International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 1 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.01.016

Antimicrobial susceptibility of bacteria isolated from abattoir, eatery and hospital effluents

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Abstract

The problems caused by effluents discharge into the environment cannot be overemphasized. This study was conducted to determine the antimicrobial susceptibility and resistance of bacteria isolated from abattoir, eatery and hospital effluents to antibiotics. The temperature and pH of the effluents were determined. Bacteria isolated were identified by biochemical method and antimicrobial susceptibility was done using disc diffusion technique. Initial temperature of abattoir, eatery and hospital effluent were 25 °C, 24 °C and 26 °C respectively. Initial pH of abattoir, eatery and hospital effluent were 6.7, 7.1 and 7.7 respectively. The bacteria from abattoir effluent were identified as *Klebsiella pneumoniae, Escherichia coli, Enterobacter amnigenus, Klebsiella planticola, Klebsiella oxytoca, Enterobacter aerogenes, Klebsiella liquefasciens, Alcaligenes faecalis, Flavobacterium gleum, Acinetobacter mallei, Pseudomonas aeruginosa, Salmonella bongori,* and *Salmonella arizonae.* Meanwhile *Klebsiella pneumoniae, Escerichia coli,* and *Klebsiella planticola* were resistant to septrin; *Enterobacter amnigenus* and *Klebsiella planticola* were resistant to chloramphenicol; *Escherichia coli* was resistant to amoxacilin; others showed susceptibility to the antibiotics. Bacteria from eatery effluent were *Acinetobacter irrovi, Enterobacter cloacae, Klebsiella aerogenes, Enterobacter cloacae, Pseudomonas mallei, Pseudomonas aeruginosa, Enterobacter agglomerans, Escherichia coli, Klebsiella oxytoca, Salmonella arizonae* and *Citrobacter freundi.* Meanwhile *Enterobacter agglomerans* was resistant to septrin and amoxacillin; *Acinetobacter irroffi* was resist to Augmentin; other bacteria were inhibited by the antibiotics. While bacteria from hospital effluent were *Escherichia coli, Enterobacter agglomerans, Citrobacter diversus, Pseudomonas aeruginosa, Citrobacter icoseri, Escherichia hermannii, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas putida, Klebsiella terrigena, Aeromonas hydrophila* and *Proteus vulgaris. Citrobacter diversus, Enterobacter cloacae* and *Klebsiella terrigena* were resistant to septrin; *Citrobacterdiversus,Pseudomonasaeruginosa,Klebsiellaterrigena,*and*Proteus vulgaris* were resistant to chloramphenicol; *Pseudomonas aeruginosa* was resistant to sparfloxacin; *Escherichia hermannii, Enterobacter cloacae, Klebsiella terrigena,* and *Aeromonas hydrophila* were resistant to amoxicillin; *Escherichia coli, Klebsiella terrigena, Aeromonas hydrophila* and *Proteus vulgaris* were resistant to augmentin; *Citrobacter diversus* and *Pseudomonas aeruginosa* were resistant to gentamicin; *Citrobacter diversus* was resistant to pefloxacin and tarivid; other bacteria were inhibited by the antibiotics.

Keywords: Resistance, bacteria, susceptibility, effluents, antibiotics.

Introduction

Environmental problems have increased over the last four decades with improper management practices being largely responsible for the gross pollution of aquatic environment with concomitant increase in water-borne diseases especially typhoid fever, cholera, diarrhoea and dysentery. Effluent is an out-flowing of water from a natural body of water, or from a human-made structure. Effluent is defined by the United States Environmental Protection Agency as "wastewater - treated or untreated - that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes discharged into surface waters" (USEPA 2006).

Bacteria associated with effluents has been extensively studied *Lactobacillus, Bacillus, Pseudomonas, Azotobacter, Arthrobacter, Zoogloea, Microbacterium, Staphylococcus, Micrococcus, Cardiobacterium, Bifidobacterium, Pasterurella, Escherichia and Eikenia*; (Selvi *et al.*, 2012) *Escherichia coli, Bacillus* sp, *Pseudomonas species, Flavobacterium species* and *Alcaligenes species* (Krishnaveni *et al.*, 2013) *Pseudomonas species, Bacillus species, Arthrobacter species* and *Micrococcus species***.**

In the livestock sector, different types of farm animals are capable of carrying a wide range of zoonotic pathogens (Swai and Schoonman, 2012). Moreover, animals brought for slaughter into urban areas more often come from villages where pathogen control regimens are weak, un-coordinated and often not available. Lack of veterinary services in these livestock rearing areas poses a substantial risk of widespread occurrence of diseases in the livestock population and concurrent human exposure to these zoonotic disease agents (Swai and Schoonman, 2012).

Health care waste consists of both organic and inorganic substance including pathogenic microorganisms. Hospital waste possess serious health hazard to the health workers, public and air flora on the area. Hazardous hospital effluents consist of parts of humans, foetus, blood, body fluid, surgery wastes, cultures and stock of infective agent from laboratory, which could cause damages to the handling persons.

In Nigeria, the situation is no better by the activities of most industries and populace towards waste disposal and management which usually leads to the increasing level of pollution of the environment. Effluent is a major menace which is compounded in areas where they are discharged without adequate treatment. This current investigation was aimed to study the effect of antibiotics on bacteria isolated from abattoir, eatery and hospital effluents.

