to antibiotics could be encoded in some strains in plasmids and in others in the chromosomes.

*Klebsiella oxytoca*, a member of the Enterobacteriaceae, is a Gram-negative pathogenic bacterium of environmental origin is becoming an important pathogen of humans, and is being implicated in increasing morbidity amongst the

patient population (Moradigaravand *et al.*, 2017). Normally found in bowel of man and animals, water and soil, infections with these bacteria are leading to prolonged stays in hospitals. *Klebsiella* species are rapidly developing multidrug resistance. *Klebsiella* are opportunistic pathogens that cause severe diseases in hospital setting (Singh *et al.*, 2016).

In this study, *K. oxytoca* showed no lysis on blood agar. Pereira and Vanetti (2015) did not observe any hemolytic activity by *K. oxytoca* while some authors documented hemolysis on horse and rabbit blood (Szramka *et al.*, 1998; Sekowska *et al.*, 2006) but no lysis on human and sheep erythrocytes (Szramka *et al.*, 1998). This is consistent with the result in this work, more so as there is lack of adequate report on the hemolytic activity of *K. oxytoca*.

*K. oxytoca* isolated in this study was found to be resistant to 50% of the antibiotics used (Table 3.2 and 3.3.). It was particularly resistant to the cephalosporins. Brisse *et al.*, (2000) reported the resistance of *K. oxytoca* to fluoroquinolones and tetracyclines. In another study, Du, *et al.*, (2014) recorded resistance of *K. oxytoca* to beta-lactam antibiotics, aminoglycosides, and fluoroquinolones. These authors support the observation recorded in this study which reports the multidrug resistance profile of this isolate. Similarly, Chakraborty *et al.*, (2016) reported the resistance of *K. oxytoca* to Amoxicillin, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Gentamicin, Nalidixic acid, Tetracycline.

The plasmid profile analysis showed that *K. oxytoca* had molecular weight plasmid as shown in Fig 5. This result is corroborated by Akanbi *et al.*, (2004) who isolated *K. oxytoca* that had plasmids of different molecular weight with sizes ranging between 1.1 and 8.0 kb.

*K. oxytoca* bore no resistance gene for the primers used in this study which are *ermB* (macrolide) and *BlaTem* (Betalactam) genes band *qnrB* gene (quinolones), (Fig 8) but the result of the antibiotic resistance to several classes of antibiotics including the betalactams, quinolones and macrolides. It is possible that the resistance factors in *K. oxytoca* are chromosome borne. Decree *et al.*, (2004) reported in their work that 25% the *K. oxytoca* isolated had their resistance factors borne on the chromosome. Mathers *et al.*, (2017) also discovered in their work that all chromosomally integrated *blaKPC* genes were from *Klebsiella* spp.

It is worthy of note that in this study, the resistance to cephalosporins is more pronounced in the isolates. Cephalosporins have proven to be of immense importance in surgery and as first line therapy for a wide variety of infections, hence its continuous relevance and usage. Unfortunately, most bacteria of clinical importance have become resistant to these antibiotics, therefore, a worldwide problem (Adesoji *et al.*, 2016). Bassetti *et al.*, (2011) identified the Enterobacteriaceae as a group of microorganisms mainly involved in conferring resistance to this antibiotic.

The gene probe of the resistance factor using primers showed that *E. faecalis* had no resistance factors for the gene primers (Fig 5-8). It is possible that the resistance gene are chromosomal and not plasmid borne. Cho *et al.*, (2020) documented that although strains of *E. faecalis* was resistant to macrolide antibiotic, *ermB* genes were not detected in them. He further affirmed that not all isolates with resistance phenotypes carried AR genes.

Certain genes may be more related to the risks of the emergence or persistence of resistance than others. For example, integrons and sulfonamide resistance genes have been used to detect anthropogenic contaminants (Wang *et al.*, 2014; Gillings *et al.*, 2015). *Erm* genes encode resistance to macrolide antibiotics, which have long been used to treat Gram-positive and certain Gram-negative pathogens infecting humans, swine and cattle. Broadly, macrolide antibiotics act by binding to the 23S subunit of the bacterial ribosome, causing premature release of peptides during translation. The *erm* genes cause resistance by methylating rRNA at the active site, reducing the ability of macrolide antibiotics to bind to the ribosome (Weisblum 1998; Vester and Douthwaite 2001). *Erm*-mediated resistance to macrolides has also been observed to confer resistance against other antibiotics, including lincosamide and streptogramin B (MLS<sub>B</sub> resistance (Leclercq and Courvalin 1991).

