Original Research Paper

Distribution and Antibiotic Sensitivity Patterns of Faecal Bacteria from Underground Faecal Storage Cisterns in Ondo State.

Aims: To determine the distribution and antibiotic sensitivity patterns of faecal bacteria from underground faecal storage cisterns in Ondo State, Nigeria. Design**:** Experimental design.

Location: Human faecal samples was collected from underground faecal storage cisterns from five major towns (Akungba-Akoko, Owo, Akure, Ondo and Ore) in Ondo State, Nigeria

Methodology: (256) human faecal samples were collected from 33 underground faecal storage cisterns across 5 major towns (Akungba, Owo, Akure, Ondo and Ore) in Ondo State, Nigeria. Salmonella-Shigella (S.S), Eosine Methylene Blue, and MaConckey agar, which are selective for

Keywords: Cisterns, Faecal, Antibiotics, Underground.

1.0 INTRODUCTION

Bacteria are single-celled microorganisms that require the use of microscope to be seen [1]. They can be classified broadly as Gram negative or positive base on their cell wall compositions. A typical example of Gram negative and positive bacteria is *Escherichia coli* and *Staphylococcus aureus* respectively. The term 'antibiotic' was coined from the word 'antibiosis', which literally means 'against life'. In the past, antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms [2]. As a result of this notion, an antibiotic was originally broadly defined as

a substance produced by one microorganism [3], of biological origin [4], which at low concentrations can inhibit the growth of, or are lethal to other microorganisms [2]. However, this definition has been modified in modern times, to include antimicrobials that are also produced partly or wholly or through synthetic means. Whilst some antibiotics are able to completely kill other bacteria, some are only able to inhibit their growth. Those that kill bacteria are termed bacteriocidal while those that inhibit bacterial growth are termed bacteriostatic [5]. Antibiotic compounds are differentiated as antibacterials, antifungals and antivirals to reflect the group of microorganisms they antagonize [6, 2].

Antibiotic resistance has been defined as the ability of bacteria to change in ways that resist the effects of drugs – "that is, the germs are not killed, and their growth is not stopped". In other words, antibiotic resistant bacteria are bacteria that are no longer susceptible to the antibiotics to which they were earlier susceptible. Antibiotic resistance in bacteria remains a major problem and the environments that help to maintain such resistance represent significant problem in the community [7]. During the past sixty years, antibiotics were extensively used in humans and in veterinary medicine, as well as in breeding practices. In high-income countries, continued high rates of antibiotic use in hospitals, the community, and agriculture have contributed to selection pressure that has sustained resistant strains [8], forcing a shift to more expensive and more broad-spectrum antibiotics. In low-income and middle-income countries (LMICs), antibioticuse is increasing with rising incomes, high rates hospitalisation, and high prevalence of hospital infections [7]. Despite the concerted efforts being made to control, through addressing its use and misuse, antibiotic resistance in bacteria, their spread continue to be an important global problem, particularly in developing and low income countries where they rapidly spread, causing morbidity and mortality with an attendant increase in the cost of treating infectious diseases due to treatment failures. In fact, the recent, and so far the first, global report on antimicrobial resistance by WHO indicates alarmingly high rates of resistance in the bacteria which cause common infections in healthcare facilities and in the community. Meanwhile more than 200,000 newborns are estimated to die each year due to infections for which effective antibiotics are unavailable [9]. This is becoming a global threat [10]. In fact, it is estimated that the global annual mortality of 700, 000 death associated with antibiotic resistance may by 2050 increase to 10 million, at a cost of 100 trillion USD to the world economy, if nothing is done [10]. For example, [11] state that patients with antibiotic-resistant infections had to pay 700 USD more than those

with infections susceptible to first-line antibiotics in India. This equates to more than a year of wages for an Indian rural male casual worker [11].

There is a large group of Gram negative bacteria called Enterobacteriaceae. They are enteric bacteria and are normal intestinal flora of human. Although they are not pathogenic under normal conditions, they are capable of causing enteric infections mostly in immunocompromised hosts. Enteric infections are the fifth leading cause of death worldwide. Nearly 70% of such infections are food-borne. About 1.5 billion cases of diarrheal disease occur annually, killing 2.2 million people, mainly children [12]. Nigeria is also confronted with the burdens of antimicrobial resistance. The Nigerian Centre for Disease Control (NCDC), in collaboration with other institutions, has made efforts to develop an approach to combat antibiotic resistance, using an evidence-based method. Meanwhile, [12] reported that Nigeria has experienced huge resistance to antimicrobials in humans, especially in sepsis, respiratory, and diarrheal infections. These include childhood-related life-threatening diseases and are supported by empirical evidence, which are replete and scattered in peer-reviewed and grey literature, as well as commissioned reports. In addition, the situation analysis and recommendations on antimicrobial resistance and drug use in Nigeria has recently been documented [12]. For example, Nigeria's diarrhoea prevalence rate is one of the worst in sub-Saharan Africa. It is 18.8% and notably higher than the continental average of 16% [12]. Failure to control the spread of diarrhoeal pathogens both resistant and non-resistant ones have greatly worsened the burden of diarrhoeal disease in Sub-Saharan Africa.