Methods

Collection of samples

Effluents of hospital, abattoir and eatery were collected aseptically from different locations in Lagos metropolis. These samples were taken the laboratory for further analysis

Microbial Analysis of samples

Eosin methylene blue agar, Nutrient agar, MacConkey agar, and Salmonella Shigella agar each were prepared according to manufacturer's direction. 1ml of each sample was taken and dispensed into 9ml of distilled water; this was used for serial dilution in ten folds. 0.1ml from selected dilutions (10^2, 10^4, 10^6 and 10^8) was inoculated into Petri dishes. Using pour plate, the media for isolation were poured onto each plate. These were incubated at 37^{o}C for 24hrs.

Sterilization of media, Glassware/other apparatus

Glasswares such as pipettes were sterilized using the oven at set temperature 180^{o}C for3hrs. Inoculating loop was sterilized by holding the nichrome wire in the Bunsen flame until it is glowed red.Hockey sticks for spreading inoculums on plates were sterilized by dipping in absolute ethanol and flaming to burn off the alcohol. Filter papers were wrapped in aluminium foil and subsequently autoclaved at 121^{o}C for 15mins.

Solid media

Solid culture media used for isolation and enumeration of microorganisms such as nutrient agar, minimal salt agar, Mac Conkey agar, were all sterilized in the autoclave at a temperature of 121^{o}C for 15min. While other media for biochemical tests such as motility medium, starch agar, gelatin agar, Christensen's urea medium, simmon's citrate agar etc. were all sterilized in the autoclave but at varying temperatures depending on the heat stability of the major constituent.

Liquid media

The enrichment medium, minimal salt broth and other liquid media such as nutrient broth, peptone water, MR-VP broth, nitrate peptone water, triptone water

were sterilized in the autoclave at 121°C for 15mins. Sugars were sterilized by tyndallisation while petroleum substrates were placed in screw caps tubes and sterilized by autoclaving at 121°C for 15mins. Distilled water was used at diluents.

Characterization and identification of bacterial isolates

Pure cultures of bacterial isolates were identified on the basis of their morphology and biochemical characteristics. The organisms were subsequently characterized according to the taxonomic scheme of Buchanan and Gibbons 1999. The following tests were performed on each isolate.

Colonial morphology

The shape, size, pigmentation, elevation and marginal characteristics of the bacterial species were examined on nutrient agar plates after appropriate incubation periods.

Gram stain

Smears of young culture (18-24 hours old) of bacterial isolates on a clean glass slide were heat fixed and stained with crystal violet for 30-60 seconds. The dye was drained and then fixed with Lugol's iodine for 30seconds. The slides were rinsed with tap water, decolorized with 95% ethanol forabout 10seconds and again washed with tap water. The slides were counter stained with safranin for 30seconds then rinsed, air dried and examined under the microscope using the oil immersion lens for gram reaction and cellular morphology.

Spore staining

Certain bacteria do produce endospores and spore staining techniques is used to detect the presence of such spores. Smears of 48hours old culture of isolates were heat fixed on different glass slides. These were flooded with malachite green stain and heated over a beaker of boiling water for 10minutess. More stain was continuously added to the slides to avoid drying. The slides were subsequently washed and counter stained with safranin for 20 seconds, washed, blot dried and examined under the oil immersion lens. While the vegetative portion of the organisms stained pink to red, the spores stained green.

Motility tests

The test was carried out using Edwards and Wing motility tests medium. The semi-solid medium was inoculated with the different bacterial isolates by stabling with a sterile inoculating needle at the centre of the medium column to over half the depth. The motility organisms grew and spread out from the line of puncture while the non-motile organisms grew only along the line of puncture.

Catalase production

Most aerobic microorganisms are capable of producing the enzyme catalase although to different extents. The principle of this test is that when organisms containing catalase enzyme are mixed with hydrogen peroxide, gaseous oxygen is released.

$$2H_2O_2 \text{ catalase } 2H_2O_2 + O_2$$

A suspension of 18hours old culture of the test organisms was made with sterile distilled water on a clean glass microscope slide. A few drops of hydrogen peroxide were added using a dropping pipette. The evolution of gas bubbles caused by the liberation of free oxygen indicated the presence of catalase enzyme.

Oxidase tests

This was carried out for the detection of cytochrome oxidase in the microorganisms. The overnight broth culture of isolates was inserted Bactident oxidase test strips. The strips were withdrawn at once and left for 10minutes for color change. Color change from yellow to dark purple confirmed the presence of oxidase.The oxidase test strips were impacted with 1% tetramethyl-p-phenyldiamine solution.

Indole production

Some microbes are capable of hydrolyzing the amino acid tryptophan and one of the end products is indole. The latter reacts with 4dimethylaminobenzaldehyde to form dark red dye stuff.This procedure involved growing the isolates in tryptone broth for 48hours at 35°C, after which 2ml of chloroform were added to the broth culture and mixed gently. About 2 ml of Kovac's reagent were later added, shaken gently and allowed to stand for 20mins. A cherry-red color at the reagent later indicated indole production.