The *TEM* β-lactamases represent one of the most clinically significant families of β-lactamases. *TEM-1*, is considered broad spectrum and hydrolyzes the early cephalosporins, in addition to many penicillins. *TEM-1* has become the most commonly encountered β-lactamase and is ubiquitous among Enterobacteriaceae. The *blaTEM* genes are located on a family of related β-lactamase plasmids. Although β-lactamase genes are thought to have originally resided solely on bacterial chromosomes, they are often found on plasmids. The mobility of these genes is related to their association with transposons and even integrons. Transposons have been implicated in the spread of *TEM* β-lactamase to plasmids in *Haemophilus influenzae* and *Neisseria gonorrhoeae* (Eilwell *et al.*, 1975).

The *qnr* gene encode the resistance factor to the quinolones. *Qnr*, the gene product, is a member of the pentapeptide repeat family of proteins and has been shown to block the action of ciprofloxacin on purified DNA gyrase and

topoisomerase IV (Tran *et al.*, 2002). A large number of *qnr* alleles have been found on plasmids or bacterial chromosome. And it is predominant in the Enterobacteriaceae. Literature has shown that bacterial strain with *qnrB* demonstrated low-level resistance to all quinolones (Jacoby, *et al.*, 2006). The *qnrA* gene has been found in plasmids with a variety of other resistance determinants but always as part of a *sul1*-type integron (Nodmann *et al.*, 2005).

---

## 5. Conclusion

Bacterial antibiotic resistance, a global health threat, is caused by plasmid transfer or genetic mutations. The development of MDR bacteria is an emerging problem worldwide, especially in developing countries. The result of this research emphasizes the fact that intestinal bacteria are prone to developing antibiotic resistance through horizontal transfer of plasmids or genetic mutation. The human gut microbiota harbors both commensals and opportunistic pathogens which can acquire resistance to antibiotics through mutation and horizontal gene transfer.

The development of antibiotic resistance in bacteria has been traced to uncontrolled use of antibiotics both in human therapy and agricultural practices. There is therefore a need to promulgate laws on the consumption and recommendation of antibiotics both in hospital and agricultural settings.

The need to understand how bacteria adapt to the antibiotic environment will lead to new therapeutic strategies for antibiotic-resistant infections. Interventions measures to minimize the abundance of antibiotic-resistant commensals and opportunistic pathogens may include faecal microbiota transplantation and the use of live biotherapeutics.

Also, with the pandemic nature of bacteria resistance to cephalosporins and the thousands of annual deaths reportedly to have been caused by plasmid transfer and perhaps more alarming, its fast emergence and spread, a restriction in the use of these antibiotics (especially the third and fourth generations), would be recommended, and then the ceftolozane – tazobactam combination encouraged since it has demonstrated an excellent activity against enterobacteriaceae

---

## Compliance with ethical standards

### *Acknowledgments*

Appreciation to the department of Biological Sciences Laboratory, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria

### *Disclosure of conflict of interest*

There is no conflict of interest in this research work.

---

## References

- [1] Adesoji, AT., Abubakar, F., Ipinloye, JS (2016). Occurrence of Antibiotic resistant Bacteria in faeces from Abattoir Waste, Processing water and Products from Dutsin-Ma, Katsina State, Nigeris. *Journal of Bacteriology and Mycology*. 3 (1) 1022.
- [2] Aibinu IE, Adenipekun, EA, Odugbemi TO. (2004). Emergence of Quinolone Resistance amongst *E. coli* Strains Isolated from Clinical Infections in Some Lagos State Hospitals, in Nigeria. *Nig J Health Biomed Sci.*; 3:73–78.
- [3] Aja, AM, Gasca, AG, Grobois, AA, Mejia CB, Roque A, Gil, BG (2002). Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. *FEMS Microbiol. Lett.* 213: 7-12,
- [4] Akanbi, AA , Onile, BA, Omonigbehin, EA and Abdulraheem, IS (2004). Plasmid profiles of *Klebsiella* isolates in Ilorin, Nigeria. *African Journal of Clinical and Experimental Microbiology*. 5; 2 .
- [5] Akingbade O, Balogun S, Ojo D, Akinduti P, Okerentugba PO, Nwanze JC, Okonko IO. (2014)Resistant plasmid profile analysis of multidrug resistant *Escherichia coli* isolated from urinary tract infections in Abeokuta, Nigeria. *Afr Health Sci.*;14(4):821-8.
- [6] Akuzawa, N.; Kurabayashi, M. (2018). Native Valve Endocarditis Due to *Escherichia Coli* Infection: A Case Report and Review of the Literature. *BMC Cardiovasc. Disord.* 18, 195.
- [7] Barron, M. (2022). The Gut Resistome and the Spread of Antimicrobial Resistance. *American Society for Microbiology*. On line article Accessed on 19th Dec, 2022.