Bacteria efficiently exchange genetic materials, particularly among related species and/or groups including the pathogenic ones. And the intestine is a suitable environment for these activities and therefore intestinal flora is a reservoir for resistance bacteria genes and they are passed out with human faeces. About 70% of the antibiotics produce globally are used in agriculture, the remaining 30% are consumed by man. And many of that which are consumed by man are excreted and passed into the environment [10]. In communities with less developed sanitation infracture, there is a higher risk that waste will not be treated, and sometimes be closer to communities, thus increasing the risk of exposure to the carriage of resistant bacteria by otherwise healthy people, and the rate of drug-resistant community-acquired infections. It is in these settings that there is an additional concern about antibiotics and resistant bacteria passing into the environment as sewage system are not often functional. Inappropriate human disposal of antibiotics, for instance by flushing them into the toilet, plays role in this

[10]. Toilet users who go into the toilet with mobile phone can leave the toilet with myriads of pathogenic bacteria. Also, by vertical and horizontal flow of faecal sludge especially during raining season, the bacteria in human underground faecal storage tanks can leak into water aquifers and wells that are not in safe distance to the faecal storage tanks. Toilets act as a vehicle for the transmission of pathogens from gut, respiratory tract and skin via hands and surfaces from one person to another [13]. Toilet handles contamination is one of the common ways by which organisms that are not resident in the hand are picked up by contact with surfaces. Due to the unhygienic use of the toilet facilities, faecal matter remains a major reservoir source of human pathogens. When hands containing faecal remnant uses a door knob, the bacteria pass on to it. Although there are numerous documented reports on antibiotic sensitivity patterns of faecal bacteria isolated from various sources, there is paucity report on antibiotic sensitivity patterns of bacteria isolated from human excreta (mixture of faeces and urine) collected from underground faecal storage cisterns. This report is therefore designed to determine the distribution and antibiotics sensitivity patterns of faecal bacteria isolated from human excreta collected from underground faecal storage cisterns in Ondo State, Nigeria.

2.0 MATERIALS AND METHOD

2.1 Description of Study Location

This research was carried out in Ondo State, Nigeria from January to November, 2019. Ondo was created on 3rd February, 1976, from the former Western State. It originally included the present Ekiti State, which was split off in 1996. Akure is the administrative capital of Ondo State. The State, with coordinates 7°10′N 5°05′E, has a land area of 14, 606 km^2 and human population of 3,460,877; the statistic of males stood at 1,745,057 while females was 1,715,820 (2006 census). However, the projected population of Ondo State as at year 2016 was 4,671,700. The State borders: Ekiti State to the north, Kogi State to the northeast, Edo State to the east, Delta State to the southeast, Ogun State to the southwest, and Osun State to the northwest.

2.2 Collection of Samples

Two hundred and fifty-six human faecal samples were aseptically collected in different screw-cap sterile bottles from thirty-three faecal storage tanks across five towns (Akungba, Owo, Akure, Ondo, and Ore) in Ondo State, Nigeria, from the month of January to May 2019. All samples were transported in an icepark box within 3 hours of collection to the Microbiology Laboratory of the Federal University of Technology Akure, where bacteriological analysis was carried out on them.

2.3 Preparation and Sterilization of Agar

Nutrient, MaConckey, Eosin Methylene Blue (EMB) and Salmonella-Shigella (S-S) agar were prepared following manufacturers recommendation. All the prepared media, except Salmonella-Shigella agar, was then autoclaved at 121^0C for 15minutes. Salmonella-Shigella was dissolved by heating on hot plate [14].

2.4 Enumeration and Agar-Dependent Isolation

Serial dilutions of the faecal samples were carried out aseptically up to 10^{-6} dilution in order to obtain countable bacteria colonies on the agar plates. Using pour plate method, 1ml aliquot from the dilution 10^{-5} and 10^{-6} was poured on separate duplicated sterile Petri dishes containing sterile nutrient and MaConckey agar. The agar was allowed to congeal on the poured plates and was then incubated invertedly for 18-24 hours at 37^0C in a bacteriological incubator. Nutrient agar was used for total bacterial count while MaConckey agar was used as selective agar for faecal bacteria. Colonies on the agal plates were counted and recorded. Dinstict colonies from the MaConckey agal plates were picked with the aid of sterilized wire loop and streaked on Salmonella-Shigella agar and Eosin Methylene Blue agar, for purification. Pure colonies were later stored at 4^oC on Nutrient Agar (NA) slant in a refrigerator [14].

2.5 Biochemical Characterization

To further identify the isolates, various biochemical and sugar fermentation tests were carried out on each isolates. Gram staining, Oxidase, Urease, Catalase, Coagulase, Citrate, SIM (Sulphur, Indole, and Motility), Ornithine, Lysine, Methyl Red, Voges Prauskeur, TSI (Triple Sugar Iron) tests were carried out. Sugar fermentation (glucose, sucrose, mannitol and Xylose) was also carried out [14]

2.5.1 Gram Staining

A loopful inoculum of 24-hour pure bacterial cultures was speared on grease-free glass slides. The smears were air-dried and then heat-fixed by rapidly passing it through flame emanating from burnsen burner. This is to maintain the cellular integrity of the bacterial cells and prevent it from washing away during flooding. The smears on the slides were then flooded with crystal violent dye (primary stain) for 60 seconds and then quickly washed off under a slow running clean water. Subsequently, the smears were covered with lugol's iodine (mordant) for 60 seconds and washed off with clean running water. 75% ethanol (decourlorizer) was poured on the smears and washed of after about 30 seconds and was again washed off under slow flowing tap water. Finally, Sefranin (secondary stain) was added for 60 seconds, and was washed off under slow running tap water. The slides were then left to air dry, after which they were viewed under 40x objective lens of a light microscope. A drop of oil immersion was placed on the stained smear and was again examined using using oil immersion objective (x100) lens of the same microscope [15, 14].

2.5.2 Oxidase test

A piece of filter paper was soaked with 2-3 drops of oxidase reagent. Using a sterile piece of stick, a colony from 24 hours culture of the test organism was picked and smeared on the filter paper; reactions was observed for 10 seconds. Oxidase positive isolates changed to blue or purple colour, while oxidase negative isolates has no colour change within 10 seconds [14].

2.5.3 Urease test

This was done by streaking overnight culture of the test organisms over the slant surface of prepared slanted urease agar in a test tube. A tube without any test organisms was used as control. Release of ammonia brings about colour change from yellow to pink or red which means a positive result; tubes with no colour change werw regarded as containing organism tested negative to urease production [15].

2.5.4 Catalase test

The test distinguished catalase producing bacteria from non-catalase producer. Catalase positive isolates breakdown hydrogen peroxide to oxygen and water. 3% hydrogen peroxide was prepared and a drop was

placed on a glass slide. Using a sterile wooden stick, a colony of the test organism from 24 hours old culture was placed in the hydrogen peroxide and mixed together gently. Copious bubbles caused by the organism by the liberation of oxygen indicated positive result [14].