Citrate utilization

Simmon's citrate medium is a nutrient substrate that offers ammonium salts as the only source of nitrogen and citrate as the only carbon source. The degradation of citrates lead to alkalinisation of the medium which is indicated by the pH indicator bromothymol blue changing color from green to deep blue.Salts of simmon's citrate agar were inoculated with light inoculum of the isolates and incubated at 35^{o}C for 5 days. Color changes from green to blue indicated a positive result.

Nitrate reduction

A biochemical characteristic of many bacteria is the ability to reduce nitrates. The product, nitrate, is then tested by a special reagent. The test organisms were inoculated separately into tubes containing nitrate peptone water and Durham tubes and incubated at 35^{o}C for 2days. The test for nitrate reduction was determined by the addition of 1ml each of reagents 1 and 2 of the modified Greiss-ilosvay's reagent. The presence of nitrate was indicated by the development of pink, purple or maroon color within a few minutes. Presence of Gas in the Durham tubes also suggested production of gaseous nitrogen and consequently a positive result.

Urease activity

Urea, a common organic nitrogen source for many microbes, can be hydrolyzed to ammonia and carbon dioxide. The latter produces an alkaline condition in the medium which is indicated by a color change of the pH indicator. Slants of Christensen's urea agar medium were inoculated with the isolates and incubated at 35^{o}C for 5-7days watching daily for any color change. The development of color change from yellow to pink showed a positive urease activity.

Methyl-red voges proskaurer test

These are actually two tests in one. In the methyl red test a medium that contains a little carbohydrate fermentable by microorganisms is used. Some microorganisms normally ferment carbohydrate accompanied with acid production and hence the color of methyl red retains its red acid color while others ferment carbohydrates without acid production and hence the methyl red changes to yellow. Some of these products include acetonic (acetyl methyl carbinol), 2,2-butanediol or diacetyl. The presence of the metabolic products is established by means of Barritts or APHA reagent. In the strongly alkaline environment of these solutions, acetone and 2,3-butanediol are oxidized to diacetyl which in turn reacts with the reagent to form guanidine. This is the basis of VP tests.The isolates were inoculated into 10ml of MRVP medium and incubated at 35^{o}C for 3 days. After incubation, the tests were performed in the following way:

MR TESTS- five drops of methyl red indicator were added to the culture. A red color indicated a positive reaction.

VP TEST-5ml of APHA reagent (mixture of 1g of copper sulphate (blue)dissolved in 40ml of saturated sodium hydroxide solution were added to the culture. A pink to red color indicated a positive reaction.

Gelatin hydrolysis

Plates of gelatine agar were inoculated with test organism and incubated at35^{o}C for 3days following incubation; the plates were flooded with 5-10ml acid mercury chloride solution. Clear zone indicated area of gelation hydrolysis.

Starch hydrolysis

Many bacteria possess enzymes called amylases which can hydrolyse complex molecules of starch to sugars. Sugar agar plates were inoculated with different bacterial isolates and incubated at 35^{o}C for 2days. After incubation, each plate was flooded with aqueous iodine and left for 30seconds. A clear zone surrounding the colonies indicated a positive test, while a blue-black coloration indicated the presence of starch meaning the latter had not been hydrolyzed.

Carbohydrate utilization

The fermentation of sugars is demonstrated by the production of acid or acid and gas (carbon dioxide and/or hydrogen). The ability of an organism to ferment several sugars can be demonstrated by incorporating the sugars into a basal medium (peptone water) and testing for acid and gas production. 1ml portion of the 10% sugar (glucose, fructose, sucrose, maltose, lactose, mannitol and sorbitol solution was added to 10ml of the basal medium containing the indicator phenol red and Durham tube. The media were inoculated with test organisms and incubated at 35^{o}C for 2-7days watching daily for color change. Acid production was indicated by the appearance of a yellow colour in the medium and gas production by

the presence of an air space in the inserted Durham tubes.

Antibiotic assay (antibiogram test)

Mueller Hinton 38g was weighed into 100ml of distilled water, homogenized on a hot plate magnetic stirrer and subsequently sterilized at 121^{o}C for 15minutes in an autoclave. The cool molten Mueller Hilton agar was poured into sterile petri dishes. The plates were allowed to set and surface dried in an oven at 45^{o}C. The Mueller Hinton agar plates were seeded with freshly test strains of about 18-24hours by swabbing using sterile swab-sticks to make cell suspensions of the organisms to give a concentration of about 10^5 cells/ml and 0.1ml aliquot test organisms suspension was placed onto the Mueller Hinton plates and with the acid of hockey stick, the bacterial suspension was aseptically spread on the agar surface. The plates were allowed to dry for 1hr at room temperature. Multi-disk containing the antibodies e.g. Streptomycin, streptrin, chloramphenicol, sparfloaxin, ciprofloaxin, amoxicillin, augumentin, gentymycine, pefloaxacin was placed onto the inoculated plates using diffusion disc methods. The plates were incubated at 35^{o}C for 2hours. After incubation period, the culture plates were examined for areas of no growth around the disc (zone of inhibition). Bacterial strains resistant to antimicrobials grow up to the edges of the disc as against the sensitive strains which are inhibited at a distance from the disc. Disc containing the streptomycin, co-trimaxazole and other antibiotics were used.