- [8] Barghouthi, SA. (2011). A Universal Method for the Identification of Bacteria Based on General PCR Primers. *Indian J Microbiol.*; 51(4): 430–444
- [9] Bassetti, M., Ginocchio, F., Mikulska, M. (2011). New treatment options against Gram Negative organisms, *Crit. Care.* 15 215 10. 1186/cc9997..
- [10] Brisse S, Milatovic D, Fluit AC, Verhoef J, Schmitz FJ. 2000. Epidemiology of quinolone resistance of *Klebsiella pneumoniae* and *Klebsiella oxytoca* in Europe. *Eur J Clin Microbiol Infect Dis.* 19:64–68.
- [11] Bud, R. (2007). Antibiotics: the epitome of a wonder drug. *BMJ.* 334 Suppl 1, s6.doi: 10.1136/bmj.39021.640255.94
- [12] Canny GO and McCormick BA. (2008). Bacteria in the intestine, helpful residents or enemies from within? *Infect Immun.* Aug;76(8):3360-73.
- [13] Chakraborty, S., Mohsina, K., Sarker, PH., Alam, MDZ., Karim, MIA and Sayem, SMA (2016). Prevalence, antibiotic Susceptibility profiles and ESBL production in *Klebsiella pneumoniae* and *Klebsiella oxytoca* among hospitalized patients. *Periodicum Biologorum.* 118(1). 53-58.
- [14] Cheesebrough, M. (2000). Microbiological tests In; Cheesebrough, M. Ed. District Laboratory Practices in Tropical Countries, Part II. Cambridge press. University, Cambridge. 105-130.;
- [15] Cho S, Barrett JB, Frye JG, Jackson CR. (2020) Antimicrobial Resistance Gene Detection and Plasmid Typing Among Multidrug Resistant Enterococci Isolated from Freshwater Environment. *Microorganisms.* 8(9):1338.
- [16] Decré, D, Burghoffer, B, Gautier, V, Petit J, Arlet, G. (2004). Outbreak of multi-resistant *Klebsiella oxytoca* involving strains with extended-spectrum  $\beta$ -lactamases and strains with extended-spectrum activity of the chromosomal  $\beta$ -lactamase, *Journal of Antimicrobial Chemotherapy*, 54, 5, 881–888.
- [17] Dethlefsen, L., and Relman, DA. (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc. Natl. Acad. Sci. U. S. A.* 108 Suppl 1, 4554–4561. doi: 10.1073/pnas.1000087107
- [18] Du, J, Li, P, Liu, H, Lü, D, Liang, H and Dou, Y (2014). “Phenotypic and molecular characterization of multidrug resistant *Klebsiella pneumoniae* isolated from a University Teaching Hospital, China,” *PLoS ONE*, 9, (4), Article ID e95181.
- [19] Elwell, L. P., J. De Graaff, D. Seibert, and S. Falkow (1975). Plasmid-linked ampicillin resistance in *Haemophilus influenzae* type B. *Infect. Immun.*12:404-410.
- [20] Gashaw M, Marame ZH, Abera M, Ali S. (2021). Assessment of Gut Bacteria Profile and Antibiotic Resistance Pattern Among Psychotropic Drug Users: Comparative Cross-Sectional Study. *Infect Drug Resist.* 2021; 14:1875-1881.
- [21] Gillings MR, Gaze WH, Pruden A (2015). Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J* ;9:1269–79.
- [22] Gupta M, Didwal G, Bansal S, Kaushal K, Batra N, Gautam V, Ray P. (2019). Antibiotic-resistant Enterobacteriaceae in healthy gut flora: A report from north Indian semiurban community. *Indian J Med Res.* 2019 Feb;149(2):276-280. doi: 10.4103/ijmr.IJMR\_207\_18. PMID: 31219094; PMCID: PMC6563735.
- [23] Hassan SH. (1995). Sensitivity of *Salmonella* and *Shigella* to antibiotics and chemotherapeutic agents in Sudan. *J Trop Med Hyg.* 188:243–248.
- [24] Holmberg SD, Wachsmuth IK, Hickman-Brenner FW, Cohen ML. (1984). Comparison of plasmid profile analysis, Phage typing, and antimicrobial susceptibility testing in characterizing *salmonella typhimurium* isolates from outbreaks. *J Clin Microbiol.*;19:100–4.
- [25] Isla, AL.; Polo, JM.; Isa, MA.; Sala, RB.; Sutil, RS.; Quintas, JJ.; Moradillo, JG.; Padilla, DAG.; Rojo, EG.; Martínez, JBP. (2017). Urinary Infections in Patients with Catheters in the Upper Urinary Tract: Microbiological Study. *Urol. Int.* 2017, 98, 442–448.
- [26] Jacoby, GA., Walsh, KE., Mills, DM., Walker, VJ., Oh, H., Robicsek, A., and Hooper, DC (2006). *qnr B* another plamid mediated gene for quinolones resistance. *Antimicrob. Agents Chemother.* 50 1178-1182.
- [27] Klein, EY., Van Boeckel, TP., Martinez, EM., Pant, S., Gandra, S., Levin, SA., (2018). Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. *Proc. Natl. Acad. Sci. U. S. A.* 115, E3463–E3E70. doi: 10.1073/pnas.1717295115