2.5.5 Coagulase test

A 24hrs old culture was emulsified in normal saline on clear grease free slide containing a drop of distilled water on both end of the slide to make two thick suspensions. A loopful of plasma was added to one of the suspensions and gently mixed. The second suspensions contain no plasma and was used as control to differentiate any granular appearing as coagulase of the organism from clumping caused by the organism's Coagulase enzyme. Clumping within 10 seconds indicated a positive result while, no clumping indicated a negative result [14].

2.5.6 Citrate test

Simon citrate agar was prepared in a beaker following manufacturer's instruction. The beaker containing the prepared agar was homongenized on electric hot plate. Using sterile syringe, about 10ml each was dispensed into different test tubes. The component was then autoclaved at 121^0C for 15 minutes. The test tubes were slanted and allowed to congeal. Overnight culture of the test organism was inoculated on the slanted portion of the medium and incubated at 37 ℃ for 2-5days. Colour change from green to bright blue colour indicated a positive citrate test while, no colour change indicated a negative citrate test of medium [16].

2.5.7 SIM (Sulphur, Indole, and Motility) test

SIM agar was prepared according to the manufacturer's specification in a conical flask. It was homogenized on electric hot plate before dispensing about 10ml each into various test tubes. The components was then sterilized in the autoclave for 15 minutes at 121℃. After the agar cooled and turned semi-solid, 24hrs cultures of the test organisms were inoculated into the tubes by making a stab on the center of the medium to a depth of about one (1) inch, and then incubated aerobically at 37 ℃ for 24hours

in a bacteriological incubator. H_2S positive test indicated by blackening of the medium along the inoculation line. H_2S negative result means there is no blackening. Positive motility test indicate by a diffused zone of growth from inoculation line whereas a negative result was infected when there is no diffuse growth. In order to examine for indole production, 3-6 drops of Kovac reagent was added into the tubes. A reddish ring means the test organism is positive to indole production [15].

2.5.8 Triple Sugar Iron (TSI) test

This is used to distinguish between member of member of Enterobacteriaceae. TSI agar was prepared according to the manufacturer's specification in a conical flask. It was homogenized on electric hot plate before dispensing about 10ml each into various test tubes. The components was then sterilized in the autoclave for 15 minutes at 121℃. After autoclaving, the test tubes containing the TSI was slanted and left to congeal. Using aseptic technique, colony from overnight pure bacterial culture was picked with a sterile straight needle and stabbed into the medium, upto the butt of the TSI tube, and then streaked the needle back and forth along the surface of the slant. The neck of the TSI tube was cocked and flamed before incubating at 37^0C for 18 to 24 hours. Alkaline slant (red) and acid butt (yellow) with or without gas production (breaks in the agar butt) indicated that glucose fermentation has occurred. The organisms preferentially degrade glucose first. Since this substrate was present in minimal concentration, the small amount of the acid produced on the slant surface was oxidized rapidly. The peptones in the medium was also used in the production of alkali. At the butt, the acid reaction is maintained because of the reduced oxygen tension and slower growth of the organisms [14]

Acid slant (yellow) and acid butt (yellow) with or without gas production indicated that lactose or sucrose fermentation has occurred. Since these substances are present in higher concentrations, they serve as substrates for continued fermentative activities with maintenance of an acid reaction in both the slant and the butt. Since these substances are present in higher concentrations, they serve as substrates for continued fermentative activities with maintenance of an acid reaction in both the slant and the butt.

Alkaline slant (red) and alkaline butt (red) or no change (orange-red) butt indicated that no carbohydrate fermentation has occurred. Instead; peptones was catabolized under anaerobic and /or aerobic conditions resulting in alkaline pH due to production of ammonia [14]. The presence of hydrogen sulphide was indicated by blackening of the TSI agar. Some bacteria utilize thiosulfate anion as a terminal electron

acceptor, reducing it to sulfide. If this occurs, the newly-formed hydrogen sulfide (H_2S) reacts with ferrous sulfate in the medium to form ferrous sulfide, which is visible as a black precipitate [14].

2.5.9 Ornithine Decarboxylation test

Decarboxylation is the process in which bacteria that possess specific decarboxylase enzyme attack amino acids at their carboxyl end (-COOH) to yield an amine or a diamine and carbon dioxide. The amino acid L-ornithine is decarboxylated by the enzyme ornithine decarboxylase to yield the diamine putrescine and carbon dioxide [17]. Ornithine decarboxyse broth was used for this test. The broth contains L-Ornithine monohydrochloride, Yeast extract, Glucose, and Bromo cresol purple. Yeast extract in the medium provides nitrogen and other nutrients necessary to support bacterial growth. The amino acid ornithine is added to detect the production of ornithine decarboxylase. Glucose is the fermentable carbohydrate, which during the initial stages of incubation, is fermented by the organisms with acid production, which results in colour change of the pH indicator (BCP) to yellow. The acidic condition also stimulates decarboxylase activity. If the organism produces the appropriate enzyme, i.e. decarboxylase, the amino acid (ornithine) in the medium is degraded, yielding a corresponding amine. Decarboxylation of ornithine yields putrescine. The production of this amine elevates the pH of the medium towards alkalinity, changing the color of the indicator from yellow to purple or violet. If the organism does not produce the appropriate enzyme, the medium remains acidic or yellow in colour. The broth was prepared according to manufacturer's specification by suspending 9.01 grams in 1000 ml distilled water. The medium was dissolved completely by heating on electric hot plate. About 5ml of the broth was dispensed in test tubes and autoclaved at 121^0C for 15minutes. The tubed broth was allowed to cool in an upright position and then asceptically incubated with the test organisms. Each innoculated tubed broth was overlayed with 2-3ml of sterile (autoclaved) parraffin oil and incubated at 37^0C for 24hours. Ornithine positive isolates changed the broth to purple while the tubes containing ornithine negative isolates has no colour change (i.e remained yellow) [17].