Casein hydrolysis

Nutrient agar (250ml) was prepared and 1%w/v (2.5g of casein) casein powder was added to nutrient agar homogenized on hot plate magnetic stirrer. The medium was sterilized in an autoclave at 115^{o}C for 10minutes, cooled to about 45-50^{o}C and poured aseptically in petridishes. The plates were allowed to set and dry at 45^{o}C. Fresh culture or isolates of 18-24hours were inoculated onto plates of casein agar, incubated at 35±2^{o}C for 5days. Plates were examined for clearing of the medium around the bacterial growth using 20% acid mercuric chloride (HCl and HgCl) solution.

Results

Physical parameters of effluent samples showed that effluent from abattoir, eatery, and hospitals had a pH of 6.7, 7.1 and 7.7 respectively (Table 1) while the temperature of these wastes ranged between 25 ºC-26 ºC (Table 1). Microbiological investigation on the effluent showed that abattoir effluent had the least bio load of mesophilic heterotrophic bacteria ($1.8x10^6$cfu/g), while eatery own effluent and hospital effluent had the highest mesophilic heterotrophic bacterial counts of $1.76x10^6$cfu/ml and $1.12x10^6$cfu/ml, respectively (Table 2). In addition, *salmonella and shigella* counts of the wastes were $1.73x10^4$cfu/g, $3.8x10^5$cfu/ml and $4.0x10^4$cfu/ml in the abattoir, eatery and hospital effluents respectively (Table 3). At dilutions above 10^4, no more strains of *Salmonella* and *Shigella* were isolated in the effluents of abattoir (Table 3).Furthermore, it was observed that the total *Enterobacteria* counts in the abattoir, eatery and hospital effluents were $7.0x10^4$cfu/ml, $4.2x10^5$cfu/ml, and $2.4x10^5$cfu/ml, respectively (Table 4).

In addition, the total viable *Escherichia coli* counts isolated using methylene blue agar were$1.0x10^4$cfu/ml, $2.6x10^5$cfu/ml and $2.12x10^4$cfu/ml, respectively (Tables 5 and 6).The probable identities of the microbial isolates were established using morphological properties on solid media and gram reaction of the abattoir, hospital and eatery isolates were as shown in Tables 7, 8 and 9 respectively.

Table 1: Physical parameters of effluent samples

S/N	Sample	Temperature (^{o}C)	pH
1.	Abattoir Effluent	25	6.7
2.	Eatery Effluent	24	7.1
3.	Hospital Effluent	26	7.7

Table 2: Total viable count of mesophilic isolates from the effluents (cfu/ml) on Nutrient Agar

Effluent sample	Dilution 10^o	Dilution 10^2	Dilution 10^4
Abattoir	Numerous	1.57×10^4	1.8×10^5
Eatery	2.48×10^2	2.44×10^4	1.76×10^6
Hospital	1.48×10^2	9.7×10^3	1.12×10^6

Table 3: Total viable count of mesophilic isolates from the effluents (cfu/ml) on Salmonella Shigella Agar

Effluent sample	Dilution 10^o	Dilution 10^2	Dilution 10^4
Abattoir	Numerous	1.73×10^4	NIL
Eatery	Numerous	1.59×10^4	3.8×10^5
Hospital	Numerous	1.80×10^4	4.0×10^4

Table 4: Total viable count of mesophilic isolates from the effluents (cfu/ml) on MacConkey Agar

Effluent sample	Dilution 10^o	Dilution 10^2	Dilution 10^4
Abattoir	Numerous	1.78×10^4	7.0×10^4
Eatery	Numerous	5.84×10^4	4.2×10^5
Hospital	Numerous	5.60×10^4	2.4×10^5

Table 5: Total viable count of mesophilic isolates from the effluents (cfu/ml) on Eosin Methylene Blue Agar

Effluent sample	Dilution 10^o	Dilution 10^2	Dilution 10^4
Abattoir	4.04×10^2	1.60×10^4	3.0×10^4
Eatery	Numerous	3.08×10^4	2.6×10^5
Hospital	8.4×10^1	2.12×10^4	4.4×10^5

Table 6: Total viable count of mesophilic isolates from the effluents (cfu/ml) on Violet Red Bile Agar

Effluent sample	Dilution 10^o	Dilution 10^2	Dilution 10^4
Abattior	Numerous	1.68×10^4	NIL
Eatery	Numerous	3.82×10^4	1.7×10^5
Hospital	Numerous	3.14×10^4	2.1×10^5

Table 7: Morphological characteristics of probable isolates from abattoir effluent

Isolate	Shape	Color	Size	Surface	Margin	Elevation
A1	Rods	Pink	Large	Smooth	Round	Convex
A2	Rods	Pink	Large	Smooth	Round	Raised
A3	Rods	Pink	Large	Smooth	Round	Raised
A4	Rods	Pink	Large	Smooth	Round	Convex
A5	Rods	Pink	Medium	Smooth	Round	Convex
A6	Rods	Pink	Medium	Smooth	Round	Raised
A7	Rods	Pink	Large	Smooth	Round	Raised
A8	Rods	Cream	Small	Smooth	Round	Raised
A9	Rods	Yellow	Medium	Smooth	Round	Raised
A10	Rods	Pink	Small	Smooth	Round	Raised
A11	Rods	Black	Medium	Smooth	Round	Raised
A12	Rods	Black	Small	Smooth	Round	Convex
A13	Rods	Black	Medium	Smooth	Round	Convex