- [28] Krumperman, PH. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high risk sources of contamination of food. *Appl. Environm. Microbiol.* 46(1). 165-170.
- [29] Leclercq R and Courvalin P. (1991). Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob Agents Chemother* ;35:1267–72.
- [30] Mathers, AJ., Stoesser, N., Chai, W., Carrol, J., Barry, K., Cherunvanky, A., Sebra, R., Kasarskis, A, Peto, TE., Walker, AS., Sifri, CD, Crook, DW and Sheppard, AE. (2017). Chromosomal integration of the *Klebsiella* Carbapenemase gene *BlakPC* in *Klebsiella pneumoniae* specie is elusive but not rare. *Antimicrob agent. Chemother.* 61. e01823-16.
- [31] Marshall, BM and Levy, SB. (2011). Food animals and antimicrobials: Impacts on human health. *Clin.Microbiol. Rev.* 24, 718–733.
- [32] Moradigaravand D, Martin V, Peacock SJ, Parkhill J. (2017). Population structure of multidrug resistant *Klebsiella oxytoca* within hospitals across the UK and Ireland identifies sharing of virulence and resistance genes with *K. pneumoniae*. *Genome Biol Evol.*;9(3):574-587.
- [33] Nordmann, P., and L. Poirel. (2005). Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J. Antimicrob. Chemother.* 56:463-469.
- [34] Park, J.; Kim, S.; Lim, H.; Liu, A.; Hu, S.; Lee, J.; Zhuo, H.; Hao, Q.; Matthay, M.A.; Lee, J.-W. (2019). Therapeutic Effects of Human Mesenchymal Stem Cell Microvesicles in an Ex Vivo Perfused Human Lung Injured with Severe *E. Coli* Pneumonia. *Thorax* (Accessed 24th December, 2022).
- [35] Peireira, SC. and Vanetti, MC (2015). Potential virulence of *Klebsiella* sp isolates from enteral diets. *Braz. J. Med. Biol. Res.* 48(9); 782-9
- [36] Ravi, A., Avershina, E., Ludvigsen, J., L’Abee-Lund, T. M., and Rudi, K. (2014). Integrons in the intestinal microbiota as reservoirs for transmission of antibiotic resistance genes. *Pathogens* 3, 238–248.
- [37] Reinthaler FF, Galler H, Feierl G, (2013). Resistance patterns of *Escherichia coli* isolated from sewage sludge in comparison with those isolated from human patients in 2000 and 2009. *J Water Health.*;11(1):13–20. doi:10.2166/wh.2012.207.
- [38] Rossi, E.; Cimdins, A.; Lüthje, P.; Brauner, A.; Sjöling, Å.; Landini, P.; Römling, U. (2018). “It’s a Gut Feeling” — *Escherichia Coli* Biofilm Formation in the Gastrointestinal Tract Environment. *Crit. Rev. Microbiol.*, 44, 1–30.
- [39] Salyers, AA, Gupta, A. and Wang, Y (2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trend in Microbiology*, 12, 9, Pages 412-416
- [40] Sarowska, J.; Koloch, BF.; Kmiecik, AJ.; Madrzak, MF.; Ksiazczyk, M.; Ploskonska, GB.; Krol, IC. (2019). Virulence Factors, Prevalence and Potential Transmission of Extraintestinal Pathogenic *Escherichia Coli* Isolated from Different Sources: Recent Reports. *Gut Pathog.* 11, 10.
- [41] Skalsa, M, Jorgensenb, NR, Jens Leipzigera, and Helle A. Praetoriusa,I (2019). -Hemolysin from *Escherichia coli* uses endogenous amplification through P2X receptor activation to induce hemolysis. *PNAS.* 106 (10). 4030 – 4035.
- [42] Singh L, Cariappa MP, Kaur M. (2016). *Klebsiella oxytoca*: An emerging pathogen? *Med J Armed Forces India.*;72 (Suppl 1): S59-S61. Doi
- [43] Song, DW.; Park, BK.; Suh, SW.; Lee, SE.; Kim, JW.; Park, J.-M.; Kim, .R.; Lee, MK.; Choi, YS.; Kim, BG. (2018). Bacterial Culture and Antibiotic Susceptibility in Patients with Acute Appendicitis. *Int. J. Colorectal Dis.* 2018, 33, 441–447.
- [44] Sekowska, A., Gospodarek, E., Janickca, G., Jachna-Sawicka, K., Sawicki, M ( 2006). Hydrolytic and heamolytic activity of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Med. Dosw Mikrobiol.* 58 (2); 135-41.
- [45] Szramka, B., Kurlenda, J and Bielawski, K (1998). Hemolytic activity of *Klebsiella pneumonia* and *Klebsiella oxytoca*. *Med Dows Mikrobiol.* 50(3-4) ; 207-13.
- [46] Tankeshwa, A. (2022). Biochemical tests for Bacterial Identification. *Microbe on line*. Accessed on 12, Jan, 2023.
- [47] Tran, JH., and GA. Jacoby. (2002). Mechanism of plasmid-mediated quinolone resistance. *Proc. Natl. Acad. Sci. USA* 99:5638-5642.
- [48] Uma, B., Prabhakar, K, Rajendran, S., Kavitha, K., and Sarayu, YL (2009) Antibiotic Sensitivity and Plasmid Profiles of *Escherichia coli* Isolated from Pediatric Diarrhea. *J Glob Infect Dis.*; 1(2): 107–110.