2.5.10 Lysine Decarboxylation test

Lysine decarboxylase broth was prepared according to manufacturer's specification by suspending 14.02 grams in 1000 ml distilled water. The medium was dissolved completely by heating on electric hot plate. About 5ml of the broth was dispensed in test tubes and autoclaved at 121^0C for 15minutes. The tubed broth was allowed to cool in an upright position and then asceptically incubated with the test organisms. Each innoculated tubed broth was overlayed with 2-3ml of sterile (autoclaved) parraffin oil and incubated at 37^0C for 2 to 4 days. Lysine positive isolates changed the broth to purple while the tubes containing ornithine negative isolates has no colour change (i.e remained yellow) [18].

2.5.11 Fermentation of Sugars

The bacterial isolates were tested for the fermentation of sugars such as glucose, sucrose, mannitol and xylose. 1.0 g of each sugar was weighed and dispended into different conical flasks and labeled. 2.5 g of peptone water was added into 100 ml of distilled water and 0.01 g of phenol red was added as the indicator. 5ml of each sugar was dispensed into 15 mls test tubes with Durham's tube introduced in upturned position into each test tube. Each test tube was corked and autoclaved for 15 minutes at 121℃. After autoclaving, the test tubes were allowed to cool after which bacterial isolates were aseptically inoculated into the sugar solution and incubated at 37 ℃ for 2 to 5 days. Changes in the colour from red to yellow indicated production of acid, which implied utilization of the sugar by the bacterial isolate. Collection of gas bubbles in the Durham's tube indicated gas production [16].

2.6 Antibiotic Sensitivity test

A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). 0.5McFarland corresponds to 1.5×10^8 , which is the bacterial turbidity required for antibiotic sensitivity test The 103 bacterial isolates isolated from human faecal storage cisterns were subjected to ten (10) commonly use commercially available antibiotics, viz: Augmentin (30μg), Gentamycin (10μg), Pefloxacin (10μg), Ofloxacin (5μg), Streptomycin (10μg), Sulfomethoxazole-trimethoprim (30μg), Chloramphenicol (30μg); Sparfloxacin (10μg); Ciprofloxacin (5μg) and Amoxicillin (20μg) [19].

Using Kirby-Bauer method, colonies from overnight culture of bacterial isolate, was aseptically picked and inoculated into test tube containing peptone water that had been autoclaved after it was prepared

according to manufacturer's specification. The inoculated test tube was then incubated in a bacteriological incubator at 37^0C for about 18hours. Mueller-Hinton agar was prepared according to manufacturer's specification, autoclaved at 121^0C for 15 minutes. The agar was allowed to cool to about 42^0C and then poured into various petri dishes, reaching 4mm. The agar was then allowed to congeal in the plates. The turbidity of the test tubes containing the incubated isolates (one isolate per tube) were compared to the prepared McFarland standard and diluted with normal saline when necessary. Sterile cotton bud was inserted into each tube and used to evenly swab the the surface of the Mueller-Hinton agar contained in the petri dishes (three petri dishes to an isolate). This was done in less than 15 minutes after comparing with McFarland standard. After few minutes, antibiotic disks were then asepticall picked and placed on the petri dishes. The plates were then incubated at 37^0C for 18 to 24 hours, after which the zone of inhibition for each isolates was then measured and recorded [20].

2.7 Statistical Analysis

Data obtained were subjected to two-way analysis of variance (ANOVA) and treatment means were separated using Duncan's New Multiple Range Test (DBMRT) at $P \leq 0.05$ level of significance with the aid of Statistical Package for Social Sciences (SPSS) version 23.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Mean Colony Forming Unit of the Bacterial Isolates on MaConckey Agar

The colony forming units of the bacterial Isolates from each location are shown (figure 1). Akure has the highest mean colony forming unit of Akure 42.44 ± 1.82^b, while Akungba has the least mean colony forming unit (35.00 ± 1.27^a).

3.1.2 Biochemical Characterization of the Isolates

All the 103 bacterial isolates were subjected to various Biochemical test. The results of the biochemical tests and corresponding characterized isolates are shown (table 1). The tests identified ten (10) bacterial

organisms, viz: EC= Escherichia *coli;* EA= *Enterobacter aerogenes*; KP= *Klebsiella pneumoniae*; ST=*Salmonella typhi*; PA= *Pseudomonas aeruginosa*; SD= *Shigella dysenteriae*; PM= *Proteus mirabilis*; CK=*Citrobacter koseri*; PF= *Providentia alcalifaciens*; and KO=*Klebsiella oxytoca*. Table 2 shows the distribution of the isolates with respect to location. The table shows that E. *coli,* (32.04%), occurred more than any oraganisms identified.

3.1.3 Antibiotic Sensitivity in the Bacterial Isolates

By following standard methods, the isolates were subject to ten (10) antibiotics, using impregnated antibiotic disks. Figure 4.2 shows the zone of inhibition of the isolates with respect to locations. Table 3 shows the mean zone of inhibition of the isolates to the antibiotics used. The list zone of inhibition (0.78 ± 0.68^{a}) was found in *C. koseri* against Augmentin; While the highest zone of inhibition (20.83 ± 0.01^{b}) was found in *P. aeruginosa* against Ciprofloxacin. Table 4 shows the antibiotic resistance patterns of the bacterial isolates: the isolates were least sensitive to Augmentin, where 98 (95.15%) of the isoltes showed resistance; the highest sensitive was found against Ciprofloxacin, where 28 (27.18%) of the isolates showed resistance. Table 5 shows degree of resistance based on the classes of antibiotics to which the isolates showed resistance. From the table, it can be seen that only 2 (1.94%) isolates showed resistance to two classes of antibiotics whereas as many 38 (36.89%) isolates showed resistance to the whole five classes of antibiotics used.