Table 8: Morphological characteristics of probable isolates from eatery effluent

Isolate	Shape	Color	Size	Surface	Margin	Elevation
C1	Rods	Black	Large	Smooth	Round	Raised
C2	Rods	Pink	Large	Smooth	Round	Convex
C3	Rods	Pink	Medium	Smooth	Round	Flat
C4	Rods	Black	Medium	Smooth	Round	Raised
C5	Rods	Pink	Medium	Smooth	Round	Raised
C6	Rods	Black	Medium	Smooth	Round	Raised
C7	Rods	Black	Large	Smooth	Round	Raised
C8	Rods	Pink	Medium	Smooth	Round	Convex
C9	Rods	Pink	Medium	Smooth	Round	Raised
C10	Rods	Black	Large	Smooth	Round	Convex
C11	Rods	Pink	Large	Smooth	Round	Convex
C12	Rods	Pink	Small	Smooth	Round	Convex
C13	Rods	Pink	Large	Smooth	Round	Flat

Table 9: Morphological characteristics of probable isolates from hospital effluent

Isolate Code	Shape	Color	Size	Surface	Margin	Elevation
C1	Rods	Black	Large	Smooth	Round	Raised
C2	Rods	Pink	Large	Smooth	Round	Convex
C3	Rods	Pink	Medium	Smooth	Round	Flat
C4	Rods	Black	Medium	Smooth	Round	Raised
C5	Rods	Pink	Medium	Smooth	Round	Raised
C6	Rods	Black	Medium	Smooth	Round	Raised
C7	Rods	Black	Large	Smooth	Round	Raised
C8	Rods	Pink	Medium	Smooth	Round	Convex
C9	Rods	Pink	Medium	Smooth	Round	Raised
C10	Rods	Black	Large	Smooth	Round	Convex
C11	Rods	Pink	Large	Smooth	Round	Convex
C12	Rods	Pink	Small	Smooth	Round	Convex
C13	Rods	Pink	Large	Smooth	Round	Flat

Investigations showed that bacteria associated with the abattoir effluent were *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter amnigenus*, *Klebsiella planticola*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Klebsiella liquefaciens*, *Alcaligenes faecalis*, *Acinobacter mallei*, *Pseudomonas aeruginosa*, *Salmonella bongory* and *Salmonella arizona* (Table 10). From the eatery effluent wastes, thirteen (13) isolates associated were *Acinetobacter iwoffi*, *Enterobacter cloacae*, *Klebsiellaaerogenes*, *Enterobacter*,*Pseudomonasmullei*, *Pseudomonas aeruginosa*, *Enetrobacteragglomerans*, *Escherichia coli*, *Klebsiellaoxytoca*, *Salmonella arizonae*, *Citrobacterfreundii* and *Acinetobacter iwofii* (Table 11).In the hospital effluent, the bacterial species associated were *Escherichia coli*, *Enterobacter agglomerans*, *Citrobacterdiversus*,*Pseudomonas aeruginosa*, *Citrobacterkoseri*, *Escherichia hermannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas putida*, *Klebsiella terrigena*, *Aeromonas hydrophila and Proteus mirabilis* (Table 12).

Out of a total of 13 isolates from abattoir effluent, 100% of all the isolates were susceptible to streptomycin. Out of a total of 13 isolates, only 30% were resistant to Septrin while 70% of the isolates were between intermediate or susceptible ranges (Table 13). Chloramphenicol was also resisted by 30% of the isolates. In addition, Augmentin were susceptible. The 13 microbial isolates from the abattoir were 100% susceptible to Ciprofloxacin, Peflacin, Oflaxacin, Gentamycin, and Sparfloxacin etc. (Table 13).

Table 10: Biochemical tests for microbial identification for abattoir effluent

Isolate code	Gram's Reaction	Catalase	Oxidase	Methyl Red	VogesProskauer	Casein	Citrate	Urease	Starch Hydrolysis	Indole	motility	Gelatin	No3 Reduction	Glucose	Fructose	Sucrose	Xylose	Galactose	Sorbitol	Lactose	Maltose	Inositol	Arabinnose	Mannitol	Probable Identity
A1	-	+	-	-	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	*Klebsiella pneumoniae*
A2	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
A3	-	+	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Enterobacter amnigenus*
A4	-	+	-	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	*Klebsielaplanticola*
A5	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	*Klebsiellaoxycota*
A6	-	+	-	-	+	-	-	+	+	-	+	+	_	+	+	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*
A7	-	+	-	+	-	-	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	*Klebsiellal iquefasciens*
A8	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	*Alcaligenes faecalis*
A9	-	+	+	-	-	-	-	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	*Flavobacterium gleum*
A10	-	+	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	+	*Acinetobacter mallei*
A11	-	+	+	-	-	-	+	+	-	-	+	+	-	+	-	+	-	-	-	-	+	-	+	+	*Pseudomonas aeruginosa*
A12	-	+	-	+	-	-	+	-	-	-	+	-	+	+	-	-	+	-	-	-	+	-	-	+	*Salmonella bongory*
A13	-	+	-	-	-	-	+	-	-			+	+	_	+	-	-	-	-	-	-	+	-	-	- *Salmonella arizonaea*