- [49] van Duin D, and Paterson DL. (2016). Multidrug-resistant bacteria in the community: Trends and lessons learned. *Infect Dis Clin North Am.* 30:377–90.
- [50] Van Schaik W (2015). The human gut resistome. *Philos Trans R Soc Lond B Biol Sci.* 370: 20140087.
- [51] Vester B, and Douthwaite S. (2001). Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob Agents;* 45:1–12.
- [52] Wang FH, Qiao M, Su JQ. (2014). High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation. *Environ Sci Technol;* 48:9079–85.
- [53] Werner G., Coque TM., Franz CM., Grohmann E., Hegstad K., Jensen L., van Schaik W., Weaver K. (2013). Antibiotic resistant enterococci-tales of a drug resistance gene trafficker. *Int. J. Med. Microbiol.;* 303:360–379.
- [54] Weisblum B. (1998). Macrolide resistance. *Drug Resist Update;* 1:29–41.
- [55] World Health Organization. (2014). Antimicrobial resistance: global report on surveillance. World Health Organization. <https://apps.who.int/iris/handle/10665/112642>
- [56] World Health Organization (2018). WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation? Available at: <http://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf?ua=1> (Accessed 24th December, 2022).
- [57] Zhang, S.; Abbas, M.; Rehman, M.U.; Huang, Y.; Zhou, R.; Gong, S.; Yang, H.; Chen, S.; Wang,
- [58] M.; Cheng, A. (2020). Dissemination of Antibiotic Resistance Genes (ARGs) via Integrins in *Escherichia Coli*: A Risk to Human Health. *Environ. Pollut.*, 266, 115260.
- [59] Zhao, WD.; Liu, DX.; Wei, J.-Y.; Miao, Z.W.; Zhang, K.; Su, ZK.; Zhang, XW.; Li, Q.; Fang, WG.; Qin, XX (2018). Caspr1 Is a Host Receptor for Meningitis-Causing *Escherichia Coli*? *Nat. Commun.* 9, 2296