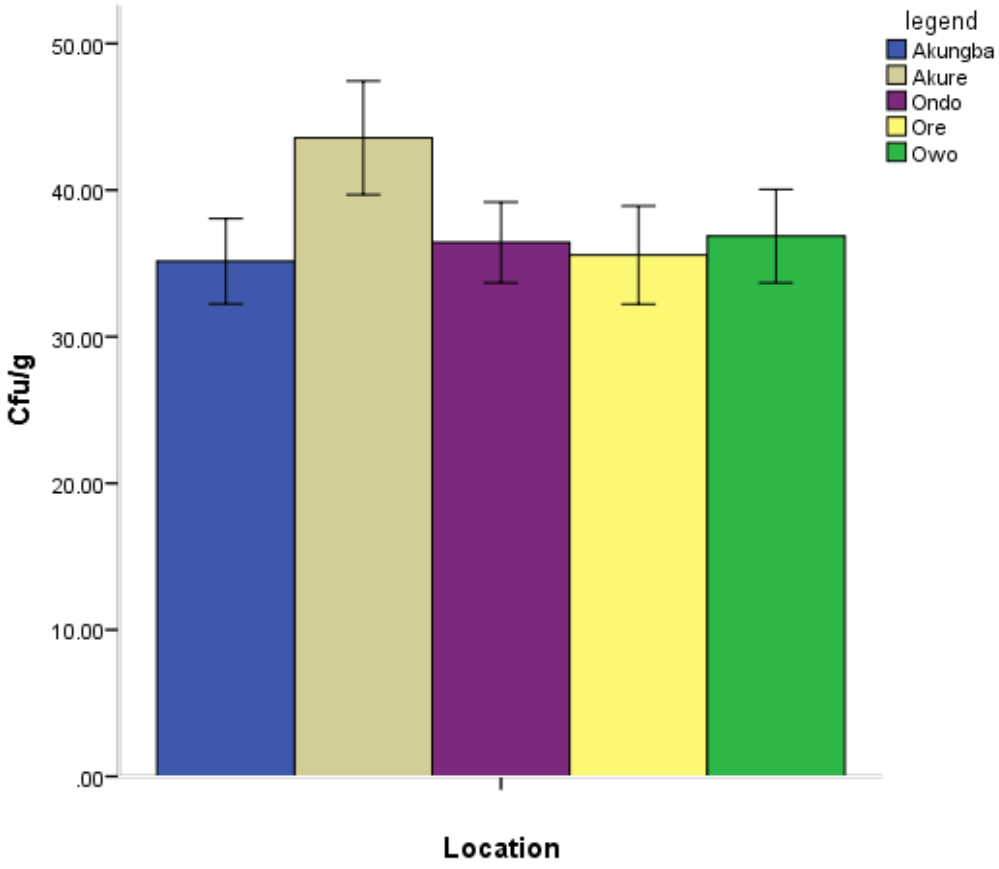

Figure 1: Mean colony forming unit per gram of sample on MaConckey agar, with respect to location.

Table 1: Biochemical Characterization of the Bacterial Isolates

PROBABLE	TRIPLE SUGAR IRON			GR	MS	CoMAC	MR	VP	CAT	OXI	COA	IND	URS	MOT	CIT	ORNITIN E
	Slant	Butt	H_2S													
EC	ACID	ACID	_	_	R	PK	+	_	+	_	_	+	_	MT	_	+

EA	ACID	ACID	_	_	R	PK	_	+	+	_	_	_	_	MT	+	+
KP	ACID	ACID	_	_	R	PK	_	+	+	_	_	_	+	NM	+	_
PA	ALK	ALK	_	_	R	CL	_	_	+	+	_	_	_	MT	+	_
ST	ACID	ALK	+	_	R	CL	+	_	+	_	_	_	_	MT	_	_
SD	ALK	ACID	_	_	R	CL	+	_	+	_	_	+	_	NM	_	_
PM	ACID	ALK	+	_	R	CL	+	_	+	_	_	_	+	MT	+	+
CK	ACID	ACID	_	_	R	PK	+	_	+	_	_	+	+	MT	+	+
KO	ACID	ACID	_	_	R	PK	_	+	+	_	_	+	+	NM	+	_
PF	ALK	ACID	_	_	R	CL	+	_	+	_	_	+	_	MT	+	_

EC= *Escherichia coli;* EA= *Enterobacter aerogenes*; KP= *Klebsiella pneumoniae*; ST=*Salmonella typhi*; PA= *Pseudomonas aeruginosa*; SD= *Shigella dysenteriae*; PM= *Proteus mirabilis*; CK=*Citrobacter koseri*; PF= *Providentia alcalifaciens*; KO=*Klebsiella oxytoca*, GR=Gram reaction, MS=Microscopic shape, CoMAC=Color on MacConkey agar; MR=Methyl Red; VP=Voges praskauer; CAT=Catalase; OXI=Oxidase; COA=Coagulase; IND=Indole; URS=urease; MOT=Motility, CIT=Citrate; PK=Pink; CL=Colourless; ALK=Alkaline; MT=Motility; NM=Non-motile

Table 2: Percentage distribution pattern of the bacterial isolates with respect to locations

ISOLATE	AKUNGBA	OWO	AKURE	ONDO	ORE
E. coli	6	5	10	5	7
E. aerogenes	5	2	3	3	2
K. pneumoniae	1	2	4	3	3
S. typhi	2	2	3	2	3
P. aeruginosa	2	1	2	2	1
S. dyseteriae	2	1	2	0	2
P. mirabilis	0	2	1	2	1
C. koseri	1	1	0	0	2

P. alcalifaciens	1	2	0	0	0
K. oxytoca	1	0	1	0	0
Total	21(20.4%)	18(17.5%)	26(25.2%)	17(16.5%)	21(20.4%)

EC= Escherichia coli; EA= Enterobacter aerogenes; KP= Klebsiella pneumoniae; ST=Salmonella typhi; PA= Pseudomonas aeruginosa; SD= Shigella dysenteriae; PM= Proteus mirabilis; CK=Citrobacter koseri; PF= Providentia alcalifaciens; KO=Klebsiella oxytoca. N/O=Number of occurrence; DIS= distribution