Table 11: Biochemical tests for microbial identification for eatery effluent

Isolate code	Gram's Reaction	Catalase	Oxidase	Methyl Red	VogesProskauer	Casein	Citrate	Urease	Starch Hydrolysis	Indole	motility	Gelatin	No3 Reduction	Glucose	Fructose	Sucrose	Xylose	Galactose	Sorbitol	Lactose	Maltose	Inositol	Arabinnose	Mannitol	Probable identity
B1	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	*Acinetobacter iwoffi*
B2	-	+	-	-	+	-	+	+	+	-	+	-	-	+	+	+	+	-	+	-	+	-	+	+	*Enterobacter cloacae*
B3	-	+	-	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Klebsiella aerogenes*
B4	-	+	-	-	+	-	+	+	+	-	+	-	-	+	+	+	+	-	+	-	+	-	+	+	*Enterobacter cloacae*
B5	-	+	+	-	-	-	+	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-	+	*Pseudomonas mullei*
B6	-	+	+	-	-	-	+	-	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	+	*Pseudomonas aeruginosa*
B7	-	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	*Enterobacter agglomerans*
B8	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
B9	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	+	+	*Klebsiella oxytoca*
B10	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
B11	-	+	-	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	+	-	-	-	*Salmonella crizonae*
B12	-	+	-	+	-	-	+	+	-	-	+	-	-	+	-	+	+	-	+	+	+	-	+	+	*Citrobacter freundii*
B13	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	*Acinetobacter irroffi*

Table 12: Biochemical tests for microbial identification for hospital effluent

Isolate code	Gram's Reaction	Catalase	Oxidase	Methyl Red	VogesProskauer	Casein	Citrate	Urease	Starch Hydrolysis	Indole	motility	Gelatin	NO_3 Reduction	Glucose	Fructose	Sucrose	Xylose	Galactose	Sorbitol	Lactose	Maltose	Inositol	Arabinnose	Mannitol	Probable identity
C1	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
C2	-	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	*Enterobacter agglomerans*
C3	-	+	-	+	-	-	+	-	-	+	+	-	+	+	-	-	+	-	+	-	+	-	-	+	*Citrobacterdiversus*
C4	-	+	+	-	-	-	+	-	-	-	+	+	+	+	-	+	-	-	+	-	+	-	+	+	*Pseudomonas aeruginosa*
C5	-	+	-	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Citrobacter koseri*
C6	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
C7	-	+	-	+	-	-	-	+	-	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	*Escherichia hermannii*
C8	-	+	-	-	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	*Klebsiella pneumoniae*
C9	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	+	-	+	-	+	+	*Enterobacter cloacae*
C10	-	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	*Pseudomonas putida*
C11	-	+	-	+	+	-	+	-	+	-	-	-	_	+	+	+	+	+	+	+	+	+	+	+	*Klebsiella terrigeria*
C12	-	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	*Aeromonas hydrophila*
C13	-	+	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	+	=	+	-	-	-	*Proteus vulgaris*

Table 12: Biochemical tests for microbial identification for hospital effluent

Isolate code	Gram's	Catalase	Oxidase	Methyl Red	VogesProskaue	Casein	Citrate	Urease	Starch	Indole	motility	Gelatin	NO_3 Reduction	Glucose	Fructose	Sucrose	Xylose	Galactose	Sorbitol	Lactose	Maltose	Inositol	Arabinnose	Mannitol	Probable identity
C1	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
C2	-	+	-	+	+	-	+	-	-	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	*Enterobacter agglomerans*
C3	-	+	-	+	-	-	+	-	-	+	+	-	+	+	-	-	+	-	+	-	+	-	-	+	*Citrobacter diversus*
C4	-	+	+	-	-	-	+	-	-	-	+	+	+	+	-	+	-	-	+	-	+	-	+	+	*Pseudomonas aeruginosa*
C5	-	+	-	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	*Citrobacter koseri*
C6	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	*Escherichia coli*
C7	-	+	-	+	-	-	-	+	-	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	*Escherichia hermannii*
C8	-	+	-	-	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	*Klebsiella pneumoniae*
C9	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	+	+	-	+	-	+	-	+	+	*Enterobacter cloacae*
C10	-	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	*Pseudomonas putida*
C11	-	+	-	+	+	-	+	-	+	-	-	-	_	+	+	+	+	+	+	+	+	+	+	+	*Klebsiella terrigeria*
C12	-	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	*Aeromonas hydrophila*
C13	-	+	-	+	+	-	+	+	-	-	+	+	-	+	-	+	+	-	+	=	+	-	-	-	*Proteus vulgaris*

Table 13: Antibiotic screening tests for identified isolates from abattoir effluent

Probable identity	S	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX
Klebsiella pneumoniae	15	**R**	12	14	13	16	14	12	16	16
E. coli	16	**R**	09	13	24	**R**	12	13	22	15
Enterobacter amnigenus	14	12	**R**	17	20	06	13	11	20	18
Klebsiella planticola	20	**R**	**R**	18	15	18	20	15	14	20
Klebsialla oxycola	12	15	13	14	20	14	16	12	21	15
Enterobacter aerogenes	13	18	14	23	22	20	20	17	18	19
Klebsiella liquefaciens	15	17	16	16	19	12	13	11	22	24
Alcaligens faecalis	16	15	14	24	28	13	23	12	25	23
Flavobacterium gleum	15	20	16	22	26	20	24	13	24	23
Acinectobacter malleir	16	18	12	24	24	10	22	18	22	24
Pseudomonas aeruginosa	14	16	10	17	20	10	20	12	20	21
Salmonella bongory	14	17	13	20	23	09	19	15	18	17
Salmonella arozonaea	16	12	12	16	22	17	20	18	21	23