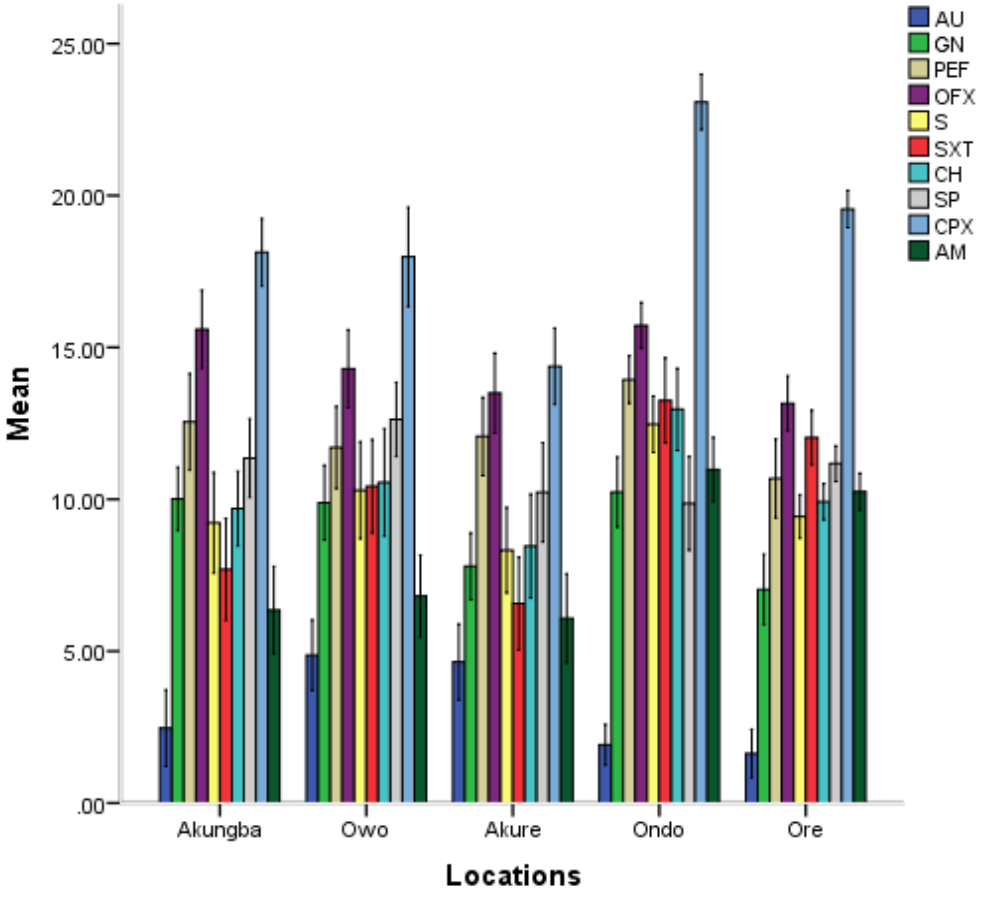

Figure 2: Mean zone of inhibition (with error bars) of the antibiotics disks to the isolates from each location. Each value is a mean of 3 replicates. AU=Augmentin; GN=Gentamycin; PEF=Pefloxacin; OFX=Ofloxacin; S=Septrin; SXT=Sulphomethazole-trimethroprim; CH=Chloramphenicol; SP=Sparfloxacin; CPX= Ciprofloxacin; AM=Ampicillin

Table 3: Zones of inhibitions of the isolated bacteria to the antibiotics used

Tested Organism	AU	GN	PEF	OFX	S	SXT	CH	SP	CPX	AM
Escherichia coli	3.27±0.02^{a}	9.76±0.03^{c}	13.87±0.01^{h}	15.06±0.03^{i}	10.88±0.01^{f}	10.06±0.06^{d}	10.52±0.04^{e}	11.03±0.03^{g}	18.90±0.09^{b}	9.38±0.08^{j}
Enterobacter aerogenes	3.16±1.16^{a}	8.25±0.01^{c}	10.03±0.03^{d}	12.69±0.01^{e}	7.28±0.01^{b}	7.07±0.01^{b}	8.49±0.01^{c}	9.49±0.00^{d}	17.89±0.10^{b}	6.80±0.00^{f}
Klebsiella pneumoniae	3.81±0.01^{a}	7.93±0.03^{b}	11.34±0.30^{g}	14.52±0.01^{h}	9.81±0.16^{e}	9.07±0.06^{d}	10.65±0.01^{f}	10.70±0.02^{f}	19.15±0.01^{c}	8.13±0.02^{i}
Salmonella typhi	1.37±0.01^{a}	7.09±0.02^{c}	10.26±0.01^{g}	12.09±0.01^{i}	8.91±0.08^{e}	8.11±0.00^{d}	10.17±0.01^{f}	10.74±0.04^{h}	17.27±0.03^{b}	6.93±0.06^{j}
Pseudomonas aeruginosa	7.50±0.03^{a}	11.45±0.05^{e}	15.71±0.03h	16.79±0.00^{i}	11.39±0.02^{d}	12.45±0.05^{g}	11.26±0.02^{c}	11.51±0.01^{f}	20.83±0.01^{b}	9.59±0.01^{j}
Shigella dysenteriae	2.52±0.01^{a}	9.06±0.01^{c}	11.85±0.01^{fg}	15.86±0.12^{h}	11.39±0.01^{e}	11.91±0.00^{g}	11.81±0.01^{f}	11.05±0.05^{d}	16.25±0.01^{b}	8.68±0.02^{i}
Proteus mirabilis	2.56±0.00^{a}	9.50±0.01^{d}	11.94±0.01^{f}	16.94±0.00^{h}	9.11±0.00^{c}	13.08±0.02^{g}	11.00±0.00^{e}	11.00±0.02^{e}	17.29±0.01^{b}	7.78±0.00^{i}
Citrobacter koseri	0.78±0.68^{a}	7.94±0.04^{b}	11.42±0.01^{e}	13.34±0.01^{h}	12.33±0.02^{f}	12.83±0.02^{g}	9.28±0.02^{d}	15.86±0.02^{i}	18.34±0.01^{c}	8.50±0.00^{j}
Providentia alcalifaciens	5.18±0.07^{b}	9.93±0.13^{g}	9.33±0.01^{e}	12.34±0.01^{h}	9.80±0.07^{f}	6.91±0.08^{c}	9.05±0.09^{d}	14.34±0.02^{i}	20.32±0.03^{a}	4.03±0.06^{j}
Klebsiella oxytoca	0.72±0.62^{a}	4.13±0.15^{c}	5.00±0.00^{d}	8.00±0.00^{f}	2.19±0.02^{b}	2.03±0.06^{b}	7.82±0.01^{f}	6.68±0.02^{e}	13.07±0.06^{a}	0.75±0.65^{g}

Each value is a mean of three replicates. Values in the same column with same superscripts are not significantly different @ P P ≤ 0.05. Key: AU=Augmentin; GN=Gentamycin; PEF=Pefloxacin; OFX=Ofloxacin; S=Septrin; SXT=Sulphomethazole-trimethroprim; CH=Chloramphenicol; SP=Sparfloxacin; CPX= Ciprofloxacin; AM=Ampicillin

Table 4: Antibiotic resistance pattern of the bacterial isolates [n (%)] to the various antibiotics used

Bacterial Isolates	I.T	AU	GN	PEF	OFX	S	SXT	CH	SP	CPX	AM
E. coli	33	30 (90.91)	21(63.64)	22(66.67)	9(27.27)	15(45.45)	14(42.42)	22(66.67)	25(75.76)	9(27.27)	27(81.82)
E. aerogenes	15	15(100)	13(86.67)	12(80.00)	7(46.67)	10(66.67)	10(66.67)	9(60.00)	13(86.67)	5(33.33)	11(73.33)
K. pneumoniae	13	12(92.31)	10(76.92)	11(84.64)	3(23.08)	8(61.54)	7(53.85)	8(61.54)	12(92.31)	2(15.38)	13(100)
S. typhi	12	12(100	9(75.00)	10(83.33)	7(53.33)	7(53.33)	9(75.00)	10(83.33)	4(33.33)	4(33.33)	11(91.67)
P. aeruginosa	8	7(87.50)	6(75.00)	5(62.50)	1(12.50)	4(50.00)	3(37.50)	5(62.50)	8(100)	2(25.00)	6(75.00)
S. dyseteriae	7	7(100)	7(100)	4(57.14)	2(28.57)	3(42.86)	1(14.29)	5(71.43)	6(85.71)	2(28.57)	5(71.43)
P. mirabilis	6	6(100)	4(66.67)	5(83.33)	0(0.00)	5(83.33)	1(16.67)	3(50.00)	4(66.67)	2(33.33)	5(83.33)
C. koseri	4	4(100)	3(75.00)	3(75.00)	3(75.00)	3(75.00)	0(0.00)	3(75.00)	2(50.00)	0(0.00)	4(100)
P. alcalifaciens	3	3(100)	3(100(	3(100)	2(66.67)	1(33.33)	2(66.67)	3(100)	2(66.67)	0(0.00)	3(100)
K. oxytoca	2	2(100)	2(100)	2(100)	1(50.00)	2(100)	2(100)	1(50.00)	2(100)	2(100)	2(100)
Total	103	98(95.15)	78(75.73)	77(74.76)	35(33.98)	58(56.31)	49(47.57)	69(66.99)	78(75.73)	28(27.18)	88(85.44)

I.T= Isolates tested; AU=Augmentin; GN=Gentamycin; PEF=Pefloxacin; OFX=Ofloxacin; S=Septrin; SXT=Sulphomethazole-trimethroprim; CH=Chloramphenicol; SP=Sparfloxacin; CPX= Ciprofloxacin; AM=Ampicillin

Table 5: Number of antibiotics group and isolates that showed resistance to (a) given group(s)

No of antibiotics group	No of resistant isolates (%)
One	2 (1.94)
Two	14 (13.59)
Three	16 (15.33)
Four	33 (32.02)
Five	38 (36.89)

3.2 Discussion

The colony forming units (on MaConckey agar) of bacterial isolates from the collected samples were found to be very similar in the samples collected from Akungba (35.00 ± 1.27^{a} cfu/g) and Ore (35.25 ± 1.49^{a} cfu/g); and Owo (36.25 ± 1.51^{a} cfu/g) and Ondo (36.43 ± 1.38^{a} cfu/g). In fact , the colony forming units of the bacterial isolates from these four locations could be regarded as similar. Only the colony forming unit of the bacterial isolates from Akure samples (42.44 ± 1.82^{b} cfu/g) was totally different with at least a difference of 7 when compared distinctly with other locations'. These similarities and differencies could be as a result the variation in sample sizes from the locations involved.

Of the 103 bacterial isolates in this study, 26 (25.2%) were isolated from faecal samples from Akure; same number of isolates, 21 (20.4%) were isolated from Akungba and Ore; 18 (17.5%) from Owo; and 17 (16.5%) from Ondo. Biochemical characterization showed that the bacterial isolates were *Escherichia coli* (32.04%), *Enterobacter aerogenes* (14.56), *Klepsiella pneumoniae* (12.62%), *Salmonella typhi* (11.65%), *Psudomonas aeruginosa* (7.77%), *Shigella dysenteriae* (6.80%), *Proteus mirabilis* (5.83%), *Citrobacter koseri* (3.88%), *Providentia alcalifaciens* (2.91%), and *Klepsiella oxytoca* (1.94%). The high number of *E. coli* is likely due to the fact that the bacterium is an aerobic intestinal organism that are passed out with fresh faeces; and it is in conformity with the work of [21], who isolated *E*. *coli* as the most occuring bacteria from human faecal samples. The presence of *Pseudomonads aeruginosa,* which is not an intestinal bacterium, in relatively large numbers can be linked to the versatility of the bacterium to adapt in various environment. The 7.77% *Pseudomonads aeruginosa* population in this work tallies with the one isolated in the work of [22]. Escherichia *coli*, *Citrobacter koseri*, *Salmonella typhi* and *Enterobacter aerogenes* (synonym: *Klepsiella aerogenes*), identified in this study, have earlier been identified by [23], who isolated same bacteria from public toilets in Nigeria. ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) pathogens are responsible for increase in antimicrobial-resistant infections worldwide, and have been rated important pathogens by WHO [24]. Therefore, the isolation of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Enterobacter aerogenes* in this research means that the toilet is an environment that can enhance the propagation of important pathogens that are inimical to human health. The work of [25], who isolated pathogenic bacteria from toilet tap waters, further lend