Key – S = Streptomycin, SXT =Septrin, CH = Chloramphenicol, SP =Sparfloxacin, CPX =Ciprofloxacin, AM =Amoxacilin, AU =Augumentin, CN =Gentamycin, PEF =Pefloxacin, OFX =Tarivid/Ofloxacin, R=Resistance

Out of 13 isolates from eatery effluent, Septrin, Amoxacillin and Augumentin were resisted 7.69% whereas the isolates were 100% susceptible to Streptomycin, Ciprofloxacin Chloramphrenicol, Sparfloxacin, Pefloxacin, Ofloxacin, Augumentin, Gentamycin (Table 14). Out of a total of 13 isolates; 15.38%1, 15.38%, 7.69% were resistance to Augumentin, Amoxaxylin and Streptomycin respectively (Table 14).Out of a total of 13 isolates from hospital effluents, 92.31% were susceptible to Streptomycin while only 7.69% were susceptible at intermediate level. No single strain resisted Septrin. In addition, 23.01%, 7.6% and 69.23% of the hospital isolates were resistant, susceptible at intermediate level and susceptible to Septrin (Table 15). Interestingly, all the isolates remained 100% susceptible to Ciprofloxacin and Streptomycin (Table 15).In addition, only about 30.06% of the hospital isolates resisted Amoxicillin while about 23.07% of the hospital isolates were slightly susceptible at intermediate level to Amoxicillin. A striking observation is 30.76% of all the hospital isolates resisted Chloramphenicol and Augumentin (Table 15). In addition, 7.69% of the isolates from hospital effluents resisted Peflacin and Ofloxacin (Table 15).

Discussion

The physico-chemical properties of the effluents showed that the abattoir effluent was within the acidic pH while eatery and hospital effluent were within the alkaline pH.

In terms of microbiological properties of the effluents, all the three effluents had similar heterotrophic bacteria counts of 10^6cfu/ml. The enteric bacteria load in effluents of hospital, abattoir and eatery were within 10^4cfu/ml, and this is not surprising since in Nigeria, the eateries are full of unwholesome practice of using non-potable water and food are carriers of pathogenic/infectious agents like *E.coli*, *Salmonella*e.t.c.

Table 14: Antibiotic screening tests for identified isolates from eatery effluent

Probable identify	S	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX
Acinetobacter iwoffi	14	16	13	17	15	11	**10**	**08**	12	**11**
Enterobacter cloacae	14	13	15	12	13	12	13	12	16	14
Klesiella aerrogenes	14	16	15	16	17	13	18	11	16	18
Enterobacter cloacae	13	16	14	15	16	13	12	**11**	17	16
Pseudomonas mullei	13	17	12	15	15	12	14	13	17	17
Pseudomonas aeruginosa	12	14	13	16	17	13	15	12	16	14
Enterobacter agglomerans	17	**R**	16	13	14	**R**	**07**	12	**09**	**08**
E. coli	14	17	14	15	14	15	16	14	15	13
Klebsiella oxytoca	13	14	15	17	13	14	13	14	13	15
E. coli	14	15	13	16	18	15	12	15	17	16
Salmonella arizonae	15	17	16	15	17	11	13	14	16	15
Citrobacter freundi	14	16	14	13	19	12	15	16	18	17
Acinetobacter iroffi	13	09	14	17	16	11	**R**	15	17	16

Key – S = Streptomycin, SXT =Septrin, CH = Chloramphenicol, SP =Sparfloxacin, CPX =Ciprofloxacin, AM =Amoxacilin, AU =Augumentin, CN =Gentamycin, PEF =Pefloxacin, OFX =Tarivid/Ofloxacin, R=Resistance

Table 15: Antibiotic screening tests for identified isolates from hospital effluent

Probable identify	S	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX
E. coli	15	18	13	18	18	13	16	15	21	20
Enterobacter agglomerans	14	16	15	15	16	12	11	16	17	18
Citrobacter diversus	13	**R**	**R**	14	16	13	12	**R**	**R**	**R**
Pseudomonas aeruginosa	14	15	**R**	**R**	13	12	14	**R**	12	11
Citrobacter koseri	14	17	12	18	17	16	15	15	19	18
E. coli	13	16	18	17	17	11	**R**	12	12	13
E. harmanni	17	16	10	17	13	**R**	11	11	16	15
Klebsiella pneumoniae	11	13	14	18	19	16	07	10	15	16
Enterobacter cloacae	12	**R**	18	19	17	**R**	08	13	15	14
Pseudomonas putida	15	14	14	16	15	10	09	12	16	15
Klebsiella terrigeria	08	**R**	**R**	15	16	**R**	**R**	10	18	16
Aeromonas hydrophila	13	09	07	16	17	**R**	**R**	11	14	12
Proteus vulgaris	12	10	R	19	20	11	**R**	13	18	07

Key – S = Streptomycin, SXT =Septrin, CH = Chloramphenicol, SP =Sparfloxacin, CPX =Ciprofloxacin, AM =Amoxacilin, AU =Augumentin, CN =Gentamycin, PEF =Pefloxacin, OFX =Tarivid/Ofloxacin, R=Resistance

In addition, presence of high enteric bacteria in the effluent from hospital origin underscores the point that there is a high level of faecal contamination either directly from sewage or poor hygiene practice. Svanstrom (2014) in Sweden reported in similar way to the findings of this work a bioload of $6.6x10^6$cfu/ml of *E. coli* and *Enterococcus* in abattoir effluents.