credence to this. *Proteus mirabilis* and *Escherichia coli* are *major* causes of urinary tract infection; *Pseudomonas aeruginosa* is known to form biofilm in indwelling medical devices like catheter; *Klepsiella pneumoniae* and *K*. *oxytoca* have been isolated from upper respiratory tract infection patients [26]; *Citrobacter koseri* has been identified as a microbial cause of menigitis and cerebral abscess [27]; while *Providentia alcalifaciens* is associated with traveller's diarrhoea [28], all of which were isolated from faecal samples used in this study. It is important to state that there is a continuing high burden of typhoid fever in many parts of the world and a rapid increase in the emergence and spread of antimicrobial resistant strains of *S. typhi* [29].

There was variations in the antibioic sensitivity profile of the bacterial isolates. All the bacteria in this study showed resistance to multiple antibiotics. For example, *Escherichia coli* has a minimum zone of inhibition of 3.27 ± 0.02^{a}, which was showed against Augmentin, and a maximum zone of inhibition of 18.90 ± 0.09^{b}, showed against Ciprofloxacin. This means that following [19] zone of inhibition interpretative criteria, none of the *E*. *coli* in this study was sensitive to any of the 10 antibiotics used. In fact, 90.91% of the *E*. *coli* tested showed resistance to Augmentin. The resistance of the isolates to the various antibiotics used varies from 27.18% to Ciprofloxacin, 33.98% to Ofloxacin, 47.57% to Sulphomethazole-Trimethroprim, 56.31% to Streptomycin, 66.99% to Chloramphenicol, 74.76% to Pefloxacin, 75.73% to Sparfloxacin, 75.73% to Gentamycin, 85. 44% to Ampicillin, and 95.15 to Augmentin. The least resistance, 27.18%, was found against Ciprofloxacin while the highest, 95.15, was found against Augmentin. This is a little more above what was observed in the findings of [30] - an indication that antibiotic resistant bacteria are on the increase. The relatively low number of Isolates which showed resistance to ciprofloxacin may be as a result of the drug's pronounced potency against Gram negative enterobacteriaceae, by interfaring with their DNA gyrase [31], and thereby hindering a complete replication. Meanwhile, [32] findings also showed that enterobacteriacea are relative sensitive to ciprofloxacin. More worrisome is the high level of multidrug resistance isolates. At least 84.46% of the isolates are multidrug resistant. That is, they showed resistance to three or more classes of antibiotics [33]. Of these multidrug resistant isolates, 43.68% showed resistance to all the five groups of antibiotics used. This is very disturbing considering the fact that O'Neill [10] has predicted a post antibiotic era against year 2050 where 10 million people are expected to die yearly due to antibiotic resistant bacteria.

The high distribution, as shown by this study, of multidrug resistant bacteria in faecal storage cisterns, is a big concern. This can be linked to many factors. For example, discarding unused drugs into toilets can contribute to increase in drug resistance among bacteria. Some of the toilets used in this study are two-in-one, that is people can also bath in the toilet. Meanwhile the waste water after bathing goes to the same tank with the faeces. Since there are many antibacterial soaps used for bathing, it is not unlikely that they will contribute to antibacterial resistance among the toilet bacteria. Bacteria in the toilets can find their way, via vertical or horizontal flow, particularly during raining seasons, into water aquifers. Waters from this aquifers will enhance the spread of pathogenic and antibiotic resistance bacteria if used without appropriate sterilization.

In the same way, cockroaches can also propagate the spread of multidrug resistance and ESBL-producing bacteria from the toilet. Cockroaches are insects with long antennae and legs, feeding by scavenging. They are one of the most significant and objectionable pests found in apartments, homes, food-handling establishments, hospitals, and health care facilities worldwide. Indoor species, especially the German cockroach, exploit conditions associated with high-density human populations and impoverished living conditions [34, 35]. Similar bacteria isolated from human faecal samples in this study have also been reportedly isolated from various parts of of cockroaches by many authors in Nigeria [36,37,38] and Ethiopia [35]. The crevices in underground faecal storage cisterns make it more easier for cockroaches to spread antibiotic resistant bacteria in communities and hospital environments. Since cockroaches can also inhabit the dark areas in water wells, they may as well spread drug resistance bacteria from the toilets to the well water. Meanwhile multidrug resistant bacteria have been isolated from wells and underground water storage tanks in Nigeria [39 40]; from surface waters [41]; as well as from toilet indoor airs and toilet door handles [42, 43].

The spread of multidrug resistant bacteria from faecal cisterns crevices within community and hospital environment is not limited to cockroach vectors. Flies, rats, and mouse are potential household vectors that can spread pathogenic and antibiotic resistant bacteria within communities. House flies (*Musca domestica*) have been known as a mechanical vector in spreading infectious diseases such as cholera, shigellosis, salmonellosis and skin infections. House flies are able to transport pathogenic agents by

attaching them to their mouth, body surface, foot, and wings [44]. [45] reported flies carrying beta-lactamase genes. In another research by [46], shiga toxin producing genes was found in *E. coli* isolated from houseflies. In yet another research carried out differently by [47], [45], [48], and [49], multidrug resistant Gram negative enteric bacteria; plasmids carrying antibiotic resistant genes; Cephalosporins resistance genes; and colistin resistance genes respectively were found in bacteria isolated from houseflies.

4.0 CONCLUSION

Findings from this study revealed that multiple-antibiotic resistant bacteria are in high distribution in faecal storage cisterns in Ondo State. And they can be transmitted to human by formite, animal vectors, and water. Consequently, adequate management of faecal storage cisterns is important. Further research is however needed to relate the bacteria isolated from the storage cisterns with bacteria present in water sources within the perimeters of the cisterns.

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