In terms of microbial diversity, this work tallies with the work of Svantrom (2014) which reported the isolation of *E.coli, Enterococcus, Salmonella* in abattoir waste water effluents. Similarly, in Ado-Ekiti (Nigeria), Oluyege and Famurewa (2015) reported the isolation of *E. coli*, *Klebsiellaspp*, and *Enterobacter spp* from eatery houses at various percentages.

Pathogens can spread from animals to man by several ways including food, water, vectors and aerosols (Centre for food security and public health, 2008). A study by Adeyemi and Adeyemo (2007) suggested that wild animals can transfer pathogens to humans and other animals from abattoir waste by feeding on the same abattoir effluent which is heavily loaded with bacteria species can accidentally/indiscriminately be discharged into water bodies and that threathen the public health via water body and aquatic life contamination.

In terms of hospital wastes microbial load observation tallies with the report of Nwachukwu*et al* (2013) which documented high load of various groups of bacteria e.t.c. however in terms of microbial spectrum, the current work did not tally with work of Nwachukwu and Orji (2012) which reported the isolation of *Listeria monocytogenes* from abbatoir wastes. This current study did not isolate any strain of *Listeria* from eatery, abattoir and hospital wastes. Studies on antibiotic susceptibility patterns of isolates from abattoir effluents showed that septrin, chloramphenicol and amoxicillin were resisted while Peflacine, Ofloxacin, Ciprofloxacin, Sparfloxacin, and Gentamycin are most effective against abattoir effluent pathogen.

The higher resistance observed in Septrin, Chloramphenicol and Amoxicillin are not surprising and even in Lagos, Nigeria, these antibiotics have been grossly abused even in veterinary practices while the guinolonoez (Sparfloxacin, Ciprofloxacin etc) are not usually abused probably because of their high cost.

Earlier work by Olayemi *et al.*, (1979) showed similar patterns of *Salmonella* isolate resistance as 46%, 40% and 3% of the *Salmonella* strains from farms in Zaria resisted Sulphonamide, Septrin and aminoglycoside(tetracycline) respectively.

Relatively recently, Etinosa and Ifeyinwa (2014) evaluated the antibiotic resistant genes in abattoir environment in Benin city and reported higher resistance (79.4%) of Amikacin and Aztreonam amongst strains of *Pseudomonas aerugmoza*.

From this study, isolates from eatery houses showed less resistance or more susceptibilities to the commonly used antibiotics in Lagos-Nigeria. However, strain B7 (*Enterobacter agglomerans*) showed peculiar characteristics of resisting antibiotics like Septrin, Amoxicillin and Peflacine (at intermediate level). This isolate is of public health importance as food infection by it will require special attention to control. The work of Oluyeye and Famurewa (2015) tallied with our report as it observed multi-drug (Amoxicillin, Cotrimaxazole and Tetracycline) resistant *E.coli,Klebsiella, Proteus spp* and *Salmonella* from various foods in Ado-Ekiti Nigeria. Tendencia*et al.,* (2002) extensively reviewed the significant roles of food and food-borne pathogens in the food chain in the epidemiology of antibiotic resistant strains of micro organism.

From this investigation, the isolates from hospital environment shows highest resistance to commonly used antibiotics. This is not surprising as most of the patients attending the hospital may have previously abused these antibiotics.

Again, most of the hospital isolates have been reported to have resistance (R) plasmids and these increases the chance of horizontal gene transfer among bacteria in a human tissue ecosystem. These resistant colonies was definitely be flushed into effluents in a hospital environment. This current investigation tallies with the work of Atata*et al*. (2013) which reported multi-drug resistant strains *of Staphylococcus epidermidis*, *Klebsiella* sp., *Proteus mirabilis*, and Bacillus cereus from hospital effluent in Nigeria. The work of Atata e*t al*. (2013), also re-emphasised the central role of resistance (R) plasmids in mediation of production of enzymes that breakdown antibiotics.

Conclusion

Bacteria isolates from hospital. Abattoir and eatery effluents are related belonging to commonly encountered physiological groups like Enterobacteriacea.

Antimicrobial drug resistance among the pathogens is highest in the hospital and effluents, but least in eatery effluents.

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DOI:10.22192/ijarbs.2017.04.01.016	

How to cite this article:
Tijani, A. A., Famotemi, A. C., Orji, F. A., Abimbola F. B., Adams, F. M. and Aba, E. (2017). Antimicrobial susceptibility of bacteria isolated from abattoir, eatery and hospital effluents. Int. J. Adv. Res. Biol. Sci. 4(1): 142-156.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.01.016